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IN VITRO CHARACTERIZATION OF THE MECHANISM OF INSULIN DEGRADATION AND THE EFFECT OF CHLOROQUINE

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Summary

The cultured rat hepatoma cell line, MH_1C_1, inactivates ^{125}I-labeled insulin by a temperature dependent mechanism. The estimated K_m for insulin degradation is $1.4 \cdot 10^{-7}$ M and the V is $2.5 \cdot 10^{-10}$ mol/10^6 cells/h. The iodocompounds released from cells preincubated at 0°C with ^{125}I-labeled insulin are immunoreactive with anti-insulin antibody, while the iodocompounds released from the cells incubated at 37°C only reacts to a very small degree with anti-insulin antibody. The degradation products were analyzed by Sephadex gel chromatography. Sephadex G-75 gel filtration of iodocompounds derived from cells incubated at 37°C with ^{125}I-labeled insulin for 5, 20 min and 1 h showed a progressive decrease in intact insulin, and an increase in the peak representing insulin breakdown products. Treatment with chloroquine, a lysosomal enzyme inhibitor, resulted in a large increase of cell-associated insulin compared to control cells. However, chromatographic studies of iodocompounds extracted from cells incubated with or without chloroquine show a similar pattern but differ in the size of the peak which represents the degradation products of ^{125}I-labeled insulin. Furthermore, the iodocompounds released from the chloroquine treated cells were not immunoreactive with anti-insulin antibody. These results suggest that chloroquine inhibits the release of insulin degradation products from the cells.

Introduction

The mechanism of insulin action on the target tissues has been extensively studied in many laboratories [1—5]. Interaction of insulin with target cells

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

involves an initial binding of the ligand to a specific surface membrane receptor [1—4]. It was shown that binding of insulin to the receptors was a reversible reaction [1—3]. Recent evidence indicates that insulin molecules may be internalized by a mechanism which involves an initial interaction with the receptor [6—8]. It has been shown that several intracellular components have the capacity to bind insulin with high affinity [9—10]. However, a portion of hormone molecules will be "degraded" by the intracellular organelles.

Insulin degradation has been shown in various preparations of cells and plasma membrane [3,4]. Although the cells isolated from various tissues by an enzyme treatment may retain many of the biological activities of the tissue origin, the membrane characteristics of the isolated cells may be altered by chemical and physical manipulation during the isolation process. Tissue culture has been utilized in recent years as an effective tool to study the interaction of hormones with target cells. Since the liver is one of the primary targets of insulin action, receptor binding, and degradation of the hormone, we studied the degradation of ^{125}I-labelled insulin in MH_1C_1 cells, a cultured rat hepatoma cell line. We have demonstrated that these cells inactivate ^{125}I-labelled insulin and may be useful for the study of insulin action.

Materials and Methods

Materials. Powdered Ham's F-10 culture media, horse serum and fetal calf serum were obtained from the Grand Island Biological Co., Grand Island, NY. Powdered Hank's balanced-salt medium was obtained from Flow Laboratories, Inc., Rockville, MD. Bovine insulin (26 IU/mg), chloroquine, and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, MO. Anti-bovine insulin antibody raised in guinea pigs were purchased from Miles Laboratories, Inc., Elkhart, IN. The protein A bearing Cowan 1 strain of *Staphylococus aureus* was kindly provided by Dr. Herbert Samuels of the New York University Medical Center.

Cell culture. The rat hepatoma cell line, MH_1C_1 cell was obtained from the American Type Culture Collection, Rockville, MD. The cells were plated at a density of $1.0—1.5 \cdot 10^5$ cells/10 cm^2 in plastic culture flasks and were grown in Ham's F-10 medium, supplemented with 15% horse serum and 2.5% fetal calf serum (v/v). The culture flasks were incubated in a humidified atmosphere of 95% air, 5% CO_2, at 37°C for 36—48 h prior to the experiments. The experimental medium consisted of Hank's balanced-salt solution supplemented with 0.5% (w/v) bovine serum albumin.

Preparation of iodinated insulin. Bovine insulin (Sigma Chemical Co., St. Louis, MO) was iodinated to a spec. act. of 100—150 Ci/g by the method of Roth with chloramine-T [11]. The iodoinsulin was characterized by (i) gel filtration with Sephadex G-75 in a solution of 6 M Urea and 1 M acetic acid [12] and (ii) immunoreactivity with anti-insulin antibody. The gel filtration pattern was very similar to that reported by other investigators. Two distinct peaks can be resolved by Sephadex chromatography, a major iodoinsulin peak and a peak representing insulin aggregates in the void volume. Iodoinsulin was freshly prepared and purified for the following experiments.

Assay of insulin degradation. Cell suspensions were removed from the cul-

ture flask with a rubber policeman and suspended in Hank's balanced-salt solution containing 0.5% (w/v) bovine serum albumin. The final cell density was adjusted to $5 \cdot 10^5$ cells/ml and incubated with 0.1 nM of ^{125}I-labelled insulin (approx. 200 000 cpm/ml) at 37°C. The reaction was terminated by sampling 100 μl aliquots of cell suspension into 1 ml of chilled 10% (w/v) trichloroacetic acid. After 10 min in an ice bath, the tubes were centrifuged and the radioactivity in the supernatant and the trichloroacetic acid-precipitated pellets were determined in a Packard auto-gamma spectrometer. The radioactivity in the acid soluble fraction represent the 'degradation' products of ^{125}I-labelled insulin. All experiments were done in triplicate. In each experiment, appropriate controls were prepared that were identical with respect to temperature and buffer, except that the cells were omitted. The percent ^{125}I-labelled insulin degradation was calculated as follows:

$$\% \text{ degradation} = \frac{\text{cpm } ^{125}\text{I in acid supernatant (experimental tube with cells)} - \text{cpm } ^{125}\text{I in acid supernatant (control tubes without cells)}}{\text{cpm of total } ^{125}\text{I added to each tube} - \text{cpm } ^{125}\text{I in acid supernatant from con trol tubes}}$$

^{125}I-labelled insulin binding to the cultured hepatoma cell. Cells suspended in Hank's balanced-salt solution +0.5% bovine serum albumin were incubated with ^{125}I-labelled insulin (final concentration of 0.1 nM; 200 000 cpm/ml) at 37°C. A parallel set of cell suspensions incubated with ^{125}I-labelled insulin and an excess of non-radioactive insulin (1.75 μM) were used to determine the non-specific binding. Triplicate samples of cell suspensions incubated in the presence and absence of non-radioactive insulin were centrifuged and washed two additional times with 5 ml of chilled buffer solution at the time indicated. The radioactivity in the final cell pellet was then determined. The amount of non-specific binding represents approx. 15% of the total binding. The results indicate the specific binding after correction for nonspecific binding.

Gel filtration studies. The nature of the cell associated radioactivity after an interaction with ^{125}I-labelled insulin has been studied by other investigators [12—14]. After incubation with ^{125}I-labelled insulin, the cells were washed and the cell associated iodocompounds were extracted by thawing and freezing 3-times with a solution of 6 M urea/3 M acetic acid and 0.1% Trition X-100 [12,13]. Greater than 95% of cell associated radioactivity can be extracted by this method. The extract was then applied to a 0.5 × 24 cm Sephadex G-75 column that was pre-equilibrated and eluted with a solution of 6 M urea and 1 M acetic acid at 4°C. 200 μl fractions were collected and radioactivity in each fraction was counted.

Results

I. Studies of ^{125}I-labelled insulin degradation on cultured hepatoma cells

The cultured MH_1C_1 cells have specific and saturable binding sites for monoiodinated insulin. Scatchard analysis shows a curvilinear insulin receptor binding (Fig. 1). We estimated the dissociation constant by assuming that there are two classes of binding sites for insulin receptors [15]. The estimated dissocia-

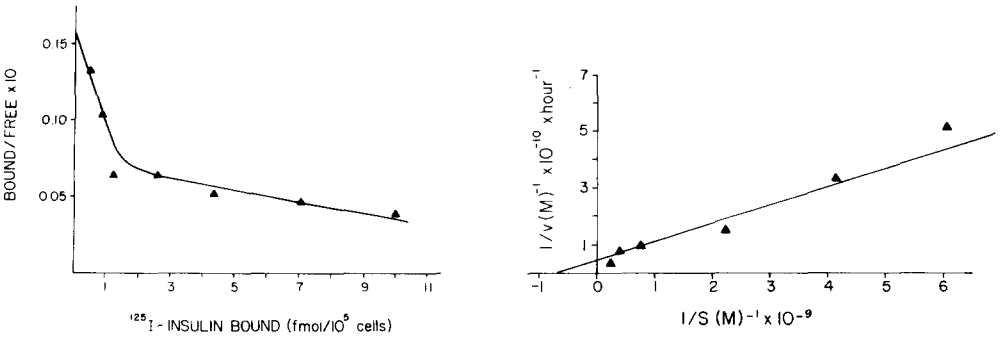

Fig. 1. Scatchard analysis of ^{125}I-labelled insulin binding to MH_1C_1 cells. The cultured hepatoma cells (final cell density $3 \cdot 10^5$/ml) suspended in Hank's balanced-salt solution +0.5% of bovine serum albumin were incubated with different concentrations of ^{125}I-labelled insulin which were prepared by adding the estimated different amount of non-radioactive insulin to ^{125}I-labelled insulin (200 000 cpm) at 37°C for 30 min. The cells were centrifuged and washed three times with chilled 0.85% NaCl and the radioactivity was counted. 200 μl of incubation buffer was removed to determine the percentage insulin degradation. The final concentrations of ^{125}I-labelled insulin bound to the cells were corrected for the spec. act. The free insulin concentrations were corrected for the percentage degradation and spec. act. Nonspecific binding was determined by incubating the cells with ^{125}I-labelled insulin and excessive non-radioactive insulin (1.75 μM). The nonspecific bindings which represent approx. 10% of total binding were subtracted from the total binding in the final calculation. The dissociation constant of the high affinity binding sites was calculated by assuming that there were two binding sites and corrected for low affinity binding component as indicated by Rosenthal [15].

Fig. 2. Estimation of the K_m and V of ^{125}I-labelled insulin degradation by the cultured hepatoma cells. Cell suspensions of (10^6/ml) were incubated with different concentrations of ^{125}I-labelled insulin at 37°C for 60 min. The amount of insulin degradation was determined and the results were analyzed by Lineweaver-Burk reciprocal plot.

tion constant for high affinity insulin binding is $7.13 \cdot 10^{-11}$ M and the calculated number of receptors per cell is approx. 4000.

Iodoinsulin degradation by hepatoma cells in culture is rapid. The rate of insulin degradation is linearly related to the cell density between $0.5—2.0 \cdot 10^6$ cells/ml. Approx. 15% of 0.1 nM of ^{125}I-labelled insulin will be inactivated by $5 \cdot 10^5$ cells/ml within 30 min of incubation at 37°C and the ^{125}I-labelled insulin degradation is inhibited by a 500-fold excess of non-radioactive insulin. The ability of hepatoma cells to degrade insulin as a function of insulin concentration was examined. The estimated K_m for insulin degradation was $1.4 \cdot 10^{-7}$ M and V was $2.5 \cdot 10^{-10}$ mol/10^6 cells/h (Fig. 2). These values were virtually identical to the observation reported by Olefsky and his coworkers in the isolated hepatocytes system [16]. It was reported that proteolytic cleavage of the B-chain of insulin was the first step of insulin degradation [17]. We have examined the effect of proteolytic inhibitors on insulin degradation in these cultured hepatoma cells. As shown in Table I, the substance which inhibit protease activities such as leupeptin and antipain fail to inhibit insulin degradation in the intact cell as well as cell homogenate. Bacitracin, *a* polypeptide antibiotic, which has been shown to block receptor mediated endocytosis by inhibiting transglutaminase activity and to prevent the protease action on the cell membrane, can prevent insulin inactivation in the intact cells as well as cell homogenate [18,19]. A similar inhibitory effect of bacitracin on insulin

TABLE I

EFFECT OF PROTEASE INHIBITORS ON ^{125}I-LABELLED INSULIN 'DEGRADATION'

Values for percentage degradation are expressed as mean ±S.D. Cell homogenates were prepared by scraping the cells from the culture flasks with a rubber policeman; they were then washed. The final cell pellets were suspended in 100 mM Hepes/120 mM NaCl/1.0 mM $MgCl_2$, pH 7.9, and homogenized with a Teflon pestle.

Experimental conditions	Addition	Percentage ^{125}I-labelled insulin 'degradation' at 30 min incubation
Whole cell	None	19.3 ± 3.6
	Antipain (10^{-3} M)	13.6 ± 4.8
	Leupeptin (10^{-3} M)	19.7 ± 3.2
	Bacitracin (2 mg/ml)	1.1 ± 1.2
Cell homogenate	None	76.7 ± 9.3
	Antipain (10^{-3} M)	61.7 ± 7.4
	Leupeptin (10^{-3} M)	59.6 ± 6.1
	Bacitracin (2 mg/ml)	2.9 ± 1.3

degradation by cultured 3T3-L 1 fibroblast has been reported by Rubin and his coworkers [12].

The kinetics of ^{125}I-labelled insulin association by the cultured hepatoma cells at different temperatures are shown in Fig. 3. Binding of ^{125}I-labelled insulin to the cells at 37°C was a rapid process. It attained a plateau within 20 min and remained constant for up to 70 min of incubation. The cells incubated at 0°C have much slower rate of ^{125}I-labelled insulin accumulation and have not

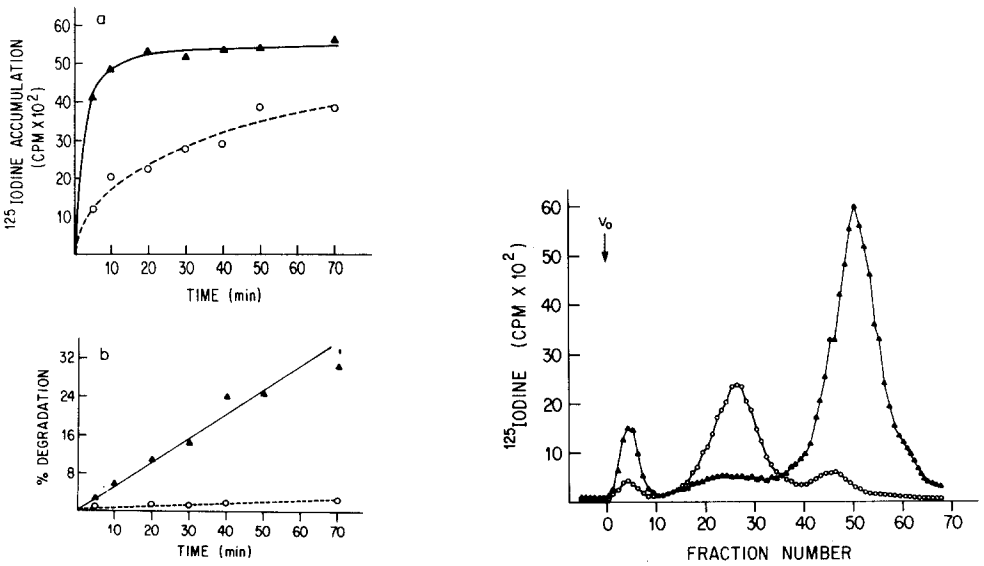

Fig. 3. Effect of temperature on ^{125}I-labelled insulin binding (a) and degradation (b) of cultured hepatoma cells. The results illustrated represent the mean of triplicated experiments. ▲———▲, 37°C; ○- - - - -○, 0°C.

Fig. 4. Sephadex gel filtration pattern of cell associated iodocompounds. ▲———▲, cells incubated at 37°C; ○- - - - -○, cells incubated at 0°C.

attained a plateau up to 60 min of incubation. In contrast with the minimal insulin inactivation by the cells which were incubated at 0°C, the cells incubated at 37°C rapidly degraded ^{125}I-labelled insulin. Although it has been shown that the magnitude of cell mediated ^{125}I-labelled insulin degradation correlated with the amount of ^{125}I-labelled insulin binding to the cells [3], the apparent difference in magnitude of ^{125}I-labelled insulin degradation by the cells incubated at different temperatures cannot be simply related to the different amount of ^{125}I-labelled insulin association with the cells.

We have examined the characteristics of radioactive labelled compounds extracted from the cells which had been incubated with ^{125}I-labelled insulin at different temperatures by gel filtration on Sephadex G-75 columns. As illustrated in Fig. 4, the cell associated iodocompounds extracted from the cells incubated with ^{125}I-labelled insulin at 37°C for 60 min were resolved by gel filtration into three different peaks. Most of the radioactivity existed as a small molecular weight component (peak III) which may represent the 'degradation' products of iodioinsulin. This gel filtration pattern is virtually similar to that observed in isolated adipocytes and hepatocytes described by other investigators [1,3,13]. However, most of the iodocompounds extracted from the cells incubated with iodoinsulin at 0°C migrated on the Sephadex to a position where intact ^{125}I-labelled insulin migrates (peak II) (Fig. 4). This demonstrated temperature dependency for cell mediated insulin degradation.

We next studied the nature of iodocompounds released from the cells. To measure the release of cell association ^{125}I-labelled insulin, we first incubated the suspension of cultured hepatoma cells with radioactive labelled insulin. After removing the unbound insulin by centrifugation, the cells were distributed into test tubes and diluted 70-fold with Hank's balanced-salt solution +0.5% bovine serum albumin, to study the dissociation of the ^{125}I radioactivity. Fig. 5 shows the loss of cell bound radioactivity from the cultured hepatoma cells preincubated with ^{125}I-labelled insulin at 37°C or at 0°C, washed and reincubated at 0°C. The cells which were preincubated at 0°C and subsequently reincubated at 0°C released cell bound radioactivity gradually until approx. 50% of initial cell bound material was released from the cells after 30 min of incubation. The kinetics of dissociation of radioactivity from the cells preincubated at 37°C and reincubated at 0°C appear to be similar to the cells preincubated at 0°C. The nature of the radioactive material released from the cell into the medium was determined by immunoprecipitation. These data indicate that the ^{125}I-labelled insulin released from the cells preincubated at 0°C remained as an intact immunoreactive insulin, whereas ^{125}I-labelled insulin was rapidly degraded by the cells at 37°C and degraded iodocompounds immunologically unreactive with anti-insulin antibody were released from the cells. It was reported that cell surface protein released into the medium may be involved in insulin inactivation [20], however, culture media which were exposed to cells for as long as 48 h do not contain any insulin degrading activity.

II. Effect of chloroquine on insulin degradation

After an initial interaction of insulin with specific receptor, the insulin is internalized as hormone-receptor complexes [6,7]. The fate of the internalized insulin molecule and the intracellular localization of insulin degradation is still

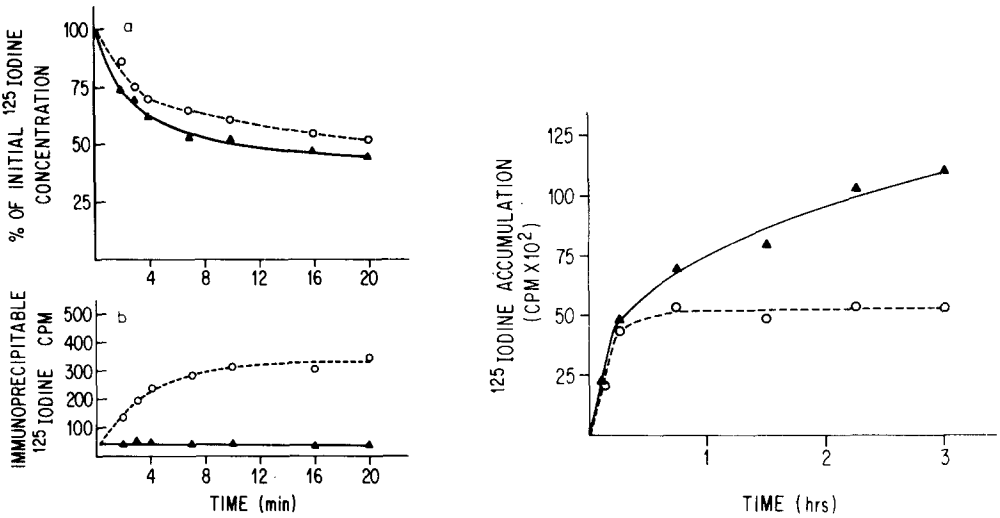

Fig. 5. Study of immunoreactivity of dissociated iodocompounds from the cells incubated at 37°C and 0°C. (a) Cell suspensions were incubated with ^{125}I-labelled insulin (30 min at 37°C, 1 h at 0°C). The cells were then washed and resuspended in incubation buffer. 50 μl aliquots were distributed into test tubes containing 3.5 ml buffer in an ice bath. At timed intervals, the cell suspensions were centrifuged in a Sorval CW-1 centrifuge for 1 min. The supernatants were transferred into other test tubes for immunoprecipitation studies. The radioactivity of the final cell pellets was counted. A parallel set of cell suspensions incubated with ^{14}C-labelled inulin and treated in the same fashion, were prepared to determine the amount of radioactive residue in the intercellular space. Less than 0.01% of the original radioactivity remains in the intercellular space. ▲———▲, cells pre-incubated at 37°C. ○- - - - -○, cells pre-incubated at 0°C. (b) The nature of dissociated iodocompounds was determined by immunoprecipitation with anti-insulin antibody. The dilution of antibody was titrated to precipitate greater than 80% of purified ^{125}I-labelled insulin in the same volume of buffer. The antibody was added into the tubes containing the buffer from the dissociation studies. The mixtures were incubated at 4°C for 1 h and 0.2 ml of washed protein-A bearing *Staphylococcus aureus* were added to each tube. The Staphylococcal protein-A-absorbed immuno-complexes were centrifuged and washed two additional times as described previously [26]. The final precipitates were then counted for radioactivity. The maximal amount of immunoprecipitable radioactivity was approx. 45% of total radioactivity dissociated from the cells. ▲———▲, pre-incubated at 37°C; ○- - - - -○, cells pre-incubated at 0°C.

Fig. 6. Effect of chloroquine on ^{125}I-labelled insulin binding. Groups of cultures were established in small Falcon culture dishes (35 X 10 mm). The media were replaced with Hank's balanced-salt solution +0.5% bovine serum albumin prior to the experiments. Chloroquine was added to a group of culture dishes (final concentration of 0.1 mM) and incubated at 37°C for 30 min prior to the addition of ^{125}I-labelled insulin to study binding and degradation. The final cellular materials were dissolved in 0.4 M NaOH to determine the amount of radioactivity. The nonspecific binding represents about 25% of total binding. The result illustrated indicated the mean of specific binding of triplicated experiments. Cells incubated with chloroquine ▲———▲; control, ○- - - - -○.

TABLE II

EFFECT OF CHLOROQUINE ON ^{125}I-LABELLED INSULIN DEGRADATION

Percentage degradation is expressed as mean ±S.D. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$; compared to control value as assessed by Student's *t*-test.

Time of incubation (min)	Percentage degradation	
	Chloroquine treated cultures	Control
20	2.2 ± 0.4	4.2 ± 0.2 *
50	3.6 ± 0.8	12.3 ± 1.2 **
90	6.2 ± 1.1	16.8 ± 2.1 ***
150	6.9 ± 1.8	26.4 ± 2.6 ***
180	7.2 ± 1.6	32.6 ± 3.4 ***

not well established. Studies on degradation of acetylated low density lipoprotein and iodinated epidermal growth factor suggest that lysosomal proteolysis may be responsible for the inactivation of these ligands [21—23]. It has been demonstrated that chloroquine, a lysosomal hydrolase inhibitor can abolish degradation of acetylated low density lipoprotein and iodinated epidermal growth factor [22,23]. We examined the effect of chloroquine on the accumu-

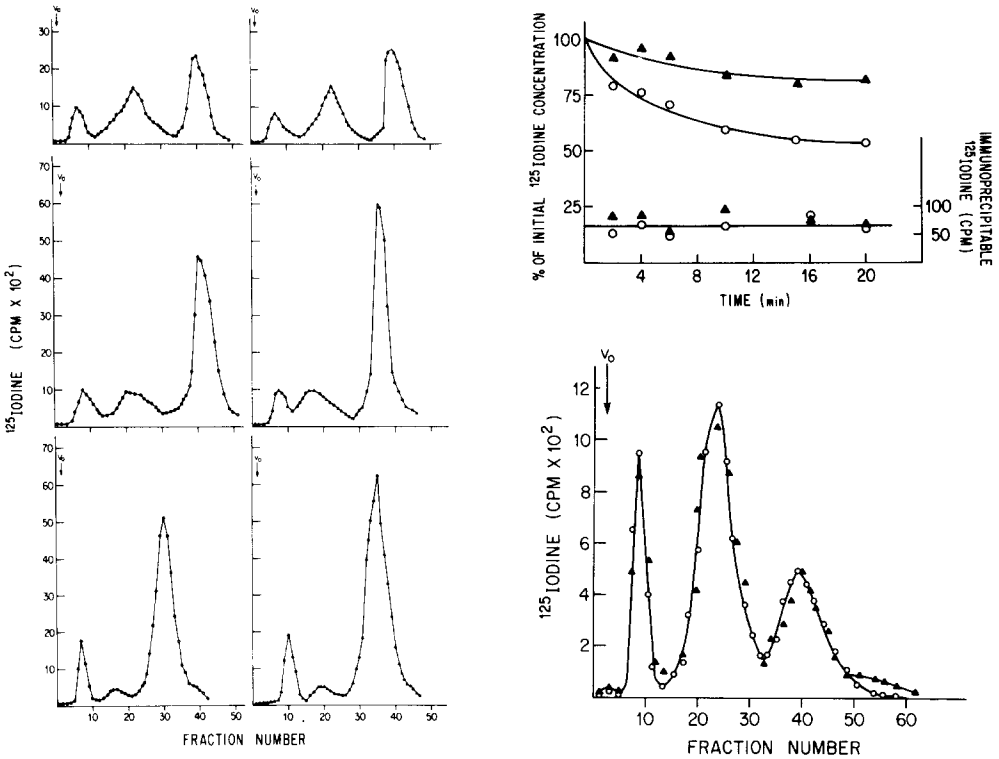

Fig. 7. Gel filtration study of cell associated iodocompounds from the chloroquine treated and control cells. (Left). Pairs of cell suspensions ($50 \cdot 10^6$ cells/10 ml) pre-incubated with or without chloroquine (0.1 mM) at 37°C for 30 min and then incubated with ^{125}I-labelled insulin in the presence or absence of chloroquine for an indicated time interval. A pair of cell suspensions was then washed and extracted for gel filtration study. Control cells, o———o (left panel); cells incubated with chloroquine, ▲———▲ (right panel). The upper panel illustrates the result of studies from cells incubated for 5 min, the middle panel, 20 min and the lower panel, 1 h with ^{125}I-labelled insulin.

Fig. 8. Effect of chloroquine on dissociation of iodocompounds from the cultured hepatoma cells. (Top right). A pair of cell suspensions was pre-incubated with or without chloroquine (0.1 mM) at 37°C for 30 min and then incubated with ^{125}I-labelled insulin at 37°C for an additional 30 min to study the dissociation of iodocompounds from the cells and immunoprecipitation of dissociated iodocompounds with anti-insulin antibody as described in Fig. 5. Cells preincubated with chloroquine, ▲———▲; Control, o———o.

Fig. 9. Sephadex gel filtration of iodocompounds in the media from the cultures incubated in the presence or absence of chloroquine. (Bottom right). A pair of culture flasks were prepared and preincubated with or without chloroquine (0.1 mM) for 30 min. An equal amount of ^{125}I-labelled insulin was added to the media (final concentration 0.1 nM) and incubated for an additional 30 min. An aliquot of medium from each culture was applied to the Sephadex G-75 column for chromatographic analysis. ▲———▲, chloroquine treated; o———o, control.

lation and degradation of ^{125}I-labelled insulin in cultured hepatoma cells. Fig. 6 shows the accumulation of ^{125}I-labelled insulin at 37°C and a linear increase in ^{125}I-labelled insulin degradation in the medium. In contrast to the controls, the cells which were preincubated with 0.1 mM of chloroquine continued to accumulate ^{125}I-labelled insulin for up to 4 h of incubation while the amount of acid soluble radioactivity in the media is not increased proportionally (Table II). We then studied the characteristics of cell associated iodocompounds by Sephadex gel filtration. As shown in Fig. 7, the gel filtration pattern of iodocompounds extracted from the cells incubated with or without chloroquine were virtually identical and three distinct peaks can be identified.

In the control cells, the percent of iodoinsulin (peak II) progressively decrease from 50% at 5 min to 15% of the total after 30 min of incubation and remained constant up to 3 h of incubation. The percent distribution of cell-associated radioactivity among the three distinct peaks on gel filtration in chloroquine treated cells was virtually identical to the control cell after 5 min incubation. The greatest amount of radioactivity accumulated in the chloroquine treated cells was recovered in peak III. These results suggest that chloroquine does not inhibit the conversion of iodoinsulin to smaller molecular weight components in the cell but inhibits the 'release' of the 'degradation' products into the medium.

In order to examine this concept further, we studied the dissociation of iodocompounds from the chloroquine treated cells compared to control cells. The dissociation rate of iodocompounds from the chloroquine treated cells is slower than that of control cells (Fig. 8). The dissociated iodocompounds were not immunoreactive to anti-insulin antibody (Fig. 8). Furthermore, the gel filtration pattern of iodocompounds in the media from the chloroquine treated and control flasks were virtually identical (Fig. 9). These results suggest that chloroquine does not alter the process of ^{125}I-labelled insulin degradation but inhibits the release of degradation product from the cells.

Discussion

We have shown that cultured MH_1C_1 cell inactivates insulin in a temperature dependent fashion. It was reported by Freychet and coworkers that ^{125}I-labelled insulin degradation is independent of binding of the hormone to its receptor [1]. Recently, using a fluorescent derivative of insulin it was demonstrated that after binding of insulin on the surface receptor, the insulin-receptor complex was rapidly internalized [7]. It was suggested that the internalization of hormone-receptor complexes might be associated with hormone degradation [7]. However, the subcellular localization of insulin inactivation has not been established. Based on the effect of chloroquine on degradation of epidermal growth factor and of light density lipoprotein by cultured fibroblast and of ^{125}I-labelled insulin by isolated adipocyte, it was suggested that a lysosomal pathway may be involved in these hormonal inactivation [22,23]. However, the electron microscopical autoradiographic study of isolated liver cells demonstrated that internalized insulin molecules are more prominent in the Golgi region and lysosomal-like vacuoles which are not cytochemically characteristic for lysosomes [25]. Our data indicate that chloroquine indeed modifies cell

mediated insulin degradation. However, the action of chloroquine on the cellular process may not be a simple inhibition of lysosomal enzyme activity.

Chromatographic studies of iodocompounds extracted from chloroquine treated and control cultured MH_1C_1 cells yielded a similar pattern. The cell associated intact iodoinsulin (peak II) was virtually identical in both chloroquine treated and control cells. However, a greater amount of insulin degradation products (peak III) was progressively accumulated in the chloroquine treated cells. This result suggests that chloroquine is not blocking the insulin degradation but preventing the degradation products being released from the cells. Marshall and Olefsky reported that conversion of intact iodoinsulin (peak II) to peak III in isolated adipocyte can be inhibited by chloroquine [24]. Therefore, their observation is paradoxical to that seen in the cultured hepatoma cells. We have further demonstrated that iodocompounds dissociated from chloroquine treated cells are not immunoreactive with anti-insulin antibody. The data also suggest that increased accumulation of iodocompounds in chloroquine treated cells is not due to an alteration of insulin degradation. Although the precise mechanism by which chloroquine affects insulin degradation is not clear and the observed discrepant action of chloroquine on cultured hepatoma cell and isolated adipocytes needs to be elucidated, chloroquine appears to affect insulin metabolism in both cultured MH_1C_1 cells and adipocyte. It was reported that chloroquine's effect on ^{125}I-labelled insulin degradation is not a generalized phenomenon on all cell types [24]. Kahn and Baird [13] have demonstrated that insulin which interacts with adipocyte will subsequently be 'compartmentalized' by the cell. The nature of subcellular compartments for insulin binding and metabolism still remain to be defined. The biological significance of the cell mediated process and functional role of each cell associated component needs to be clarified. Since the cell will metabolize and convert insulin into different size molecules, caution should be taken to interpret the results of insulin receptor binding studies.

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