Acute Effects of Adrenaline on Hepatic Lipase Secretion by Rat Hepatocytes

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Catecholamines are responsible for the daily changes in hepatic lipase (HL) expression associated with feeding and fasting. We have studied the mechanism by which adrenaline decreases HL secretion in suspensions of freshly isolated rat hepatocytes. Adrenaline acutely inhibited HL activity through activation of the α_1-adrenergic pathway. The cells had significantly less HL activity in the presence of adrenaline versus cycloheximide, where protein de novo synthesis is completely blocked. The specific enzyme activity of secreted HL was not affected. Intracellular HL activity was decreased by adrenaline treatment. Pulse-labeling with [^{35}S]methionine showed that de novo synthesis of the 53-kd endo-β-*N*-acetylglucosaminidase H (Endo H)–sensitive HL protein was unaffected by adrenaline. During subsequent chase of the control cells, the 53-kd form was converted to a 58-kd Endo H–resistant HL protein, which was rapidly secreted into the medium. In the presence of adrenaline, formation of the 58-kd protein was markedly reduced, whereas the 53-kd protein disappeared at a rate similar to the rate in controls. This suggests that part of the HL protein was degraded. In contrast to adrenaline, inhibition of HL secretion by colchicine was accompanied by an intracellular accumulation of HL activity and of the 58-kd protein. We conclude that adrenaline inhibits HL secretion posttranslationally by retarding the maturation of the 53-kd HL precursor to an active 58-kd protein, possibly by stimulating degradation of newly synthesized HL protein.

HEPATIC LIPASE (HL) is synthesized and constitutively secreted by liver parenchymal cells and subsequently bound extracellularly in the liver.[1-3] Here, it plays an important role in lipoprotein metabolism. HL hydrolyzes phospholipid and triacylglycerol present in high- and intermediate-density lipoproteins and chylomicron remnants, and facilitates the selective uptake of cholesterol from high-density lipoproteins and the removal of remnant particles by the liver (see Jackson[4] for review). Thus, by contributing to reverse cholesterol transport and to the decrease of atherogenic remnant particles in the circulation, HL is thought to protect against the development of premature atherosclerosis. Indeed, postheparin HL activity was lower in patients with versus those without stenotic coronary arteries.[5] In addition, HL activity was inversely correlated with progression of coronary atherosclerosis in patients on a lipid-lowering diet.[6] The factors that determine the level of HL activity in the liver are poorly understood.

The amount of HL activity present in the liver of rat and man is under hormonal and dietary control.[7-10] An important role is played by the "stress" hormones, glucocorticoids and catecholamines, which all reduce HL expression.[11,12] Catecholamines and long-term treatment with corticosteroids are associated with profound changes in plasma lipoprotein turnover and the development of atherosclerosis.[11-15] Decreased HL may contribute to the changes in lipoprotein metabolism. The catecholamines were recently shown to be responsible for the daily changes in HL expression in the rat.[16] Depending on the feeding condition, HL activity in the liver changes over a twofold to threefold range, and is lowest during fasting periods.

Whereas most hormones, including glucocorticoids, have been shown to alter HL expression at the level of transcription,[12] the mechanism by which catecholamines decrease HL expression is less clear. In contrast to the other hormones, catecholamines have an acute effect on HL secretion, which can be demonstrated in suspensions of freshly isolated hepatocytes.[11,16] Cells treated with adrenaline secrete less HL than cells in which protein de novo synthesis is completely blocked with cycloheximide.[16] This observation led to the hypothesis that at least part of the inhibitory effect occurs at the posttranslational level. In the present study, the mechanism by which adrenaline decreases HL activity was addressed directly. We show here that HL de novo synthesis is unaffected by adrenaline. Instead, intracellular processing of newly synthesized HL into a mature protein is inhibited and degradation is increased.

MATERIALS AND METHODS

Materials

Cycloheximide and 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate (CHAPS) were purchased from Boehringer (Mannheim, Germany). Benzamidine and amino acids were from Merck (Darmstadt, Germany). Adrenaline was obtained from Centrafarm (Etten-Leur, The Netherlands), and prazosin from Pfizer (Brussels, Belgium). Trasylol was from Bayer (Mijdrecht, The Netherlands), and heparin from Leo Pharmaceutical Products (Weesp, The Netherlands). Ham's F10 medium and methionine-free minimal essential medium were from Gibco (Paisley, UK). Endo H was from Genzyme (Cambridge, MA). Glycerol tri[9,10(*n*)-^{3}H]oleate was purchased from Amersham (Amersham, UK), and Tran-^{35}S-label (1,100 Ci/mmol) was from ICN (Costa Mesa, CA). All other chemicals were from Sigma (St Louis, MO).

Hepatocyte Isolation and Incubation

Male Wistar rats (250 to 300 g body weight) were fed ad libitum with a standard chow diet (Hope Farm, Wilnis, The Netherlands). Hepatocytes were isolated by in situ perfusion with collagenase type I, and nonparenchymal cells were removed by differential centrifugation according to the method of Seglen.[17] The cells were washed with Ham's F10 medium containing 25 U/mL heparin to remove residual extracellularly bound HL. The cells were resuspended at 3 to 5 × 10^6 cells/mL Ham's F10 medium containing 25 U/mL heparin and 20% heat-inactivated dialyzed bovine serum.[1] The cell suspensions were incubated at 37°C under an atmosphere of 95% O_2/5% CO_2 in a shaking water bath. Cell viability ranged from 85% to 95% as determined by

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Trypan blue exclusion, and remained essentially unaltered throughout the incubation.

At the indicated times, 0.5-mL samples were collected from the incubation media and put on ice. Cells and media were separated by centrifugation (5 seconds at 10,000 × *g* and 4°C), and the cell-free media were used for analysis of secreted HL. For analysis of intracellular HL, the cells were washed twice with Ham's F10 medium and then resuspended in the original volume of phosphate-buffered saline (PBS) containing CHAPS 4 mmol/L, heparin 25 U/mL, and the protease inhibitors leupeptin 1 μg/mL, antipain 1 μg/mL, chymostatin 1 μg/mL, pepstatin 1 μg/mL, benzamidine 1 mmol/L, Trasylol 10 IU/mL, and EDTA 1 mmol/L.[18,19] The cells were lysed by sonication (10 seconds at amplitude 14 μ with the MSE Soniprep 150) either immediately or after overnight storage at −80°C. Thereafter, the homogenates were centrifuged (10 minutes at 10,000 × *g* and 4°C), and the supernatants were used for further analysis.

HL Activity and Protein

HL activity was determined by a triacylglycerol hydrolase assay at pH 8.5 in 0.6 mol/L NaCl using a gum acacia–stabilized glycerol [^{3}H]trioleate emulsion as substrate.[1] Assays were performed for 30 minutes at 30°C. Enzyme activities are expressed as milliunits (nanomoles of free fatty acids released per minute). Lipase activity in cell-free media was completely inhibited by goat anti-HL IgGs.[20] Of the lipase activity present in the cell homogenates, approximately 0.17 ± 0.03 mU/10^6 cells was resistant to immuno-inhibition with anti-HL; this value was not affected by any of the incubation conditions used (data not shown). For determination of intracellular HL activity, this value was subtracted from the total lipase activity in the cell homogenates.

The amount of HL protein in cell-free media was measured by a solid-phase enzyme-linked immunosorbent assay (ELISA), in which HL is sandwiched between goat polyclonal and a mixture of monoclonal anti-HL IgGs, as described previously.[20] Absorbance was read against a standard curve prepared by serial dilutions of rat HL. The latter was prepared from postheparin rat liver perfusates by affinity chromatography on Sepharose-heparin. HL activity was eluted with a linear 0.2- to 1.0-mol/L NaCl gradient in 1% bovine serum albumin; peak fractions were pooled and kept at −80°C until use.

Protein De Novo Synthesis

Freshly isolated hepatocytes were preincubated for 30 minutes in methionine-free minimal essential medium containing 25 U/mL heparin and 20% heat-inactivated dialyzed bovine serum in the absence of adrenaline. Then, 50 μCi/mL Tran-^{35}S-label was added with or without adrenaline. After 10 minutes, the incubation was stopped on ice, and cold methionine was added at a final concentration of 1 mmol/L. The cells were collected by centrifugation (2 minutes at 50 × *g* and room temperature). After washing once in Ham's F10 medium, the cells were lysed in lysis buffer (1% Triton X-100, 0.1% sodium deoxycholate, 25 U/mL heparin, 1 mmol/L methionine, and 1 mmol/L cysteine, and the cocktail of protease inhibitors described above).[18] After 30 minutes on ice, the lysates were centrifuged for 10 minutes at 10,000 × *g* and 4°C, and the supernatants were used for immunoprecipitation of [^{35}S]HL (see below). After separation of the immunoprecipitated proteins by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), the HL bands were quantified by overnight exposure to a phosphor screen in the GS363 Molecular Imager system from Bio-Rad (Richmond, CA).

To determine overall protein de novo synthesis, 25 μL of the lysates were spotted onto Whatman 3MM filter paper (Whatman, Maidstone, UK). After boiling in 5% trichloroacetic acid (TCA), the filters were washed successively with ethanol:ether (1:1) and ether, and radioactivity in the TCA-precipitable material was determined by a 1-hour exposure in the GS363 Molecular Imager system.

Pulse-Chase Experiments

Cells were pulse-labeled in the absence of adrenaline with Tran-^{35}S-label 80 μCi/mL for 5 to 10 minutes as described earlier. After washing and resuspending the cells, the incubation was continued in Ham's F10 medium containing 25 U/mL heparin, 20% bovine serum, and 1 mmol/L cold methionine in the absence or presence of adrenaline. After the indicated chase times, the incubations were stopped on ice and lysis buffer was added. In some experiments, cells and media were first separated by centrifugation and then the cells were lysed in lysis buffer as described earlier.

Immunoprecipitations

HL protein was immunoprecipitated by goat polyclonal anti-HL IgGs immobilized onto Sepharose.[20] Twenty milligrams of the goat antibody preparation was coupled per 1 g CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Similarly, nonimmune IgGs from a control goat were also immobilized onto Sepharose. Samples (0.5 to 1 mL) of cell-free media and cell lysates were first incubated for 2 hours at 4°C with 50 μL of a 50% slurry of immobilized nonimmune IgGs. After removal of the beads by centrifugation (20 seconds at 10,000 × *g* and 4°C), the samples were incubated overnight at 4°C with 50 μL of a 50% slurry of the immobilized anti-HL IgGs. The beads were collected by centrifugation and then washed twice with 1 mL of, successively, PBS, 1 mol/L NaCl in PBS, 0.2% Tween 20 in PBS, and finally PBS (all at 4°C). The bound proteins were released by boiling in Laemmli sample buffer without β-mercaptoethanol. After removal of the beads, the proteins were reduced with β-mercaptoethanol and then resolved by SDS-PAGE on 10% gels. Radioactive bands were visualized by fluorography using Amplify (Amersham), and the molecular masses were estimated using broad-range markers from Bio-Rad electrophoresed in parallel. To quantify radioactivity in the protein bands, the dried gels were analyzed by a 24-hour exposure in the GS363 Molecular Imager system.

Transferrin was immunoprecipitated from the cell-free media as outlined earlier, using 10 μL of a 1:10 diluted antiserum against rat transferrin (a kind gift from Dr H.G. van Eijk, Rotterdam, The Netherlands) followed by 20 μL of a 50% slurry of protein A–Sepharose (Pharmacia).

Deglycosylation With Endo H

Following pulse-chase labeling and immunoprecipitation, [^{35}S]HL was removed from the Sepharose beads and denatured by heating for 5 minutes at 95°C in 50 mmol/L NaP_i buffer (pH 6.0) containing 0.5% SDS. Aliquots (10 μL) were incubated with or without 80 mU/mL Endo H for 16 hours at 37°C in NaP_i buffer with 0.2% SDS. Samples were heated for 5 minutes at 95°C in Laemmli sample buffer and subjected to SDS-PAGE and phosphor imaging with the molecular imaging system. Sensitivity to Endo H was evidenced by increased electrophoretic mobility.[18]

Statistics

Statistical significance was determined by two-way ANOVA followed by the Student-Newman-Keuls test.[21]

RESULTS

Secretion of HL Activity and Protein

HL activity in suspensions of freshly isolated rat hepatocytes was reduced by adrenaline in a concentration-dependent manner (Fig 1). In the presence of 1 μmol/L adrenaline, secretion was inhibited by 30%; a maximal inhibition of 50% was obtained with 100 μmol/L adrenaline. This effect was completely abolished by coincubation with 1 μmol/L of the α_1-

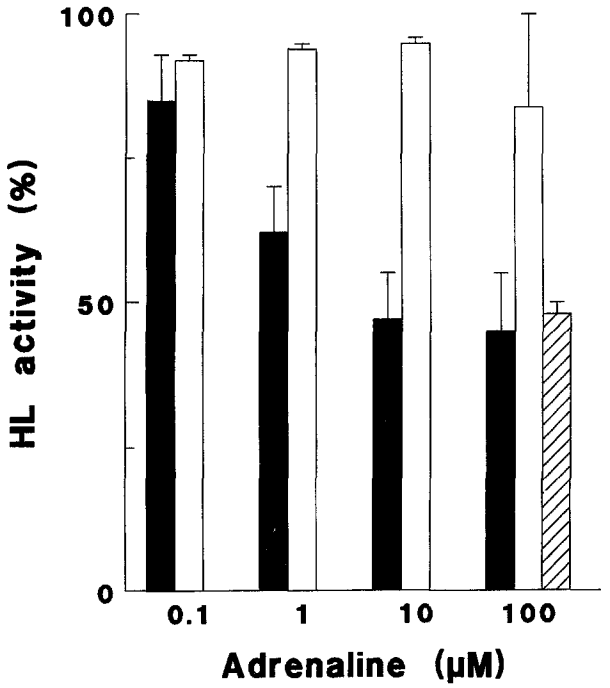


Fig 1. Effects of different adrenaline concentrations on secretion of HL activity. Freshly isolated hepatocytes were incubated in control medium or in the presence of different amounts of adrenaline either alone (■) or with 1 μmol/L prazosin (□) or 1 μmol/L propranolol (▨) added at time 0. After 1 hour, samples were collected on ice and the cell-free media were assayed for HL activity. Data are expressed as a % of the control value (1.31 ± 0.53 mU/10⁶ cells) and are the mean ± SD for 3 independent experiments.

blocker prazosin, but not with 1 μmol/L of the β-blocker propranolol. A 50% inhibition of HL secretion was also obtained with 10 μmol/L phenylephrine, a selective α_1-agonist. Therefore, the effect of adrenaline was mediated by α_1-adrenergic receptors.

Throughout the first 90 minutes of incubation with adrenaline, HL activity was secreted at a constant rate of approximately 0.63 ± 0.18 mU/h/10^6 cells (mean ± SD, n = 5), versus 1.16 ± 0.26 mU/h/10^6 cells in control suspensions (Fig 2). Already 30 minutes after addition of adrenaline, HL activity in the medium was significantly lower than in parallel controls, indicating that adrenaline acted almost immediately. This time course of secretion differed markedly from that observed with cycloheximide (10 μg/mL), which completely and instantaneously blocked protein de novo synthesis.[19] With cycloheximide, the secretion of HL activity initially continued unaffected, but plateaued after 60 minutes (Fig 2). This lag period apparently reflects the intracellular transport time of newly synthesized HL protein. At the 30- and 60-minute time points, extracellular HL activity was significantly lower with adrenaline than with cycloheximide ($P < .05$). Under all conditions tested, secretion of HL protein as measured by ELISA paralleled the extracellular appearance of HL activity. Hence, the specific enzyme activity of secreted HL remained unaffected by incubation with adrenaline (Fig 2, insert).

Intracellular HL Activity and Protein

Freshly isolated hepatocytes contained 0.57 ± 0.17 mU HL activity/10^6 cells (mean ± SD, n = 4), which was constant throughout the 90-minute incubation (Fig 3). In the presence of adrenaline, HL activity decreased during the first 30 minutes to 60% of control values. Upon prolonged incubation, intracellular HL activity remained constant at this low level. Hence, the adrenaline-induced reduction in HL secretion was accompanied by a loss rather than an increase in intracellular HL activity. In contrast, HL activity in cycloheximide-treated cells continued to decrease to negligible levels after 90 minutes (Table 1). An intracellular accumulation of HL activity could be induced by treating cells with 0.1 mol/L colchicine, which inhibits protein secretion posttranslationally.[22,23] Herein, intracellular HL activity almost doubled in about 90 minutes of incubation (Fig 3). secretion of HL activity (and protein) was inhibited to a similar extent as obtained by 10 μmol/L adrenaline (Table 1).

Since the ELISA for HL protein proved unreliable on cell lysates, we used ^{35}S-labeling followed by immunoprecipitation with anti-HL IgGs as a relative measure for intracellular HL protein. Cells that had been pulsed for 10 minutes with [^{35}S]methionine were chased for 60 minutes in the absence or presence of adrenaline (Fig 3, insert). In control cells, intracellular [^{35}S]HL mainly migrated at the 53-kd position, whereas a faint band was visible at 58 kd (Fig 3, insert). The 58-kd band comigrated with HL present in extracellular media, and therefore represents mature HL. In contrast to the 58-kd band, the 53-kd band was Endo H–sensitive (see later) and thus corresponds to the high-mannose–type HL precursor.[18] Intracellular [^{35}S]HL was not affected by adrenaline, despite the inhibition of HL secretion. In the presence of adrenaline, intracellular [^{35}S]HL mainly migrated at the 53-kd position. In colchicine-treated cells, inhibition of HL secretion and the increase in

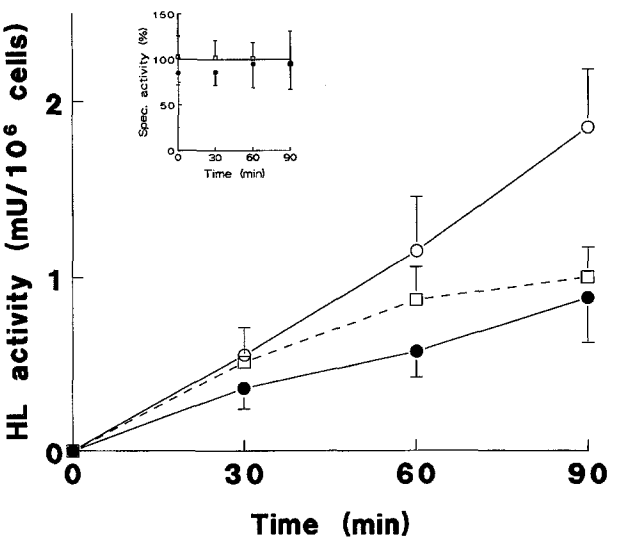


Fig 2. Acute inhibition of HL secretion. Hepatocytes were incubated in control medium (○) and in medium containing 10 μmol/L adrenaline (●) or 10 μg/mL cycloheximide (□) added at the start of the incubation. At the times indicated, samples were collected and cell-free media were assayed for HL activity and HL protein. The data for HL activity are the mean ±SD of 5 independent experiments. At all time points, the difference between adrenaline and control data was statistically significant ($P < .05$). At 30 and 60 minutes, the adrenaline data were also significantly different from cycloheximide data ($P < .05$). Insert: Effect on specific HL activity, calculated from HL activity and amount of HL protein in cell-free media. Data are expressed as % of control and represent 3 similar experiments.

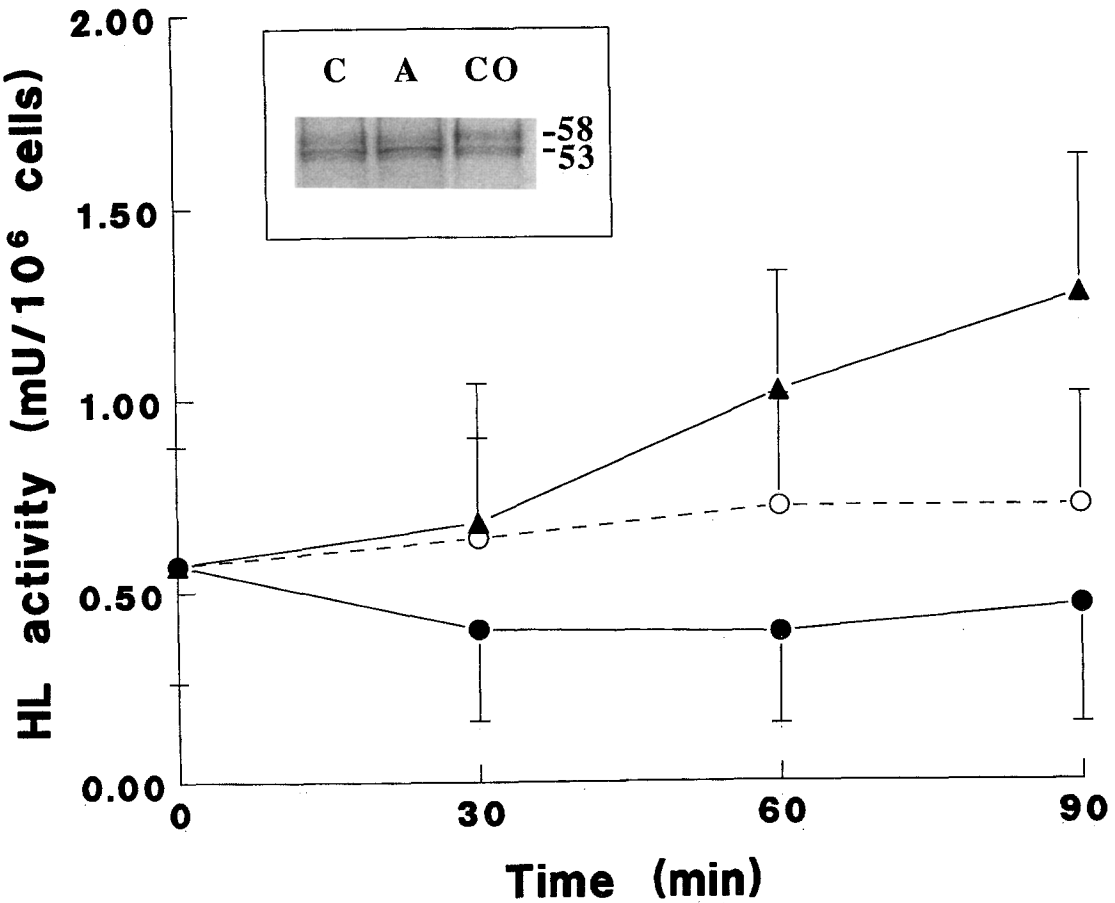


Fig 3. Effect of adrenaline on intracellular HL. Cells were incubated in control medium (○) or in medium containing 10 μmol/L adrenaline (●) or 100 μmol/L colchicine (▲). At the indicated times, samples were taken for determination of intracellular HL activity. Data are the mean ± SD (n = 3). Statistically significant differences were found between control and adrenaline-treated cells at 60 and 90 minutes ($P < .05$). Insert: Immunoprecipitated HL from cells labeled for 10 minutes with [^{35}S]methionine and chased for 60 minutes in the absence (C) or presence of 10 μmol/L adrenaline (A) or 100 μmol/L colchicine (CO). At the end of the incubation, HL was immunoprecipitated from the cell lysates and resolved by SDS-PAGE and fluorography; the molecular weight of the bands is indicated in kd.

intracellular HL activity coincided with the accumulation of [^{35}S]HL at the 58-kd position. These observations suggest that intracellular HL activity is associated with the mature 58-kd HL protein rather than with the 53-kd precursor protein.

Effect on HL Translation

Parenchymal cells were incubated with [^{35}S]methionine for 10 minutes with or without adrenaline. Thereafter, the incorporation of radioactivity into HL protein and into total TCA-precipitable material was determined. In control cells, approximately 0.02% of ^{35}S-radioactivity in total TCA-precipitable material was present in HL protein (the 53- plus 58-kd bands) (Table 2). With 10 μmol/L adrenaline, incorporation of ^{35}S-label into HL protein was slightly but not significantly lower than in control cells. Similar values were found in cells incubated with

Table 1. Distinct Effects on Intracellular and Secreted HL Activity

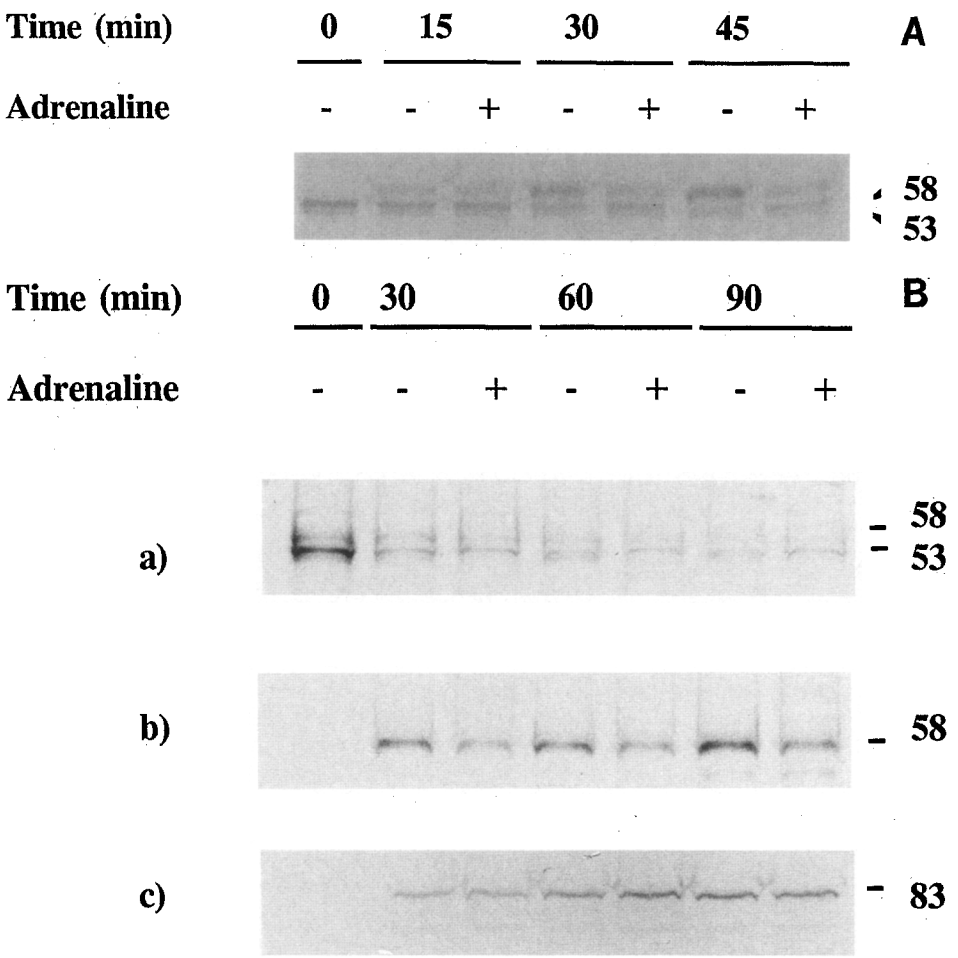
	Medium		Cells	
Treatment	mU/10^6 Cells	%	mU/10^6 Cells	%
Control	1.87 ± 0.63	100	0.55 ± 0.26	100
Cycloheximide	1.16 ± 0.41	62	0.10 ± 0.14	18
Adrenaline	0.94 ± 0.45	50	0.29 ± 0.28	52
Colchicine	0.56 ± 0.13	30	1.10 ± 0.33	200

NOTE. Cells were incubated in control medium or in medium containing either 10 μg/mL cycloheximide, 10 μmol/L adrenaline, or 100 μmol/L colchicine. After 90 minutes, samples were collected on ice, and HL activity in the cell-free media and cell homogenates was measured. Data are expressed as the mean ± SD (n = 3) or as a percentage of the control. HL activities in the treatment groups were all significantly different from control levels ($P < .05$).

Table 2. Effect of Adrenaline on Protein and HL De Novo Synthesis

	Control	Adrenaline	Adrenaline + Prazosin
HL	33 ± 13	31 ± 12	30 ± 10
Total protein (× 10^3)	144 ± 46	145 ± 43	131 ± 43

NOTE. Hepatocytes were pulsed with [^{35}S]methionine for 10 minutes with or without 10 μmol/L adrenaline either alone or in combination with 1 μmol/L prazosin. Thereafter, radioactivity in total TCA-precipitable material and immunoprecipitated HL (the 53- plus 58-kd bands on SDS-PAGE) was determined. Data represent the amount of ^{35}S-labeled protein, which was calculated in terms of cpm/mL cell suspension by taking into account the different times of exposure to the phosphor screens. Data are the mean ± SD of 3 independent experiments, each performed in duplicate. No statistically significant differences were found between treatment values and control values ($P > .05$).



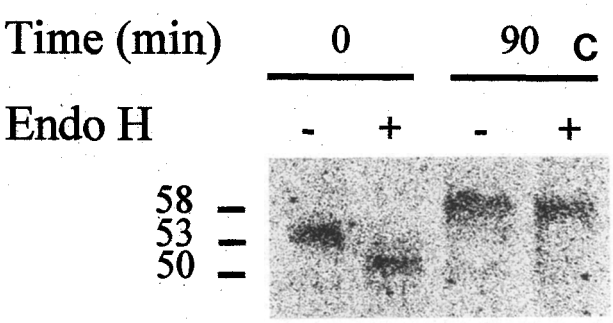


Fig 4. Chase experiments in the presence or absence of adrenaline. Hepatocytes were pulsed with [^{35}S]methionine for 5 minutes in the absence of adrenaline. After washing the cells in fresh medium, the cell suspension was divided in two. One part was incubated in control medium, and the other part in medium containing 10 μmol/L adrenaline. At the times indicated, samples were collected and HL was immunoprecipitated from the whole suspensions (A). (B) Suspensions were first separated into cells and media, and then HL was immunoprecipitated from the cell lysates (a) and cell-free media (b); then, transferrin was immunoprecipitated from the same cell-free media (c). Samples were resolved by SDS-PAGE and fluorography. Data represent 4 similar experiments. (C) Effect of Endo H on mobility of the 53-kd and 58-kd bands in SDS-PAGE. The molecular weight of the bands is indicated in kd.

the combination of adrenaline and prazosin. Overall protein synthesis was not affected by adrenaline. This demonstrated that the short-term effects of adrenaline on inhibition of HL expression are not mediated at the level of translation.

Effect on Intracellular HL Processing

In [^{35}S]methionine pulse-chase experiments, cells were pulsed in the absence of adrenaline and then chased with or without adrenaline. After a 5-minute pulse, a protein band with an apparent molecular mass of 53 kd was immunoprecipitated with anti-HL IgGs from the total cell suspensions (Fig 4A). During the chase, this band gradually shifted toward the 58-kd position. In similar pulse-chase experiments, when HL was immunoprecipitated separately from cell lysates and cell-free media, we observed that the 53-kd band was the major intracellular protein. The ^{35}S-labeled 53-kd protein was never observed extracellularly. The 58-kd protein was rapidly secreted into the medium (Fig 4B, a and b). Upon incubation of the 53-kd band with Endo H, the apparent molecular weight decreased to about 50 kd, demonstrating that the 53-kd protein represented Endo H–sensitive HL protein bearing high-mannose–type oligosaccharide chains. In contrast, the apparent molecular weight of the 58-kd band was not affected by Endo H treatment, and thus represents the mature, complex-type HL protein. When adrenaline was included in the chase medium, less of the 58-kd band appeared (Fig 4A). ^{35}S-labeled HL remained predominantly in the 53-kd form. Secretion of [^{35}S]HL was markedly reduced in the presence of adrenaline compared with control levels (Fig

4B, b). Concomitantly, the secretion rate of [^{35}S]transferrin, a glycoprotein unrelated to HL, was not affected by adrenaline, or even slightly increased (Fig 4B, c). This finding argues against a general effect of adrenaline on glycoprotein secretion.

Figure 5 shows the quantitative analysis of the pulse-chase experiments. Although the appearance of the 58-kd band was markedly reduced by adrenaline compared with control levels, the disappearance of radioactivity from the 53-kd band was hardly affected. As a result, the total radioactivity in HL protein gradually decreased, so that at the end of the chase, total ^{35}S-radioactivity in HL protein was approximately 25% lower in adrenaline-treated suspensions than in controls. The radioactivity lost from adrenaline-treated suspensions was not recovered in any immunoreactive protein, and may reflect complete degradation. This observation suggests that adrenaline induces the degradation of newly synthesized HL protein.

DISCUSSION

Our study confirms previous reports that adrenaline acutely inhibits secretion of HL by rat hepatocytes.[11,16,24] Up to 50% inhibition was observed within 60 minutes of exposure to 1 to 100 μmol/L adrenaline, which was mediated through activation of the α_1-adrenergic pathway. We show here that the acute inhibition of HL expression occurs mainly posttranslationally, but that HL de novo synthesis is not affected by adrenaline. This contrasts with the effect of adrenaline on lipoprotein lipase expression in 3T3 adipocytes, which was recently reported to be mediated at least in part by a reduced translation of the lipoprotein lipase mRNA.[25,26]

Adrenaline has been previously hypothesized to affect HL secretion at the posttranslational level.[16,24] In line with this, we found that HL activity in the presence of adrenaline was significantly lower than with cycloheximide, where protein de novo synthesis is completely blocked. Which posttranslational event in the maturation of HL protein is sensitive to inhibition by adrenaline is unknown. Neither the specific catalytic activity of secreted HL protein, as determined by ELISA, nor its mobility on SDS-PAGE were affected by adrenaline. Pulse-chase experiments in which adrenaline was added after the pulse showed that the extracellular appearance of [^{35}S]HL was retarded compared with control levels. After a 30-minute chase, secretion of [^{35}S]HL was 25% to 30% lower than in the controls, similar to the observed effect on secretion of HL activity. In the presence of adrenaline, maturation of the 53-kd Endo H–sensitive protein into the 58-kd Endo H–resistant HL protein occurred at a lower rate than in control cells; after 45 minutes, intracellular [^{35}S]HL was mainly in the immature form. At this time, recovery of total ^{35}S-label in HL protein was only 75% of the level in parallel controls, thus suggesting that adrenaline increases intracellular degradation of HL protein. Retardation of HL maturation may render the 53-kd HL more susceptible to degradation.

The decreased secretion of HL into the extracellular medium was not accompanied by an intracellular increase in HL activity. On the contrary, intracellular HL activity was markedly lower than in control cells. We recently showed that in rat hepatocytes, HL is initially synthesized as an inactive protein in the rough endoplasmic reticulum (RER). Subsequent oligosaccharide processing by RER glucosidases is necessary for the protein to leave the RER and become catalytically active.[19,20] Here, we show that intracellular HL activity is mainly associated with the presence of the 58-kd protein (Fig 3). In adrenaline-treated

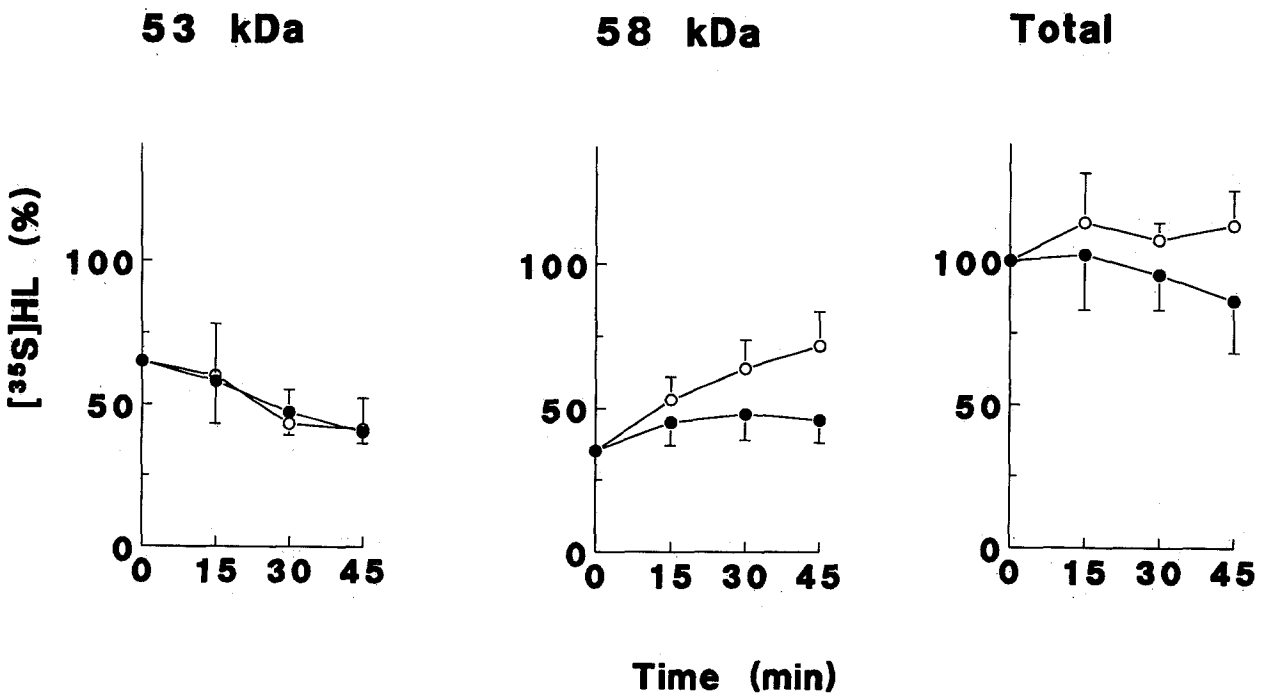


Fig 5. Effect of adrenaline on the maturation of HL into a 58-kd protein. Experiments were performed as outlined in Fig 4A. ^{35}S-labeled bands of immunoprecipitated HL on SDS-PAGE were analyzed quantitatively. The data show the disappearance of the 53-kd protein, the appearance of the 58-kd protein, and the sum of both signals for the chase in the absence (○) or presence of 10 μmol/L adrenaline (●). Data are expressed as a % of total radioactivity in the HL bands at the start of the chase (14 ± 5 cpm/mL cell suspension) and are the mean ± SD of 3 similar experiments.

cells, HL protein was predominantly in the 53-kd precursor form, which was found to be associated with a low specific enzyme activity. Taken together, our data indicate that adrenaline acts at an early stage during the maturation of HL by inhibiting the processing of the inactive precursor into the mature active protein.

How the extracellular presence of adrenaline is signaled to the posttranslational modification of HL remains unclear, but the mechanism presumably involves binding to α_1-adrenoceptors and the subsequent increase in intracellular calcium. Indeed, secretion of HL is also inhibited by other Ca-mobilizing agonists such as vasopressin and angiotensin II, as well as by the Ca ionophore A23187.[11,27] These calcium signals may affect vesicular transport from the RER to the Golgi complex, thus inhibiting maturation and secretion of HL. However, this mode of action would be expected to affect overall constitutive secretion. In light of our results with transferrin, this possibility appears unlikely. Alternatively, a factor required for the maturation of a limited number of glycoproteins, including HL, may be involved. Such a factor has been implicated in mice with combined lipase deficiency.[28,29] In these mice, HL and lipoprotein lipase are normally synthesized in the liver and nonhepatic tissues, respectively, but the protein accumulates in an inactive, high-mannose–type form within the RER without being secreted.[30] No other glycoproteins appear to be affected in this syndrome. A factor that specifically interacts with HL in the RER and is impaired by the *cld* mutation may be the target of adrenaline. Further studies are required to delineate the mechanism by which catecholamines interfere with posttranslational processing of HL.

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