Maturation and secretion of rat hepatic lipase is inhibited by α_{1B}-adrenergic stimulation through changes in Ca^{2+} homoeostasis: thapsigargin and EGTA both mimic the effect of adrenaline

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In rats, the daily changes in hepatic lipase (HL) activity in the liver follow the diurnal rhythm of the catecholamines. To study the underlying mechanism, the effect of adrenaline on maturation and secretion of HL was determined in freshly isolated rat hepatocytes. Adrenaline (10 μM) acutely inhibited the secretion of HL. This effect was abolished by 0.1 μM prazosin, but not by 1 μM propranolol, indicating the involvement of the α_1-adrenergic pathway. Prazosin was at least 1000-fold more potent than WB4101, a selective α_{1A}-antagonist. Adrenaline had no effect on HL secretion in hepatocytes pretreated with chloroethylclonidine, an irreversible α_{1B}-selective antagonist. Inhibition of HL was not induced by 10 μM methoxamine, a α_{1A}-selective agonist. Thus, adrenaline inhibited HL secretion through activation of the α_1-adrenoceptors subtype B, which have been shown to signal through Ca^{2+} as well as cAMP. A similar reduction in HL secretion was induced by the Ca^{2+}-mobilizing hormones angiotensin II (100 nM) and vasopressin (12 nM), the Ca^{2+} ionophore A23187 (2 μM), and by thapsigargin (1 μM), which inhibits the ER Ca^{2+}–ATPase pump. HL secretion was unaffected by elevating cAMP with 10 μM forskolin or 1 μM 8-Br-cAMP. These results suggest that the α_{1B}-adrenergic effects on HL expression are mainly mediated through elevation of intracellular Ca^{2+}. Chelation of extracellular Ca^{2+} and subsequent lowering of intracellular Ca^{2+} with EGTA also inhibited HL secretion. In pulse-chase experiments, adrenaline was shown to inhibit the maturation of HL from the 53 kDa, Endo H-sensitive precursor to the Endo H-resistant, catalytically active protein of 58 kDa. In addition, adrenaline induced intracellular degradation of newly synthesized HL. Similar post-translational effects, both qualitatively and quantitatively, were observed with A23187, thapsigargin and EGTA. We conclude that the inhibition of HL maturation and increase in intracellular degradation induced by catecholamines, A23187, thapsigargin and EGTA is evoked by changes in Ca^{2+} homoeostasis, possibly through lowering ER Ca^{2+}.

INTRODUCTION

Hepatic lipase (HL) plays an important role in lipid metabolism. HL hydrolyses phospholipid and triacylglycerol present in high- and intermediate-density lipoproteins, and facilitates the selective uptake of cholesterol from high-density lipoproteins and the removal of remnant particles by the liver (for review see [1]). HL may affect lipoprotein metabolism via its enzymic activity [2], or by its ligand function towards lipoproteins [3–6]. By contributing to reverse cholesterol transport and by lowering atherogenic remnant particles in the circulation, HL is thought to protect against the development of premature atherosclerosis. A low HL has been shown to be a risk factor for premature atherosclerosis [7,8]. In addition, HL activity inversely correlated with progression of coronary atherosclerosis in patients on a lipid-lowering diet [9]. In line with this, HL transgenic mice were shown to accumulate less cholesterol in the aortic wall than non-expressing littermates [10].

Catecholamines are responsible for the diurnal changes in HL activity observed in rat liver, with HL being low when plasma catecholamines are elevated [11]. In freshly isolated hepatocytes adrenaline decreases HL secretion via activation of α_1-adrenoceptors [11–13], which are generally thought to exert their action via intracellular Ca^{2+}. Recently, several subclasses of this receptor have been identified and shown to signal differentially [14–17]. The α_{1A}-adrenergic receptor signals by stimulating Ca^{2+} influx from the extracellular medium and mediates a tonic response [18,19]. The α_1-subclasses B, C and D induce a rapid IP_3 formation and subsequent release of Ca^{2+} from intracellular stores [20,21]. In addition to calcium mobilization, the latter subclasses were also shown to elevate cAMP [21,22]. Hepatocytes from different species express different subclasses of α_1-adrenergic receptors. Rats, mice and hamsters mainly express α_{1B}-adrenoceptors [23]. In isolated rat hepatocytes, the α_{1B}-adrenoceptors were reported to directly stimulate cAMP accumulation, rather than indirectly in response to elevated Ca^{2+} [22]. The cAMP accumulation, induced by the α_1-receptors, depends on the maturity of the rats from which the hepatocytes are isolated [24]. This may indicate age-related changes in α_1-adrenoceptor subclass expression. The isolation procedure may also cause changes in expression of α_{1B}-adrenoceptors by rat liver cells [25]. Therefore, we decided to re-investigate the signalling pathway involved in the adrenaline-induced reduction of HL secretion in isolated hepatocytes.

In earlier studies, we showed that adrenaline acutely decreases the maturation of HL and increases degradation of newly synthesized HL protein [13]. To distinguish between the various α_1-adrenoceptors, we incubated hepatocytes with compounds known to have different affinities for the α_1-subtypes [26]. We

Abbreviations used: HL, hepatic lipase; 8-Br-cAMP, 8-bromo-cAMP; ER, endoplasmic reticulum; PAGE, polyacrylamide gel electrophoresis; Endo H, endo-β-*N*-acetylglucosaminidase H.

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show here that the α_1-adrenoceptor subclass B mediates the inhibitory effects of adrenaline on HL expression. Although α_{1B}-stimulation induces elevated Ca^{2+} and cAMP levels, the post-translational effects of adrenaline on HL expression appear to be only mediated by Ca^{2+}. In order to study the mechanism of action, the role of intracellular Ca^{2+} in the inhibition of HL secretion by freshly isolated hepatocytes was studied.

EXPERIMENTAL

Materials

Adrenaline was obtained from Centrafarm BV (Etten-Leur, The Netherlands) and prazosin from Pfizer (Brussels, Belgium). Propranolol, thapsigargin, angiotensin II, vasopressin, 8-bromo-cAMP and EGTA were purchased from Sigma (St. Louis, MO, U.S.A.). Methoxamine, WB4101 and chloroethylclonidine were from Research Biochemicals International (Natick, MA, U.S.A). A23187 was from Boehringer–Mannheim (Mannheim, Germany). Benzamidine and amino acids were purchased from Merck (Darmstadt, Germany). Trasylol was from Bayer BV (Mijdrecht, The Netherlands) and heparin from Leo Pharmaceutical Products BV (Weesp, The Netherlands). Protein A-Sepharose and CNBr-activated Sepharose 4B were obtained from Pharmacia (Uppsala, Sweden). Ham's F10 and methionine-free MEM were from Gibco–BRL (Paisley, U.K.). Glycerol tri[9,10(n)-^{3}H]oleate (5–20 Ci/mmol) was purchased from Amersham (Amersham, U.K.), Tran-^{35}S-label (1100 Ci/mmol) was from ICN (Costa Mesa, CA, U.S.A.). Broad range markers for SDS/PAGE came from Bio-Rad (Richmond, CA, U.S.A.). All other chemicals were from Sigma.

Hepatocyte isolation and incubation

Hepatocytes were isolated from male Wistar rats (200–300 g) by *in situ* perfusion with collagenase type I, whereafter non-parenchymal cells were removed by differential centrifugation according to Seglen [27]. The cells were washed with Ham's F10 medium containing 25 U/ml of heparin to remove residual extracellularly bound HL. Then the cells were resuspended at $3–5 \times 10^6$ cells/ml in Ham's F10 medium containing 25 U/ml of heparin and 20 % heat-inactivated, dialysed bovine serum [28]. 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 Trypan Blue exclusion and remained essentially unaltered during all incubations. At the indicated times, 0.5 ml samples were collected from the incubations and put on ice. Cells and media were separated by centrifugation (5 s, 10000 ***g***, 4 °C) and the cell-free media were used for analysis of secreted HL.

HL activity and protein

HL activity was determined by a triacylglycerol hydrolase assay at pH 8.5 in 0.6 M NaCl using a gum acacia-stabilized glycerol [^{3}H]trioleate emulsion as substrate [28]. Assays were performed for 45 min at 30 °C. Enzyme activities are expressed as mU (nmol of free fatty acids released per min). The lipase activity in the cell-free media was completely inhibited by goat anti-HL IgGs [29].

The amount of HL protein in cell-free media was measured by a solid-phase ELISA as described previously [29], with minor modifications. HL was sandwiched between goat polyclonal and rabbit polyclonal anti-HL IgGs. Absorbances were read against a standard curve prepared by serial dilutions of partly purified rat HL. The latter was prepared from post-heparin rat liver perfusates by affinity chromatography on Sepharose-heparin. HL activity was eluted with a linear 0.2–1.0 M NaCl gradient in 1 % BSA; peak fractions were pooled and kept at −80 °C until use.

Pulse-chase experiments with [^{35}S]methionine

Pulse-chase experiments were performed as described before [13]. In short, freshly isolated hepatocytes were pre-incubated for 30 min in methionine-free MEM containing 25 U/ml of heparin and 20 % heat-inactivated, dialysed bovine serum. After a 5 min pulse with 80 μCi/ml of Tran-^{35}S-label, cold methionine was added at a final concentration of 1 mM. After washing twice by centrifugation (2 min, 50 ***g***, room temperature) and resuspending in Ham's F10, the cells were incubated further in Ham's F10 medium containing 25 U/ml of heparin, 20 % serum and 1 mM cold methionine. After the indicated chase times the incubations were stopped on ice. The whole cell suspension was lysed with 1 % Triton X-100, 0.1 % sodium deoxycholate, 25 U/ml heparin, 1 mM methionine and the protease inhibitors leupeptin (1 μg/ml), antipain (1 μg/ml), chymostatin (1 μg/ml), pepstatin (1 μg/ml), benzamidine (1 mM), Trasylol (10 IU/ml) and EDTA (1 mM). After a 30 min incubation on ice, the lysates were centrifuged for 10 min at 10000 ***g*** at 4 °C, and the supernatants were collected. These post-nuclear supernatants were used for immunoprecipitation.

Immunoprecipitations

HL protein was immunoprecipitated by goat polyclonal anti-HL IgGs immobilized onto Sepharose [29]. Twenty mg of the goat antibody preparation was coupled per 1 g of CNBr-activated Sepharose 4B according to the manufacturer's instructions. The post-nuclear supernatants (1 ml) 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 M NaCl in PBS, 0.2 % Tween-20 in PBS, and finally PBS (all at 4 °C). The bound proteins were released by a 5 min incubation at 95 °C 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. Protein bands were visualized by staining with Coomassie Brilliant Blue, and their molecular masses were estimated using broad range markers run in parallel. To visualize and quantify the radioactivity in the bands, the dried gels were analysed by phosphor imaging using CS screens and the GS363 Molecular Imager (Bio-Rad, Richmond, CA, U.S.A.).

Transferrin was immunoprecipitated from the cell free media as outlined above, 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 [13].

Statistics

Statistical significances were determined by one-way analysis of variance (one-way ANOVA), followed by the Student–Newman–Keuls test [30].

RESULTS

Characterization of the α_1-adrenoceptor subtype involved in HL secretion

In the presence of heparin, freshly isolated hepatocytes secreted 1.34 ± 0.19 mU/10^6 cells of HL activity (mean $\pm$ S.D.; $n = 3$) into the medium in 1 h. When adrenaline (10 μM) was present from the start of the incubation, the secretion of HL activity and

Table 1 Secretion of HL activity in the presence of different α_1-adrenergic (ant)agonists

Freshly isolated hepatocytes were incubated with 1 μM propranolol in control medium and in medium containing the indicated compounds. Part of the hepatocytes were preincubated with chloroethylclonidine for 20 min and then adrenaline was added. After 60 min, samples of the cell-free media were assayed for HL activity and mass. Data are expressed as percentage of control (1.34 ± 0.19 mU/10^6 cells and 20.4 ± 4.9 mU/μg respectively) and represent mean $\pm$ S.D. ($n = 3$). Statistically significant differences from control are indicated by the asterisks ($P < 0.05$).

	HL activity (%)	HL mass (%)	HL specific activity (%)
Control	100	100	100
Adrenaline (10 μM)	57 $\pm$ 6*	48 $\pm$ 6*	119 $\pm$ 15
Adrenaline (10 μM) + prazosin (1 μM)	98 $\pm$ 6	91 $\pm$ 24	108 $\pm$ 31
Adrenaline (10 μM) + WB4101 (10 μM)	67 $\pm$ 7*	51 $\pm$ 13*	131 $\pm$ 30
Adrenaline (10 μM) + chloroethylclonidine (100 μM)	97 $\pm$ 11	103 $\pm$ 10	94 $\pm$ 14
Methoxamine (10 μM)	91 $\pm$ 12	96 $\pm$ 18	95 $\pm$ 11

Table 2 Secretion of HL activity is inhibited by increasing intracellular Ca^{2+} but not cAMP levels

Hepatocytes were incubated for 60 min in the presence of the compounds indicated, whereafter the cell-free media were assayed for HL activity and mass. Data are expressed as percentage of control (1.47 ± 0.62 mU/10^6 cells and 20.4 ± 4.9 mU/μg respectively) and represent mean $\pm$ S.D. for 3–5 independent experiments. Asterisks indicate statistically significant differences from control ($P < 0.05$).

	HL activity (%)	HL mass (%)	HL specific activity (%)
Control	100	100	100
Adrenaline (10 μM) + propranolol (1 μM)	57 $\pm$ 6*	48 $\pm$ 6*	119 $\pm$ 15
A23187 (2 μM)	67 $\pm$ 17*	56 $\pm$ 5*	120 $\pm$ 19
Thapsigargin (1 μM)	57 $\pm$ 10*	56 $\pm$ 2*	102 $\pm$ 27
Angiotensin II (100 nM)	72 $\pm$ 15*	63 $\pm$ 4*	114 $\pm$ 28
Vasopressin (12 nM)	70 $\pm$ 10*	59 $\pm$ 3*	119 $\pm$ 20
Forskolin (10 μM)	96 $\pm$ 6	77 $\pm$ 19	125 $\pm$ 32
8-Br-cAMP (1 μM)	92 $\pm$ 18	74 $\pm$ 6	124 $\pm$ 22

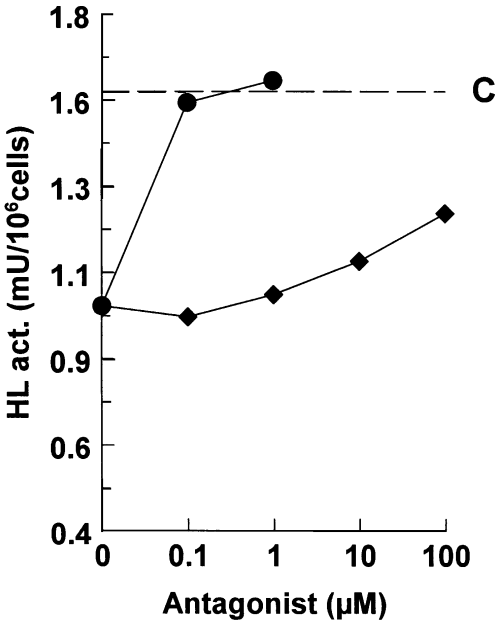

Figure 1 Secretion of HL activity in the presence of different concentrations of α_1-adrenergic antagonists

Freshly isolated hepatocytes were incubated with 1 μM propranolol in control medium (C) and in medium containing 10 μM adrenaline either alone or in combination with different concentrations of WB4101 (◆) or prazosin (●). After 60 min of incubation the cell-free media were assayed for HL activity.

HL mass decreased to approx. 60% of control (Table 1). Propranolol (1 μM), which was included in these experiments to exclude possible interference by the β-adrenergic pathway, did not alter the secretion of HL activity and mass in control, nor in adrenaline-treated hepatocytes [13]. On the other hand, the response to adrenaline was abolished by co-incubation with 1 μM prazosin, confirming the involvement of the α_1-adrenergic receptor pathway.

To distinguish between the various α_1-adrenoceptor subtypes, we incubated freshly isolated hepatocytes with adrenaline and propranolol in combination with antagonists that have different affinities for the α_1-adrenoceptor subtypes [26]. In the presence of 1 μM WB4101, an α_{1A}-antagonist, the effect of adrenaline on secretion of HL activity and HL mass was hardly affected (Table 1). Adrenaline was less effective when higher concentrations (up to 100 μM) of WB4101 were added (Figure 1). Adrenaline was inactive in the presence of only 0.1 μM prazosin, demonstrating that prazosin was at least 1000-fold more potent than WB4101. Adrenaline had no effect on HL secretion when hepatocytes were pre-incubated with 100 μM chloroethylclonidine, an irreversible α_{1B}-selective antagonist (Table 1). Moreover, the effect of adrenaline on secretion of HL activity and HL mass was not mimicked by 10 μM methoxamine, an α_{1A}-selective agonist. Under all conditions tested, the specific enzyme activity of secreted HL was not significantly changed (Table 1). These findings indicate that adrenaline inhibited HL secretion by hepatocytes through activation of the α_1-adrenoceptors subtype B.

The effect of adrenaline is mediated by changes in intracellular calcium

In hepatocytes of adult rats α_{1B}-adrenoceptors are shown to signal simultaneously through Ca^{2+} mobilization and cAMP accumulation [22]. We therefore studied through which signal adrenaline induces inhibition of HL secretion. When hepatocytes were incubated with the Ca^{2+} ionophore A23187 (2 μM), the HL activity and HL mass secreted into the cell-free medium was reduced to levels comparable to that with adrenaline (Table 2). Thapsigargin (1 μM), which elevates intracellular Ca^{2+} by mobilizing Ca^{2+} stores from the ER [31], also induced a 40% fall in HL activity and HL mass in the medium. In addition, the Ca^{2+}-mobilizing hormones angiotensin II (100 nM) and vasopressin (12 nM) reduced secretion of HL similar to adrenaline. On the other hand, secretion of HL activity and HL mass was not affected by incubation with 10 μM forskolin or 1 μM 8-Br-cAMP. Under all conditions tested, the specific enzyme activity of the secreted HL was not significantly affected (Table 2). These results imply that the α_{1B}-adrenergic effect of adrenaline on HL secretion is mainly mediated by the Ca^{2+} signal.

Opposite changes in intracellular Ca^{2+} levels inhibit the secretion of HL

As shown above, elevation of intracellular Ca^{2+} by A23187, thapsigargin, angiotensin II or vasopressin results in a similar

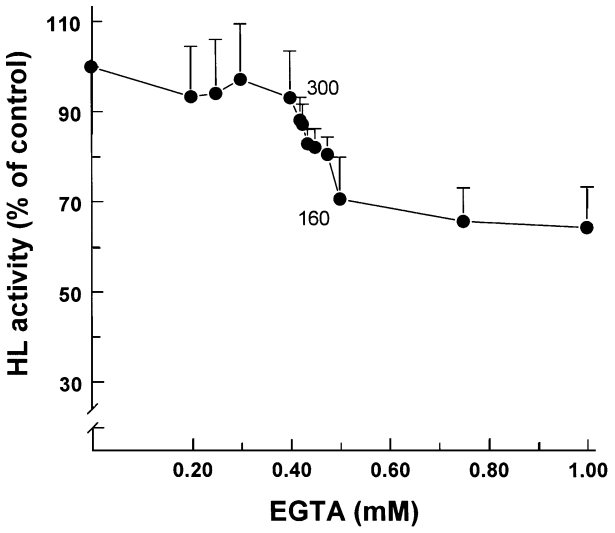

Figure 2 Effect of chelating extracellular Ca^{2+} on secretion of HL activity

Freshly isolated hepatocytes were incubated in Ham's F10 medium containing 0.3 mM Ca^{2+} and different concentrations of EGTA. After 60 min the cell-free media were assayed for HL activity. Data are expressed as percentage of control (1.54 ± 0.80 mU/10^6 cells) and represent mean $\pm$ S.D. for 2–6 independent experiments. The numbers indicate the free extracellular Ca^{2+} concentrations (nM) calculated for these conditions [33,34].

inhibition of HL secretion as with adrenaline. Chelation of extracellular Ca^{2+} with EGTA has also been shown to inhibit secretion of HL activity [32]. To establish at which concentration of extracellular free Ca^{2+} HL secretion is affected, freshly isolated hepatocytes were incubated for 1 h in medium containing 0.3 mM Ca^{2+} and different concentrations of EGTA. Secretion of HL activity (Figure 2) and HL protein (not shown) was unaffected by EGTA up to 0.4 mM. Between 0.4 and 0.5 mM EGTA, secretion of HL abruptly fell by 40%. A further increase in EGTA concentration (up to 1 mM) had no additional inhibitory effect. Calculation of free Ca^{2+} concentrations [33,34] in the media show that the threshold value was between 300 and 160 nM free extracellular Ca^{2+}. Half-maximal reduction in HL secretion was observed at approximally 200 nM free Ca^{2+}, which is close to intracellular Ca^{2+} concentrations reported for rat hepatocytes [22,35–37]. Apparently, the inhibitory effect of EGTA on HL secretion occurs when the free extracellular Ca^{2+} concentration falls to levels close to or below intracellular Ca^{2+}.

Effects of adrenaline, thapsigargin and EGTA on HL maturation

The inhibitory effect of the Ca^{2+}-mobilizing hormones, as well as of A23187, thapsigargin and EGTA are evident within the first hour of incubation. During this period, mainly presynthesized HL is secreted [13,29]. Thus, the acute effects on HL secretion are mainly post-translational. In pulse-chase experiments with [^{35}S]-methionine, the mechanism by which adrenaline, thapsigargin, A23187 and EGTA lower HL secretion by rat hepatocytes was compared. To study post-translational processing of HL, hepatocytes were pulsed with [^{35}S]methionine for 5 min in control medium. During the chase, equal parts of the cell suspension were incubated without any addition, or with either adrenaline, A23817, thapsigargin or EGTA.

After a 5 min pulse, HL was immunoprecipitated as 53 kDa, Endo H-sensitive protein [13,38]. During the chase in control suspensions, the 53 kDa protein gradually decreased (Figures 3A and 3B). In parallel the mature, Endo H-resistant HL protein of 58 kDa was formed. Total [^{35}S]HL, which was determined by the sum of the radioactivity in the 53 and 58 kDa protein, was not altered during the 45 min of chase, indicating that there was no degradation of HL under these conditions. When adrenaline (10 μM) was present during the chase, the intracellular 53 kDa protein disappeared at a rate similar to control (Figures 3A and 3B; Adr). However, the appearance of the mature 58 kDa protein was retarded compared with control cells. After 45 min of incubation, total [^{35}S]HL was 30% less than in control incubations. The loss in total [^{35}S]HL during incubation with adrenaline was not recovered in any immunoreactive protein, and may reflect complete degradation. Overall, the results indicate that adrenaline inhibited the maturation of HL protein and increased the degradation of HL.

When hepatocytes were chased in the presence of 1 μM thapsigargin, the ^{35}S-labelled 53 kDa protein disappeared at a similar rate to that in control or adrenaline-treated cells (Figures 3A and 3B; TSG). The formation of the 58 kDa protein was retarded compared with control cells to an extent similar to adrenaline-treated cells. At the end of the chase with thapsigargin, total [^{35}S]HL was decreased to about 70% of control. With 2 μM A23187 (not shown) or with 0.5 mM EGTA (Figures 3A and 3B) the effects on the disappearance of the 53 kDa protein, the appearance of the 58 kDa protein, and loss of total [^{35}S]HL were superimposable to those observed with thapsigargin and adrenaline. In contrast to secretion of [^{35}S]HL, the secretion of [^{35}S]transferrin was unaffected after up to 45 min of incubation with either adrenaline, thapsigargin or EGTA (Figure 3C).

DISCUSSION

In freshly isolated hepatocytes secretion of HL was shown to be inhibited by adrenaline through activation of the α_1-adrenoceptor pathway [11–13]. Rat liver was reported to be enriched in the α_{1B}-subtype [23,39]. In our isolated rat hepatocytes WB4101, an α_{1A}-antagonist, was at least 1000-fold less potent than prazosin in preventing the effect of adrenaline on HL secretion. When pre-incubated with chloroethylclonidine, an α_{1B}-antagonist, the adrenaline-induced inhibition of HL secretion was completely abolished. The effect of adrenaline was mimicked by phenylephrine [13], but not by the selective α_{1A}-agonist methoxamine. Thus, adrenaline inhibited the secretion of HL via activation of the α_{1B}-adrenergic receptors. This adrenoceptor subtype was recently described to signal simultaneously through elevation of intracellular Ca^{2+} and cAMP [22]. Several observations suggest that the effect of adrenaline is mediated via Ca^{2+} rather than cAMP. First, similar inhibition of HL secretion was observed with the Ca^{2+}-mobilizing hormones vasopressin and angiotensin II. In addition, the Ca^{2+} ionophore A23187 and thapsigargin had similar effects on the secretion of HL. Second, the effect of adrenaline could not be mimicked by elevating cAMP with forskolin or 8-Br-cAMP. cAMP may be important for potentiating the IP_3-induced Ca^{2+} release from the ER [40,41]. However, co-incubation of hepatocytes with forskolin and adrenaline showed that the forskolin-mediated elevation of cAMP did not modulate the inhibitory effect of adrenaline on HL secretion (not shown).

The acute effects of the Ca^{2+}-mobilizing hormones, A23187, thapsigargin and EGTA on HL secretion are mainly post-translational. They are evident within the first hour, during which mainly pre-synthesized HL is secreted [13,29]. Pulse-chase experiments with [^{35}S]methionine showed that adrenaline, A231-87, thapsigargin, as well as EGTA retard the maturation of the 53 kDa, high mannose precursor to the mature 58 kDa HL

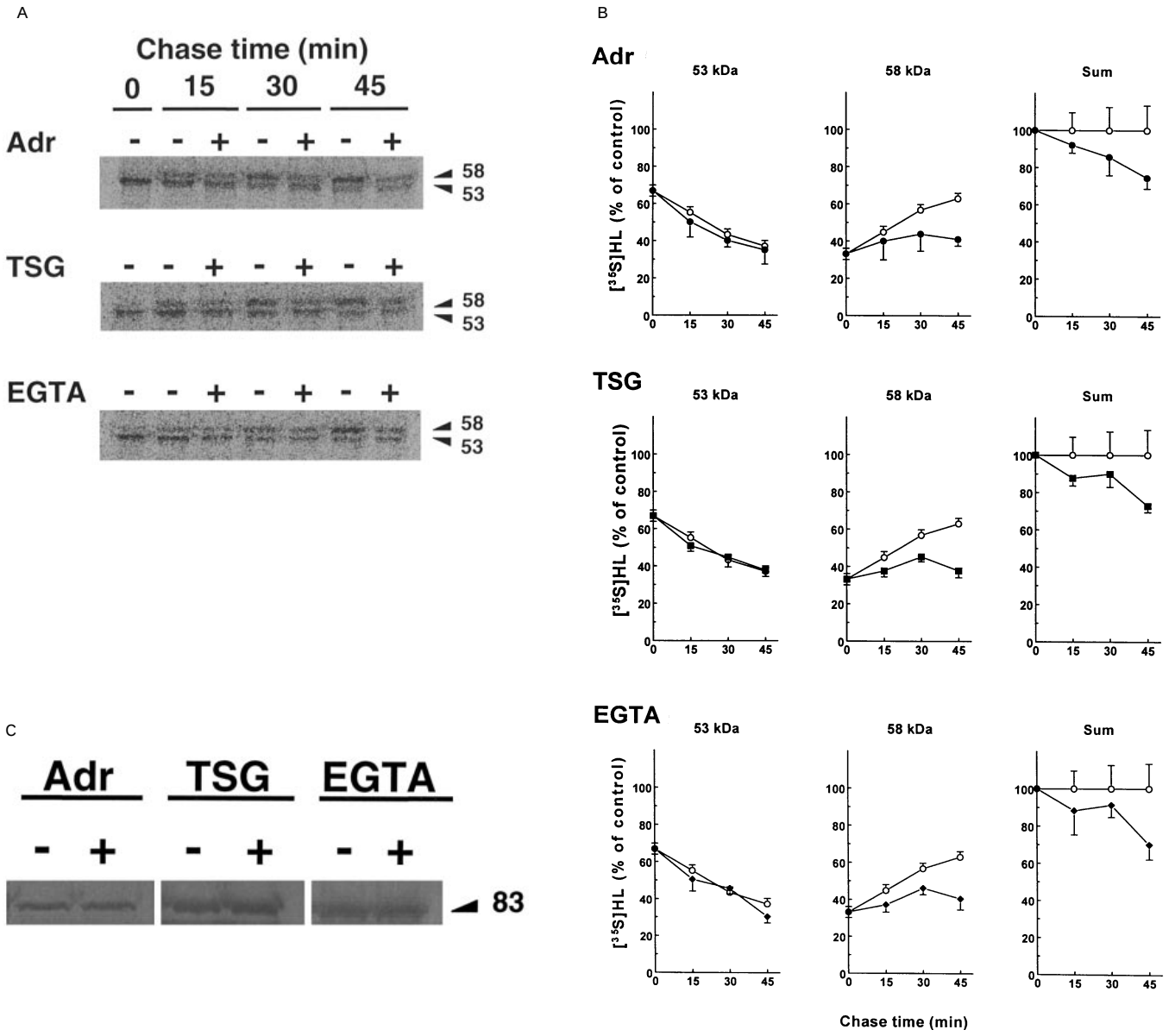

Figure 3 Maturation of HL in the presence of adrenaline, thapsigargin or EGTA

Hepatocytes were pulsed with [^{35}S]methionine for 5 min in the absence of any agent. After washing the cells in fresh medium, part of the cell suspension was incubated in control medium, and another part was incubated in medium containing either 10 μM adrenaline (Adr), 1 μM thapsigargin (TSG) or 500 μM EGTA. At the times indicated samples were collected and HL was immunoprecipitated from the whole cell suspensions. The [^{35}S]HL was analysed by SDS/PAGE followed by phosphor imaging. (**A**) A representative image for a chase in the absence (−) and presence (+) of Ca^{2+}-modulating agents. The apparent molecular weight of the bands is indicated in kDa. The quantified data in (**B**) are expressed as percentage of total radioactivity in the HL bands at the start of the chase (mean ± S.D. $n = 3$). They show, from left to right, the disappearance of the 53 kDa protein, appearance of the 58 kDa protein, and total radioactivity in the 53 plus 58 kDa bands in the absence (○) or presence of either adrenaline (Adr, ●), thapsigargin (TSG, ■), or EGTA (◆). (**C**) Shows a fluorograph of [^{35}S]transferrin, which was immunoprecipitated from the extracellular medium after 45 min of chase in the absence (−) or presence (+) of either adrenaline (Adr), thapsigargin (TSG) or EGTA.

protein, and stimulate the intracellular degradation of HL. Thus, the mechanisms by which HL secretion was inhibited by these agents were highly similar.

Maturation and secretion of HL was inhibited when the intracellular Ca^{2+} concentration was increased by incubation with Ca^{2+}-mobilizing hormones, A23187 and thapsigargin, suggesting that the intracellular Ca^{2+} concentration itself may influence the processing of HL. However, HL maturation was inhibited similarly when the free extracellular Ca^{2+} was reduced with EGTA to concentrations close to or below intracellular Ca^{2+}. Under these conditions, intracellular Ca^{2+} may be reduced, and EGTA may inhibit HL processing by lowering intracellular Ca^{2+}. These results imply that, besides elevation of intracellular Ca^{2+}, also the lowering of intracellular Ca^{2+} mimics the effect of adrenaline on maturation and secretion of HL. EGTA may induce a different process than the Ca^{2+}-mobilizing agents, which leads to a similar inhibition of HL maturation and increase in HL degradation. Alternatively, this apparent contradiction may be resolved by considering the effects on Ca^{2+} levels in the ER. The α_{1B}-adrenoceptor stimulates the release of Ca^{2+} from

IP_3-sensitive stores, such as the ER [20–22]. The Ca^{2+} ionophore A23187 will carry Ca^{2+} across the ER membrane along the gradient of Ca^{2+}, thereby lowering ER Ca^{2+} [42,43]. Thapsigargin increases intracellular Ca^{2+} by inhibition of the Ca^{2+}–ATPase in the ER membrane; Ca^{2+} is no longer retrieved from the cytosol and the Ca^{2+} level in the ER decreases [31,43]. Incubation of hepatocytes with EGTA depletes non-mitochondrial Ca^{2+} pools, including the ER [43]. Therefore, the decreased Ca^{2+} level in the ER, induced by incubation of hepatocytes with adrenaline, A23187, thapsigargin and EGTA, may evoke the effects on HL processing.

In which order the retardation of HL maturation and degradation of HL protein occurs is unclear. Lowering ER Ca^{2+} may increase the degradation process by increasing the activity of Ca^{2+}-sensitive proteases in the ER. Maturation of α_1-antitrypsin and the asialoglycoprotein receptor was also reported to be decreased by lowering Ca^{2+} in the ER [44,45]. In contrast, secretion and maturation of the glycoprotein transferrin was not affected by adrenaline, thapsigargin or EGTA. Depletion of ER Ca^{2+} was also shown to selectively increase the degradation transfected T-cell antigen receptor-β and CD3-δ, but not of CD3-γ and CD3-ϵ-β dimers in CHO cells [46]. Alternatively, the effects on HL processing may be mediated by processes other than the Ca^{2+}-dependent stimulation of proteases in the ER. A low Ca^{2+} level in the ER may change the affinity of HL for possible chaperones, whereafter HL may become more susceptible to ER degradation. Several chaperones resident in the ER are reported to be calcium-dependent [47]. The existence of a chaperone with selectivity towards HL and other lipases was implicated from mice suffering from combined lipase deficiency [48,49]. Thus, a specific Ca^{2+}-sensitive chaperone may be involved in HL maturation. We conclude that the inhibition of HL maturation and increase in intracellular degradation by catecholamines, A23187, thapsigargin and EGTA are mediated by changes in Ca^{2+} homoeostasis, possibly through lowering ER Ca^{2+}.

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