Involvement of Calcium in the Mevalonate-accelerated Degradation of 3-Hydroxy-3-methylglutaryl-CoA Reductase*

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3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in the biosynthesis of cholesterol and isoprenoids, is subject to rapid degradation which is regulated by mevalonate (MVA)-derived metabolic products. HMG-CoA reductase is an integral membrane protein of the endoplasmic reticulum, the largest nonmitochondrial pool of cellular Ca\(^{2+}\). To assess the possible role of Ca\(^{2+}\) in the regulated degradation of HMG-CoA reductase, we perturbed cellular Ca\(^{2+}\) concentration and followed the fate of HMG-CoA reductase and of HMGal, a fusion protein consisting of the membrane domain of HMG-CoA reductase and the soluble bacterial enzyme \(\beta\)-galactosidase. The degradation of HMGal mirrors that of HMG-CoA reductase, demonstrating that the membrane domain of HMG-CoA reductase is sufficient to confer regulated degradation (Skalnik, D. G., Narita, H., Kent, C., and Simon, R. D. (1988) J. Biol. Chem. 263, 6836-6841; Chun, K. T., Bar-Nun, S., and Simon, R. D. (1990) J. Biol. Chem. 265, 22004-22010). In this study we show that the MVA-dependent accelerated rates of degradation of HMG-CoA reductase and HMGal in cells maintained in Ca\(^{2+}\)-free medium are 2-3-fold slower than the rate of degradation in cells grown in high (1.8-2 mM) Ca\(^{2+}\) concentration. This effect is reversed upon addition of Ca\(^{2+}\) to the medium. Furthermore, when cells maintained in high Ca\(^{2+}\) are treated with cobalt, a known antagonist of Ca\(^{2+}\)-dependent cellular activity and is sufficient to support growth when expressed in an HMG-CoA reductase-deficient cell line (4); (ii) a membrane-bound NH\(_2\)-terminal domain which contains several stretches of hydrophobic residues that are predicted to span the ER membrane seven times (5). Although this membrane domain of HMG-CoA reductase lacks a catalytic role, it is sufficient to confer ER localization onto HMGal, a fusion protein consisting of the membrane domains of HMG-CoA reductase and the bacterial enzyme \(\beta\)-galactosidase (6).

The cellular levels of HMG-CoA reductase are regulated by cholesterol as well as by a MVA-derived nonsterol product(s) of the pathway (1). This regulation is at the level of transcription of the reductase gene (7, 8), translation of its mRNA (9-11), and via regulated degradation of the enzyme molecules (11-14). The enzymatic activity of HMG-CoA reductase is also regulated by a reversible phosphorylation/dephosphorylation cascade (15-17) and through thiol/disulfide exchange (18, 19). In animals and in cultured cells, HMG-CoA reductase has a short half-life relative to most cellular proteins and to other ER proteins in particular. When cells are supplied with sterols or high concentrations of MVA, HMG-CoA reductase is degraded at a 2-5-fold faster rate (1, 11, 13, 14). Conversely, when endogenous production of MVA or sterols is blocked by drugs, such as compactin, the reductase is degraded at a several-fold slower rate (1, 11-12, 13).

It has been shown that the membrane-spanning domain of the reductase is necessary for its rapid regulated degradation and is sufficient to confer regulated degradation of HMGal (6, 20). Deletion of this domain results in soluble proteins with long half-lives and which no longer respond to MVA/sterols (6, 14, 20, 21). These results demonstrate the crucial role of the membrane domain of HMG-CoA reductase in regulating the degradation of the enzyme. However, little is known about additional factors that participate in this process such as the signal(s) evoked by MVA/sterols, the "tagging" of the reductase to become a substrate for accelerated degradation, the intracellular site at which this degradation takes place, and the protease(s) involved.

The regulated degradation of HMG-CoA reductase appears
to involve neither lysosomes (11, 22) nor the ubiquitin-dependent degradative pathway (23). Taking advantage of HMGal which mirrors the fate of HMG-CoA reductase, we have recently provided evidence indicating that ongoing protein synthesis is required for the regulated degradation and that this process takes place in the ER (20). The ER has recently emerged as a degradative compartment for luminal (24) and membrane (25–28) proteins and is thought to be the first “quality control” checkpoint for newly synthesized proteins which are destined to exit the ER (29). This process, which is best illustrated by the degradation of individual subunits of plasma membrane protein complexes, such as the T-cell antigen receptor (30) and the asialoglycoprotein receptor (26), appears to be determined, in part, by the state of assembly of these proteins. Thus, in the absence of their complementary subunits, these proteins are retained in the ER and are rapidly degraded (30). Although the ER degradation of different proteins may proceed at various rates, this process is generally rapid and does not appear to be regulated. In this respect, the ER degradation of HMG-CoA reductase and HMGal is unique in that steroids and/or MVA regulate the rate of this process. Taken together with the ER residency of these proteins, we propose that MVA/steroids may, in part, regulate the rate of HMG-CoA reductase degradation indirectly by modulating some ER function(s). An important function of the ER is to serve as the major intracellular reservoir of Ca2+ and as a dominant organelle regulating cytosolic Ca2+ levels (31, 32). Ca2+ in the ER has also been implicated in the proper folding and secretion of proteins via the secretory pathway and in retention of resident proteins in the lumen of the ER (31, 33, 34). In the current study we demonstrate that perturbation of cellular Ca2+ impairs the MVA-dependent accelerated degradation of HMG-CoA reductase.

EXPERIMENTAL PROCEDURES

**Materials**—We obtained ionomycin from Calbiochem. Minimal essential medium (MEM, Cat. number 410-1100, [Ca2+]i = 1.8 mM), Ca2+-free MEM (Cat. number 410-1800) and G418 sulfate were from Gibco-Bethesda Research Laboratories. [35S]Methionine (>100 Ci/mmol) was from Amersham, and [3H]MVA (40 Ci/mmol) was purchased from Du Pont-New England Nuclear. Compactin was a generous gift of A. Endo (Tokyo Noko University, Japan). Antibodies against synthetic peptides in the membrane domain of HMG-CoA reductase were raised in New Zealand White rabbits. The characterization of these antibodies will be described elsewhere.

**Cells**—Stock cultures of CHO-HMGal cells (6, 20) were maintained in 5% CO2 atmosphere in regular MEM supplemented with 5% fetal calf serum and 250 µg/ml active G418. For experiments shown in Figs. 1 and 3, CHO-HMGal cells were plated on Day 1 in 24-well dishes in MEM supplemented with 5% lipid-poor serum (LPS) (35) and 250 µg/ml G418. On Day 2, compactin and MVA were added to the medium to final concentrations of 1 and 100 nM, respectively. Under these conditions, HMGal activity in the cells is maximal (20). The medium was removed and the cells were washed once with Ca2+-free PBS and transferred to 1 ml of either regular (1.8 mM Ca2+) MEM or Ca2+-free MEM, both supplemented with 5% LPS, 250 µg/ml G418, 1 µM compactin (without MVA), and 100 µM MVA. The cells were pulsed for 2 h with [35S]methionine (0.5 mM; 0.3 ml/dish in starvation medium) and then chased for the indicated periods of time in Ca2+-free or regular MEM supplemented with 5% LPS/1 µM compactin in the absence or presence of 20 mM MVA and/or 1 µM ionomycin. Cells were lysed and processed for immunoprecipitation, as previously described (20), using anti-membrane domain antibodies. Immune complexes were resolved by 5-15% SDS-PAGE (36).

**Incorporation of [3H]MVA into Sterols**—CHO-HMGal cells were plated on 50-mm dishes on Day 1 and grown in MEM supplemented with 5% LPS and 250 µg/ml of G418 for 48 h. On Day 3, the medium was replaced by the same fresh medium containing 1 µM compactin and 100 µM MVA. On Day 4, the medium was removed and the cells were washed once with Ca2+-free PBS and transferred to 1 ml of either regular (1.8 mM Ca2+) MEM or Ca2+-free MEM, both supplemented with 5% LPS, 250 µg/ml G418, 1 µM compactin (without MVA). After 4 h later, [3H]MVA (dissolved in 30 mM KP, pH 4.5) was added to a final concentration of 31 µCi/ml, and the cells were labeled for the indicated time periods. At each time point, the medium was removed and the cells were washed three times in 5 ml of ice-cold PBS, and lysed by 15-min incubation on ice with 0.5 ml of 1% Triton X-100. The cell lysate was transferred to a glass tube, and the dish was washed once more with 0.5 ml of 1% Triton X-100. An aliquot was withdrawn for protein determination, and the lysates were supplemented with 50 µg each of unlabeled squalene, lanosterol, and cholesterol. The mixture was saponified with 5% methanolic KOH for 60 min at 65 °C, and extracted three times with 2 ml of hexane. The combined hexane extracts were dried under N2, redissolved in a minimal volume of hexane, and spotted, along with standards, onto a 0.25-mm Silica Gel G thin layer chromatography plate. The plate was developed with ethyl acetate:hexane (12:88) and the standards were visualized in I2 vapor. Sections (1 cm) of the silica were scraped into scintillation vials and counted for incorporated [3H]MVA. The results given are the counts recovered in the cholesterol spot. At all time points, and in either media, the radioactivity in the lanosterol spot constituted about 13% of the radioactivity incorporated into total nonsaponifiable lipids. Under the above conditions, no radioactivity was recovered in the squalene spot.

**RESULTS**

To assess a possible role of Ca2+ in the degradation of HMG-CoA reductase, we perturbed Ca2+ homeostasis in CHO cells expressing the fusion protein HMGal (CHO-HMGal cells) by adding Ca2+ ionophores to their growth medium. In preliminary experiments, we established that ionomycin, at a concentration of 1 µM, had no effects on the morphology or viability of cells maintained in MEM containing high (1.8 mM) Ca2+. Yet, at this concentration, ionomycin reduced by 50–60% the MVA-dependent decrease in HMGal activity (Fig. 14; see below). At higher concentrations of ionomycin, cells began to round up and detach from the dish. We therefore used 1 µM ionomycin throughout this study.

The data shown in Fig. 1 demonstrate the time course of disappearance of HMGal enzymatic activity following addition of MVA and/or ionomycin to the medium of CHO-HMGal cells. In Ca2+-containing MEM, addition of 20 mM MVA caused a time-dependent decrease in HMGal activity, with an apparent t1/2 of about 6 h (Fig. 1A). As the transfected gene for HMGal is transcribed from the constitutive SV40 early promoter, we have previously shown that this decrease in activity reflects a net MVA-dependent accelerated rate of HMGal degradation (6, 20). Treatment with ionomycin alone resulted in a slight decrease in HMGal activity, with an apparent t1/2 value of about 34 h. However, when added together, ionomycin effectively attenuated the MVA-dependent accelerated decrease in HMGal activity (Fig. 1A). Under these conditions, the activity declined with an apparent t1/2.

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value of 13.5 h, reflecting more than 2-fold decrease in the MVA-accelerated degradation rate. Neither MVA, nor ionomycin, had any effect on β-galactosidase activity, as observed in CHO cells expressing the soluble β-galactosidase (CHO-Gal (6, 20); data not shown). This demonstrates that the observed effects of MVA and/or ionomycin are restricted to the ER-bound fusion protein and reflect changes in the steady-state levels of HMGal.

The data shown in Fig. 1B depicts the results of a similar experiment, performed in Ca2+-free MEM. The decline in HMGal activity following addition of MVA progressed with an apparent t1/2 of 18 h, 3-fold slower than in Ca2+-containing MEM. Surprisingly, when only ionomycin was added, HMGal activity increased more than 2-fold by the 22-h time point, with a calculated doubling time of 19 h (Fig. 1B). Since HMGal gene is transcribed from the constitutive SV40 promoter, this indicates that, in cells exposed to ionomycin in Ca2+-free medium, the degradation rate of HMGal is even slower than the rate of its synthesis, resulting in net accumulation of the enzyme. Addition of MVA along with ionomycin prevented this increase in activity (t1/2 >180 h) indicating that, under these conditions, the rate of HMGal degradation equals the rate of its synthesis, as it does in untreated control cells.

To demonstrate directly that the observed changes in HMGal activity following these treatments result from altered degradation rates of the enzyme, pulse-labeled CHO-HMGal cells were chased either in Ca2+-free or Ca2+-containing medium. Yet, in Ca2+-free medium, MVA was ineffective in accelerating the degradation rate of both proteins. Addition of ionomycin to the chase extended the half-life of HMGal and HMG-CoA reductase about 2-fold (Table I). Taken together with the results presented in Fig. 1, we conclude that the observed changes in the activity of HMGal can be fully accounted for by respective changes in the degradation rates of both HMGal and HMG-CoA reductase proteins.

We next examined whether the inability of MVA to accelerate HMGal degradation in Ca2+-free medium could be reversed by adding Ca2+ back to the medium. The data in Fig. 3 show that without added Ca2+, only about 55% of HMGal was degraded after 22-h incubation with 20 mM MVA, as compared to the 100% found in cells incubated without MVA. This residual degradation in Ca2+-free medium was not affected by inclusion of up to 0.5 mM EGTA (data not shown), suggesting that the partial effectiveness of MVA is not due to trace amounts of Ca2+ in the medium. Addition of as little as 0.1 mM of Ca2+ to the medium restored the effect of MVA, resulting in the typical 80–85% of HMGal being degraded during this time period, and this level of degradation was maintained at higher Ca2+ concentrations. The data in Fig. 3 also show that, when ionomycin was included in the medium, added Ca2+ no longer restored the effect of MVA. In Ca2+-free medium, the residual HMGal in MVA-treated cells was about 55% that of found in ionomycin-treated cells which were not exposed to MVA. Although addition of 0.1 mM of Ca2+ to the ionomycin-containing medium did not affect this value significantly, added Ca2+ at the 0.25–0.5 mM range reproducibly diminished the effectiveness of MVA. Under these conditions, only about 25% of HMGal was degraded (Fig. 3). At higher Ca2+ concentrations, the MVA-dependent degradation returned to the typical value of 50% of HMGal remaining, even at concentrations as high as 8 mM (data not shown). Interestingly, ionomycin-treated cells that are maintained in low Ca2+ (0–0.1 mM) for 22 h become round and lose their characteristic fibroblast morphology. Yet, these morphological changes are largely prevented when MVA is also included in the medium (data not shown).

The striking effect of Ca2+ perturbation on the MVA-accelerated degradation of HMGal raised the possibility that MVA uptake or its incorporation into sterols might require extracellular Ca2+. To examine this possibility, we labeled CHO-HMGal cells with [3H]MVA for various lengths of time, in Ca2+-containing or Ca2+-free medium, and determined the radioactivity incorporated into cholesterol, the bulk product of cellular MVA metabolism (Fig. 4). As can be seen, the incorporation of MVA into cholesterol proceeded linearly with time for up to 6 h, regardless of the presence of Ca2+ in the medium. Under the conditions employed, most (~87%) of the radioactivity incorporated into the nonsaponifiable lipid fraction migrated as cholesterol on thin layer chromatography. The remaining 13% of the radioactivity was recovered as lanosterol. These results demonstrate that the attenuated effectiveness of MVA in accelerating the degradation of HMG-CoA reductase/HMGal in cells maintained in Ca2+-free medium results neither from reduced uptake of MVA nor from diminished capacity to synthesize sterols. These results are consistent with a previous study reporting that MVA enters CHO cells by passive diffusion (37) which is Ca2+-independent and that the rate of incorporation of MVA is limited by the rate of its uptake.

Additional support for the requirement for Ca2+ in the MVA-dependent accelerated degradation of HMGal was demonstrated by blocking Ca2+-dependent cellular functions.
Degradation of HMG-CoA Reductase

A. Calcium-containing medium

B. Calcium-free medium

FIG. 2. Perturbation of cellular calcium attenuates the MVA-dependent accelerated degradation of HMG-CoA reductase and HMGA. CHO-HMGA cells were pulse-labeled with [35S]methionine and chased in Ca²⁺-containing (A) or Ca²⁺-free (B) medium in the absence (○) or the presence of MVA (20 mM, △), ionomycin (1 μM, ■), or both (□, ○, △ only). At the indicated time points, cells were lysed and HMGA-CoA reductase (HMGCR) and HMGA were immunoprecipitated simultaneously using anti-HMG-CoA reductase membrane domain antibodies. The immunoprecipitates were resolved by 5-15% SDS-PAGE. The fluorograms (insets) were quantified by densitometry, and the results presented are those for HMGA. The results for both HMGA-CoA reductase and HMGA are summarized in Table I.

TABLE I

Half-lives of HMGA and HMGA-CoA reductase $t_{1/2}$

Values were calculated from pulse-chase experiments similar to those shown in Fig. 2. Results are means ± S.D. of three independent experiments.

| Medium Addition | HMGCR t₁/₂ (h) | HMGA t₁/₂ (h) |
|-----------------|----------------|---------------|
| +Ca²⁺ None      | 6.6 ± 0.4      | 7.3 ± 2.1     |
| MVA             | 3.0 ± 0.0      | 2.5 ± 0.7     |
| Ionomycin       | 8.7 ± 0.6      | 7.8 ± 1.6     |
| MVA + Ionomycin | 6.5 ± 0.7      | 6.5 ± 0.7     |
| -Ca²⁺ None      | 6.7 ± 0.6      | 7.5 ± 2.3     |
| MVA             | 5.0 ± 0.0      | 7.5 ± 0.7     |
| Ionomycin       | 14.7 ± 2.5     | 14.0 ± 2.0    |

CHO-HMGA cells, maintained in high Ca²⁺ medium, were exposed to increasing concentrations of cobalt chloride. Co²⁺ is a potent Ca²⁺ antagonist known to inhibit Ca²⁺ influx to a variety of cells by blocking Ca²⁺ channels in the plasma membrane and was implicated to interfere with intracellular vesicular movement (38-44). The data in Fig. 5 show that Co²⁺, although partially affecting the basal activity, totally inhibits the MVA-dependent accelerated degradation of HMGA (Fig. 5A), with an IC₅₀ = 520 μM (Fig. 5B). Since Co²⁺ is able to totally abolish the accelerated degradation of HMGA, while omission of extracellular Ca²⁺ only attenuates degradation by about 50%, we suggest that Co²⁺, in addition to perturbing Ca²⁺ uptake, must also interfere with intracellular Ca²⁺-dependent processes operating in the regulated degradation of HMGA-CoA reductase.

FIG. 3. Effect of extracellular calcium on the degradation of HMGA. Cells were set up for experiments in media containing the indicated concentrations of Ca²⁺. HMGA activity was assayed 22 h after addition of MVA (20 μM) to cells treated without (○) or with 1 μM ionomycin (■). The results presented are given as percentage of HMGA activity without MVA and are the mean of five to 14 independent experiments, each performed in hexaplicates.
Degradation of HMG-CoA Reductase

![Graph](image)

**FIG. 4.** Incorporation of [3H]MVA into cellular cholesterol is independent of external calcium. CHO-HMGal cells, maintained in Ca²⁺-containing (●) or Ca²⁺-free (▲) MEM, were labeled with [3H]MVA (31 μCi/ml, 40 Ci/mmol) for the indicated periods of time, as described under "Experimental Procedures." Cellular [3H]cholesterol content was determined, as described under "Experimental Procedures." The results are the mean of duplicate determinations.

**FIG. 5.** Cobalt inhibits the MVA-dependent accelerated degradation of HMGal. A, CHO-HMGal cells, maintained in Ca²⁺-containing MEM, were treated with the indicated concentrations of cobalt chloride in the presence (●) or absence (▲) of 20 mM MVA. HMGal activity was assayed after 22-h incubation. B, the results are presented as percentage of HMGal activity in the absence of MVA and are the mean of two experiments, each performed in hexaplicates.

**DISCUSSION**

The results presented here demonstrate that maintenance of cellular Ca²⁺ homeostasis is required for the regulated degradation of the endoplasmic reticulum proteins HMG-CoA reductase and HMGal. Specifically, perturbation of cellular Ca²⁺ by ionomycin largely attenuates the MVA-dependent accelerated degradation of both proteins when cells are maintained in Ca²⁺-containing medium. These phenomena are amplified when the cells are transferred to Ca²⁺-free medium. Under these conditions, MVA is much less effective in accelerating the degradation of HMGal and HMG-CoA reductase, and ionomycin totally abrogated the response to MVA (Figs. 1 and 2 and Table I), such that for HMGal the rate of its degradation is even slower than the rate of its synthesis, resulting in a net accumulation of active enzyme molecules (Fig. 1). Addition of as little as 0.1 mM Ca²⁺ to the external medium fully restored the effectiveness of MVA and, again, in the presence of ionomycin, this restoration was prevented even at high extracellular Ca²⁺ concentrations (Fig. 3). Moreover, exposing the cells to Co²⁺, a potent antagonist of Ca²⁺-dependent functions, completely abolished the response to MVA. Yet, Ca²⁺ does not appear to influence the basal turnover rate of HMGal and HMG-CoA reductase measured in the absence of MVA.

There are several mechanisms that might link between the MVA-dependent accelerated degradation of HMGal and HMG-CoA reductase and Ca²⁺ homeostasis as follows.

(i) **HMGal and HMG-CoA Reductase Are Degraded by a Ca²⁺-dependent Protease**—Indirect evidence suggests that degradation of HMG-CoA reductase takes place in the ER (20, 45), yet the protease(s) involved in this process in vivo has not been identified. *In vitro*, the cytoplasmic domain of the reductase can be proteolyzed by a Ca²⁺-dependent cysteine protease, probably calpain II, to generate a 53–55-kDa soluble enzymatically active fragment (2, 5, 46). Calpains are Ca²⁺-dependent neutral cysteine proteases that have been detected in many tissues (47–50). There are mainly two types of calpains, calpain I (or μCAND) and calpain II (or mCAND), which differ in their affinities for Ca²⁺ ions. Both types are found predominantly in the cytoplasm, though significant proportions of these proteases are associated with membranes. The activity of the calpains is thought to be regulated by Ca²⁺ concentrations and by an endogenous protein inhibitor calpastatin (47–50). We have recently shown that synthetic peptides, designed to inhibit the activity of calpains, totally block the basal and the MVA-dependent accelerated degradation of HMGal and HMG-CoA reductase in vivo (51). However, we could neither detect any changes in the amounts of calpains or calpastatin in cells challenged with MVA, nor could we detect changes in Ca²⁺-dependent proteolytic activities in extracts prepared from such cells (51). Thus, although calpain(s), or other Ca³⁺-dependent calpain-like protease(s), may be involved in the degradation of HMGal and HMG-CoA reductase in vivo, the observation that ionomycin diminished the effect of MVA even in the presence of high Ca²⁺, argues against the possibility that the protease(s) is devoid of Ca²⁺ ions required for its activity.

(ii) **Degradation of HMGal/HMG-CoA Reductase Requires Ca²⁺-dependent Exit from the ER**—There is a growing body of evidence for the involvement of Ca²⁺ in the retention of proteins in the lumen of the ER (31, 34), transport of secretory proteins out of the ER (33, 52), and in secretion of hormones via the regulated secretory pathway (53, 54). In most cases, perturbation of cellular Ca²⁺ by ionophores or by Ca²⁺ antagonists leads to inhibition of these transport events. Therefore, the inhibition of the MVA-accelerated degradation of HMGal and HMG-CoA reductase, observed when cells were transferred to Ca²⁺-free medium or treated with ionomycin or Co²⁺, might also be viewed in the context of inhibition of a Ca²⁺-dependent transport event whereby these proteins are transported to a post-ER degradative compartment. Although this possibility awaits a direct demonstration, the facts that the MVA-dependent accelerated degradation of HMGal and HMG-CoA reductase occurs when exit of proteins from the ER is arrested by brefeldin A (20), and that this degradation in brefeldin A-treated cells could be blocked by calpain inhibitors (51), strongly argue against such a mechanism.

(iii) **Oscillations in Cytoplasmic Ca²⁺ Are, in Part, the Trigger for the MVA-dependent Accelerated Degradation**—The distri-
bution of Ca\(^{2+}\) in the cell is not uniform. The concentration of Ca\(^{2+}\) in the ER, the largest nonmitochondrial Ca\(^{2+}\) pool, is estimated to be about 3 mM, whereas in the cytoplasm is three orders of magnitude lower (31). In many nonexcitable cell types, Ca\(^{2+}\) is discharged into the cytoplasm in an oscillatory pattern in response to external stimuli which are mediated intracellularly by Ins-1,4,5-P\(_3\) (55–57). It has been suggested that releasable intracellular Ca\(^{2+}\) is stored in two pools, of which only one, presumably the ER, is Ins-1,4,5-P\(_3\)-sensitive. The Ins-1,4,5-P\(_3\)-insensitive pool is proposed to be discharged by a Ca\(^{2+}\)\(-\)dependent process with Ca\(^{2+}\) originating from the Ins-1,4,5-P\(_3\)-sensitive pool and from the extracellular milieu (55–57). The recharging of these pools depends on extracellular Ca\(^{2+}\) and Ins-1,4,5-P\(_3\), and is thought to be regulated by Ins-1,3,4,5-P\(_4\) and GTP (32, 58). Our finding that ionomycin, MVA still partially affects the rate of HMGal and ionomycin at low extracellular Ca\(^{2+}\).

The substrates and products of the HMG-CoA reductase reaction are water-soluble and are formed in the cytosol. Therefore, it is not surprising that the catalytic activity of the reductase is carried out by the cytosol-facing domain of the enzyme. Yet, about one-third of the NH\(_2\)-terminal end of the native HMG-CoA reductase is embedded in the endoplasmic reticulum. It has been shown that this membrane domain of the reductase, which lacks any catalytic activity, is necessary for the regulated degradation of the enzyme (14). It seems likely that the localization of HMG-CoA reductase in the membrane of the ER is necessary for the regulatory response since the regulatory molecules, sterols for example (45), are themselves hydrophobic and membrane-bound. The results presented in this report might give an additional reason for the ER localization of HMG-CoA reductase. Since calcium is required for the regulated degradation of reductase, the enzyme is located in the compartment which stores the largest pool of cellular calcium.

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