Decreased Synthesis and Increased Intracellular Degradation of Newly Synthesized Collagen in Freshly Isolated Chick Tendon Cells Incubated with Monensin†

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ABSTRACT: The synthesis and secretion of procollagen in embryonic chick tendon fibroblasts in suspension culture were inhibited with the carboxylic ionophore monensin. The synthesis of procollagen was inhibited by 50% in a 2-h exposure to 0.1 μM monensin and was inhibited by 70% in a 6-h exposure to 0.1 μM monensin. Secretion of procollagen was inhibited by >90% in the 0.1 μM monensin-treated cultures and was totally inhibited by higher doses of the reagent. A cellular pool of collagenase-digestible peptides was demonstrated in the control cells, the level of which was elevated 3–4 times in the monensin-treated cultures. In order to determine whether the secretory and synthesis block caused by monensin inhibited intracellular degradation of newly synthesized collagen, the hydroxy[^{14}C]proline in degraded collagen fragments present in control and monensin-treated cultures was determined and compared to the total hydroxy[^{14}C]proline synthesized in each culture. The intracellular degradation of newly synthesized, pulse-labeled collagen was shown to proceed at rates comparable to those seen in the control cultures. The monensin-treated cells degraded pulse-labeled newly synthesized collagen nearly twice as long as the controls, resulting in an overall increase in the fraction of newly synthesized collagen that was degraded. The results indicate that inhibiting the synthesis and blocking the secretion of procollagen did not inhibit the intracellular degradation of a significant fraction of newly synthesized collagen. The results also indicate that intracellular degradation is not dependent on continuing synthesis nor does it require the participation of elements of the secretory pathway distal to the monensin block.

The intracellular degradation of newly synthesized collagen is a phenomenon that has now been described in a variety of collagen-producing cell lines and tissues (Rennard et al., 1982; Bienkowski, 1984a,b; Berg, 1986). It has been shown that 10–20% of all newly synthesized collagen is degraded by cells actively involved in its synthesis, and this degradation occurs prior to secretion.

It is currently recognized that intracellular degradation may occur in two modes. The 10–20% levels of degradation observed in cells synthesizing collagen under normal culture conditions constitute what has been termed the basal level (Bienkowski et al., 1978a,b; Berg et al., 1980). In addition, certain culture conditions have been shown to result in levels of intracellular degradation significantly elevated above the basal level to 25–50% of all newly synthesized collagen (Berg et al., 1980, 1983, 1984; Neblock & Berg, 1982a,b; Baum et al., 1980; Bienkowski, 1984b; Palotie, 1983; Steinmann et al., 1981), depending on the cell type and culture conditions. This increased intracellular degradation has been shown to differ from basal level with respect to susceptibility to inhibitors of cellular proteolysis (Berg et al., 1980, 1984). This has been designated the enhanced mode of intracellular degradation (Berg et al., 1980). When cells are exposed to conditions or agents that lead to the synthesis of non-triple-helical collagen, the enhanced mode of degradation has been shown to be activated (Berg et al., 1980, 1984; Neblock & Berg, 1982a,b), and indirect evidence based on the use of lysosomal protease inhibitors and on the identification of hydroxyproline in lysosomes suggests that the enhanced intracellular degradation occurs in lysosomes (Berg et al., 1984).

While some gains have been made in the elucidation of the involvement of lysosomal proteinases in the enhanced degradation mode, little is known about the cellular mechanism responsible for the basal level of intracellular degradation. A recent report has indicated that the kinetics of basal intracellular degradation in embryonic chick tendon cells differ sufficiently from the kinetics of collagen production and secretion and suggests the existence of a degradation pathway that is at some point divergent from the collagen secretory route (Bienkowski et al., 1986). On the basis of the kinetic studies alone, however, it is still not possible to establish the point in the secretory pathway where newly synthesized collagen may be degraded or shuttled to another compartment where the degradation may occur.

In order to provide a clue as to the location of the basal mode of intracellular degradation, a reagent that blocks the secretion of procollagen was employed. The carboxylic ionophore monensin has been shown to block the translocation of procollagen (Uchida et al., 1979, 1980; Nishimoto et al., 1982a,b) and a variety of other secretory proteins (Tartakoff & Vassili, 1977, 1978; Nagase et al., 1983; Ledger & Tanzer, 1984; Tartakoff, 1983) in the cis face of the Golgi apparatus. While the ability of monensin to insert into membranes and facilitate the transmembrane exchange of Na^+ for K^+ or H^+ ions has been characterized (Tartakoff, 1983; Pressman & Fahim, 1982), how this perturbation of ion gradients in cellular vesicles leads to the inhibition of secretory processes is not known.

The purpose of the present investigation was to determine if basal intracellular degradation is altered by the presence of monensin. Freshly isolated, embryonic chick tendon cells devote 20–40% of their total protein synthesis to procollagen in short-term suspension cultures and demonstrate basal levels of intracellular degradation of approximately 10–15% of their

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newly synthesized collagen (Neblock & Berg, 1982a,b; Curran et al., 1983). Because they synthesize a substantial amount of collagen, chick tendon cells provide an ideal model system in which to study intracellular degradation of collagen (Berg, 1986).

Experimental Procedures

Materials. L-[U-^{14}C]Proline was purchased from New England Nuclear, purified clostridial collagenase was purchased from Advanced Biofactures, Inc., and culture supplies were purchased from Gibco, Inc. Monensin was purchased from Sigma, and all other chemicals were purchased from Sigma or Fisher Scientific.

Suspension Cultures of Embryonic Chick Tendon Cells. Freshly isolated tendon fibroblasts were obtained from the leg tendons of 17-day embryonic chicks and were grown in suspension cultures according to the procedure of Dehm and Prockop (1971) as modified by Kao et al. (1977). The growth and labeling medium for the cultures of up to 6 h consisted of modified Krebs(II) medium containing 0.2% glucose, 2% fetal bovine serum, and 10 μg/mL sodium ascorbate. Cells were grown in culture tubes in a shaking water bath at 37 °C for the culture periods as indicated in the descriptions of individual experiments. Except as indicated, all variables and controls were incubated in identical, triplicate cultures. Cells were incubated at 7.5 × 10^6 cells/mL as indicated for individual experiments.

Radioisotopic Labeling of Protein Synthesis. The synthesis of collagenous and noncollagenous polypeptides by the suspension cultures was labeled by the incorporation of [^{14}C]-proline included in the growth medium at a concentration of 2 μCi/mL. The specific activity of the [^{14}C]proline was 273 mCi/mmol. Radiochemical contamination with hydroxy-[^{14}C]proline has been shown to be less than 0.01% (Bienkowski & Engels, 1981). At the end of labeling periods, the synthesis and degradation of proteins were terminated by placing culture tubes in a 100 °C water bath for 10 min. The samples were then frozen at −20 °C pending further analysis. After thawing the samples, each was sonicated with a microprobe sonicator (Kontes Instruments) in order to ensure the release of cellular contents.

Determination of Collagen and Noncollagen Protein Production. Aliquots were removed from each suspension sample and dialyzed against several changes of deionized H_2O at 4 °C for no more than 48 h, until less than 100 cpm remained in the final dialysate. The nondialyzable material was then lyophilized and resuspended in a calcium-containing collagenase digestion assay buffer as detailed by Berg (1982). The amount of [^{14}C]proline present in collagenous (CDP) and noncollagenous (NCP) peptides was determined by digestion of parallel aliquots of the resuspended material with bacterial collagenase (Peterkofsky & Diegelman, 1971) as described (Berg, 1982). The cpm per sample in CDP and NCP were calculated, and the proportion of total protein synthesis devoted to collagen production was calculated according to Breul et al. (1980).

Analysis of Protein Secretion in Suspension Cultures. When the secretion of newly synthesized collagen or NCP was to be analyzed, the cultures were radiolabeled and processed as indicated above with the exception that at the end of the incubation period, the cells and medium from each culture were separated by centrifugation for 5 min at 1000*g*. Medium fractions were removed from the culture tubes and the resulting cell pellets were washed with an equal volume of cold Krebs medium, vortexed, and recentrifuged. After the second centrifugation step, the cell pellet was resuspended in a volume of deionized H_2O equal to the volume of culture medium. The cell and medium fractions were then processed identically according to the method outlined above. The production of cellular and medium polypeptides was analyzed by polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) according to Laemmli (1970). The radioactivity present in the slab gels was visualized by radiography according to standard procedures as described by Bonner and Laskey (1974). Samples for polyacrylamide slab gel electrophoresis were SDS-treated as described by Kao et al. (1977). Protein secretion was also analyzed by performing the bacterial collagenase digestion assay on cell and media fractions according to the procedures outlined above.

Determination of Intracellular Degradation of Collagen. Intracellular degradation of newly synthesized collagen was determined according to the procedure of Bienkowski and Engles (1981). After incubation of the suspension with [^{14}C]proline two 0.375-mL aliquots were removed from suspensions containing 7.5 × 10^6 cells/mL and sonicated. One aliquot was immediately frozen and lyophilized while the second was precipitated with 1.125 mL of ice-cold absolute ethanol and allowed to precipitate at 4 °C for 2 h. The samples were then centrifuged in a microcentrifuge (Eppendorf) (9000*g*) for 15 min at 4 °C, and the supernatant solution was saved. The precipitates were washed with 1.0 mL of ice-cold ethanol and centrifuged again as described above. The two ethanol supernatant solutions were combined and evaporated with a vortexing evaporator (Buchler Instruments). Both aliquots were then hydrolyzed for 11 h in 6 N HCl at 116 °C, evaporated, and resuspended in 1.5 mL of deionized H_2O. The redissolved samples were filtered through a 0.45-μm centrifugal filter (Schleicher and Schuell) by centrifuging at 1000*g* for 5 min. The filtrates were collected in microcentrifuge tubes. The filtered solutions were then concentrated to dryness in a Speed-Vac (Savant) and resuspended in 0.150 mL of 0.2 N sodium citrate buffer (Pierce) at pH 2.20 immediately prior to high-pressure liquid chromatography (HPLC) analysis. [^{14}C]Proline was separated from *trans*-4-hydroxy-L-[^{14}C]-proline on an amino acid hydrolysate cation-exchange HPLC column (Varian Instruments). The HPLC apparatus consisted of a Waters WISP 710 automatic sample injector, two Model 6000 pumps, and a Model 600 gradient controller (all from Waters Associates). Hydroxy[^{14}C]proline was eluted isocratically in 12 min by 0.2 N sodium citrate buffer at pH 3.28 at 45 °C. The flow rate was 0.3 mL/min. After the elution of the hydroxy[^{14}C]proline, the column was washed for 15 min in 0.2 N NaOH and reequilibrated. The hydroxy[^{14}C]proline region of the chromatogram was detected and quantitated by collecting six 1-min fractions between minutes 9 and 14 of each run. As described in individual experiments, the degradation of newly synthesized collagen was measured both as the absolute quantity of hydroxy[^{14}C]proline (Hyp) present in the ethanol-soluble fraction and as the percentage of total collagen degraded. The extent to which newly synthesized collagen was degraded was defined as

$$\% \text{ degradation} = ([^{14}\text{C}]\text{Hyp in ethanol-soluble fraction} \times 100) / ([^{14}\text{C}]\text{Hyp in ethanol-soluble} + [^{14}\text{C}]\text{Hyp in precipitated fractions})$$

Pulse–Chase Analysis of Intracellular Degradation Kinetics. Pulse–chase studies of intracellular degradation were performed according to a modification of the procedure described by Kao et al. (1977). Newly synthesized proteins were pulse-labeled in the presence of [^{14}C]proline at a concentration

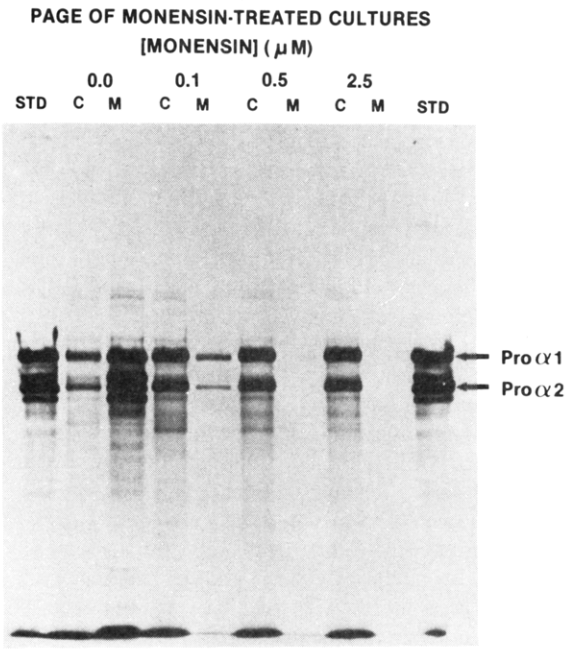

FIGURE 1: Separation of cellular and medium [^{14}C]proline-labeled peptides by polyacrylamide slab gel electrophoresis in the presence of SDS. Two milliliters of cell suspension (7.5×10^6 cells/mL) was labeled with 2 μCi/mL [^{14}C]proline for 6 h at 37 °C. The samples were prepared for electrophoresis as described under Experimental Procedures. The positions of the type I proα 1 and proα 2 polypeptides are indicated in the first and last lane, which was loaded with a ^{14}C amino acid mix labeled procollagen standard. Doses of monensin applied to the suspension cultures are as indicated at the head of the figure; 0 = untreated controls; 0.1, 0.5, and 2.5 refer to the respective micromolar doses of monensin used. At the end of the experiment, each sample was split into cell (C) and media (M) fractions, processed for SDS–PAGE as described by Kao et al. (1977), and samples corresponding to 7.5×10^5 cells were run per lane.

of 10 μCi/mL. At the end of the 15-min pulse period, the incorporation of radiolabel was stopped by the addition of an equal volume of cold chase medium containing 1 mg/mL unlabeled proline. The cultures were rapidly centrifuged (1000g for 5 min) and washed with an equal volume of cold chase medium and recentrifuged. The chase period was initiated by the resuspension of the pelleted cells in chase medium that had been prewarmed to 37 °C. The cultures were then incubated at 37 °C in a shaking water bath for the times indicated in the figures. One culture was chased under control conditions, while a parallel culture was chased in the presence of 0.1 μM monensin. At the indicated time points during the chase, 0.375-mL aliquots were withdrawn in triplicate from each suspension and pipetted into 1.125 mL of ice-cold ethanol. The ethanol precipitates were sonicated with a probe sonicator for 30 s and allowed to precipitate at 4 °C for at least 2 h. The samples were centrifuged according to the procedure described above and ethanol supernatant fractions were pooled. The ethanol-precipitated pellet was used as a source of the total level of insoluble hydroxy[^{14}C]proline synthesized in each culture. The ethanol supernatants were used as the source of hydroxy[^{14}C]proline that was degraded by intracellular degradation (see above). The amount of hydroxy[^{14}C]proline in the supernatant and the pellets was constant at each time period during the chase period as was previously shown for the synthesis and secretion of procollagen in these cells (Kao et al., 1977).

RESULTS

The kinetics for the secretion of procollagen from freshly isolated chick tendon cells has previously been shown to be

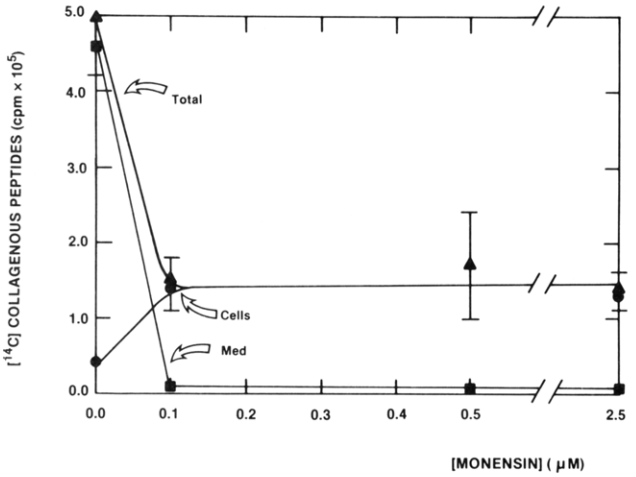

FIGURE 2: Determination of the production and secretion of collagenous polypeptides by a quantitative collagenase digestion. Cell suspensions of 2 mL were labeled for 6 h with 2 μCi/mL [^{14}C]proline and exposed during the labeling period to monensin at concentrations from 0.0 to 2.5 μM. The [^{14}C]proline radioactivity that was digestible with bacterial collagenase was determined for cells (●) and medium (■) from each culture. The total amount of collagenase-digestible radioactivity (▲) was calculated by the addition of the values obtained for the cells and media. Each point represents the average of values obtained for three cultures. Error bars designate ±SD when that value was graphically larger than the symbols used.

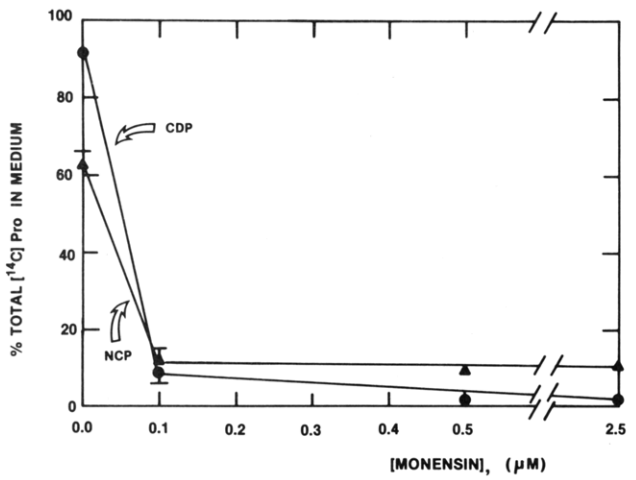

FIGURE 3: The secretion of collagenous (CDP, ●) and noncollagenous (NCP, ▲) peptides from tendon fibroblasts in suspension culture treated with 0.0–2.5 μM monensin. Two milliliters of suspension was labeled with 2 μCi/mL [^{14}C]proline for 6 h. Samples of cells and medium from each culture were assayed for the production of CDP and NCP by using the bacterial collagenase digestion assay and the percentage of CDP or NCP present in the medium was calculated. Each point represents the average of values obtained from three identically treated cultures ±SD.

biphasic, with the bulk of the procollagen being secreted with a half-time of 15 min. Monensin concentrations from 0.1 to 2.5 μM significantly inhibited the secretion of the newly synthesized procollagen, as demonstrated by the retention of the proα 1 and proα 2 polypeptides in the treated cells (Figure 1). These results are in agreement with those results reported by Uchida et al. (1979, 1980), that monensin inhibited the secretion of procollagen in cultured fibroblasts and caused a shift to the slow phase of secretion (Uchida et al., 1980). As shown in Figures 2 and 3, collagen secretion dropped from control levels of 90% to less than 10% in the cultures treated with 0.1 μM monensin and total inhibition of secretion was demonstrated in cultures exposed to 0.5 or 2.5 μM monensin. Also shown in Figure 2 is the retention by the cell fraction

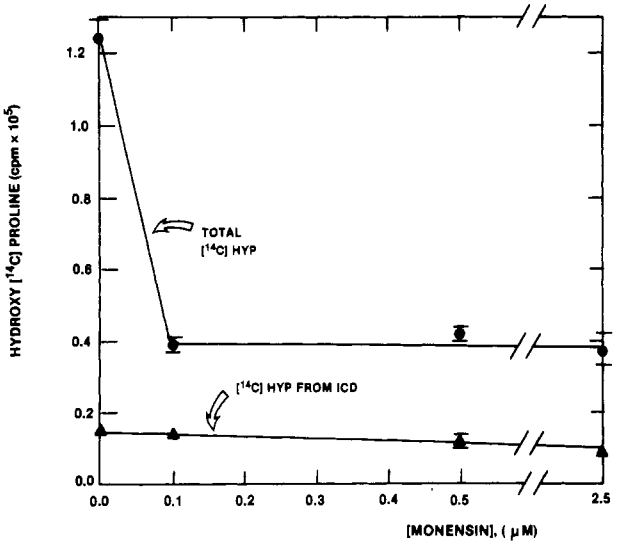

FIGURE 4: The intracellular degradation of newly synthesized collagen was determined for tendon cultures treated with up to 2.5 μM monensin. The suspensions were incubated and labeled as described in the legends to Figures 1 and 2. Hydroxy[^{14}C]proline determinations for total (●) or the ethanol-soluble fraction (▲) from equal aliquots of each undialyzed sample were performed by cation-exchange HPLC as described under Experimental Procedures. Each point represents the mean ±SD for values from three independent cultures.

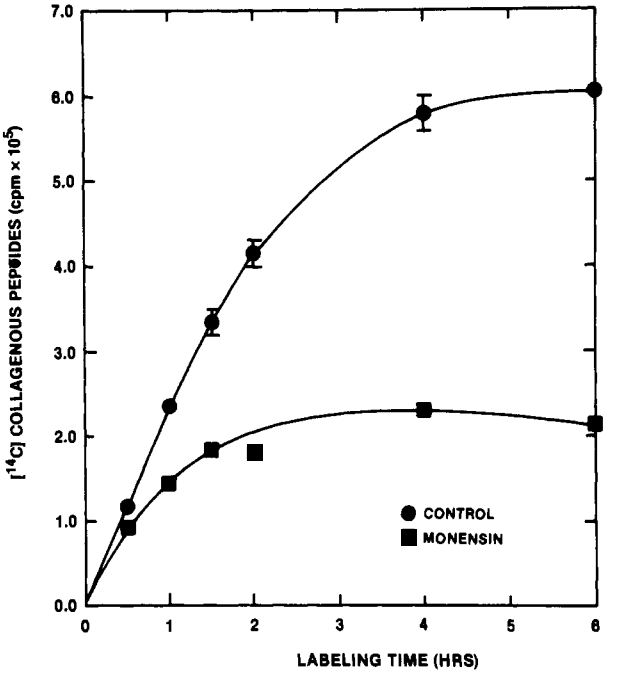

FIGURE 5: Continuous labeling analysis of the production of collagenous polypeptides in suspension cultures of tendon cells incubated under control conditions (●) or in the presence of 0.1 μM monensin (■). Samples of 2 mL of suspension were cultured as described in the legends to Figures 1 and 2 for periods of up to 6 h in order to analyze the kinetics of collagen production under these conditions. Nondialyzable, [^{14}C]proline-labeled CDP was determined by digestion with bacterial collagenase as described under Experimental Procedures.

of ~3–4 times as much collagenase-digestable polypeptides as was present in the control cells. This cellular collagenous pool was shown to be maximal with as little as a 0.1 μM dose of monensin.

The presence of monensin caused 75% inhibition of total procollagen synthesis at doses of 0.1 μM or higher (Figure 2), and the total amount of collagenous polypeptides synthesized by the monensin-treated cultures was found in the cells exclusively (Figures 1 and 2). The appearance of this effect was gradual, requiring at least 2 h for total inhibition to occur (see below, Figure 5).

The inhibition of the secretion of newly synthesized procollagen was not limited to procollagen since the secretion of noncollagen proteins was also inhibited (Figure 3). Treatment with 0.1 μM monensin caused the secretion of noncollagen proteins to decrease to approximately 10–15%. The secretion of noncollagen proteins was not further decreased after treatment with 0.5 or 2.5 μM monensin (Figure 3). The incorporation of [^{14}C]proline into noncollagen proteins was also inhibited about 70% by monensin at levels of 0.1 μM or greater (not shown).

The intracellular degradation of newly synthesized collagen was examined in the monensin-treated cultures. As shown in Figure 4, the total production of hydroxy[^{14}C]proline produced in 0.1 μM or greater monensin-treated cultures dropped to a level equal to approximately 30–40% of that seen in the control cultures. In contrast to the sharp decrease in the total amount of the hydroxy[^{14}C]proline produced in monensin-treated cultures, the amount of ethanol-soluble hydroxy[^{14}C]proline that was demonstrated in the monensin-treated cultures did not drop significantly. The data indicate that the actual amount of hydroxy[^{14}C]proline present in collagen degradation products decreased only slightly from control levels. However, since a decrease in total hydroxy[^{14}C]proline synthesized was shown in the cultures treated with 0.1 μM or greater monensin (Figure 4), the percentage of total hydroxy[^{14}C]proline solubilized by the intracellular degradation of newly synthesized collagen was increased by the presence of monensin. The control level of approximately 12% degradation was increased to 35% in the presence of 0.1 μM monensin (Figure 4).

It was of interest to further examine the relationship between the effects of 0.1 μM monensin on the synthesis and intracellular degradation of collagen. As shown in Figure 5, , the continuous labeling of total, nondialyzable collagenous polypeptides in both the cells and media remained linear in the control cultures for the first 4 h of suspension culture in these experiments. In the cultures treated with 0.1 μM monensin, the inhibition of total collagen production was apparent at 30 min, and after 60 min of labeling, the level of collagen production in the monensin-treated cultures was equal only to ~60% of the control levels. Thus the effect of monensin on collagen synthesis was clearly demonstrated within 60 min after the start of the incubation period, and this effect continued until total inhibition of collagen production occurred between 2 and 4 h of incubation.

To test the effect of monensin on intracellular degradation independent of its effect on collagen synthesis, collagen was pulse-labeled in the absence of monensin for 15 min with [^{14}C]proline. The labeled collagen was then tested for intracellular degradation during chase periods of up to 6 h in the presence or absence of 0.1 μM monensin. The intracellular degradation of newly synthesized collagen chased in the presence of monensin was increased to levels of about 25% of the total collagen synthesized (Figure 6). The greatest increase in the levels of degradation relative to the control cultures was shown to occur during the periods between 2 and 4 h of chase. These data demonstrate that the presence of 0.1 μM monensin caused increased intracellular degradation of newly synthesized collagen (Figure 6) independent of effects on collagen synthesis (Figure 5).

The initial rate of degradation in the presence of 0.1 μM monensin closely approximated the rate demonstrated in the controls and was maintained for at least 2 h longer than in the control cultures (Figure 6). At different time points in

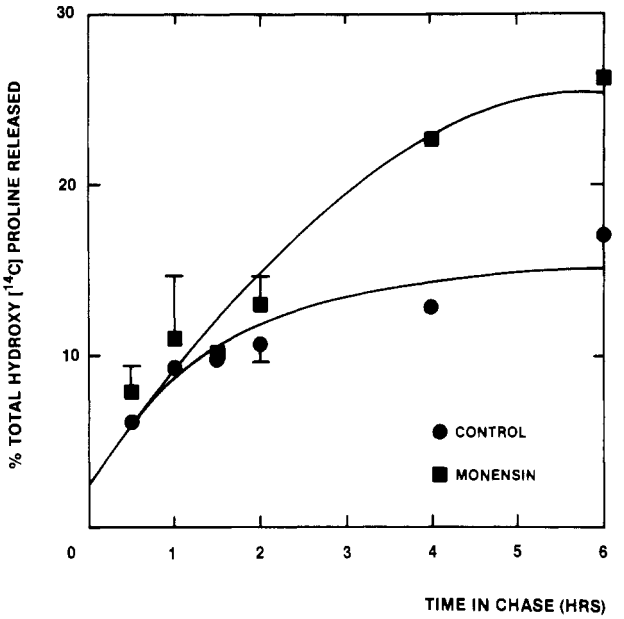

FIGURE 6: Intracellular degradation of newly synthesized collagen during a chase in the presence of 0.1 μM monensin. Collagen synthesized in 20 mL of suspension culture was pulse-labeled in control cultures with 10 μCi/mL of [^{14}C]proline for 15 min. The culture was chased in the presence of unlabeled proline and in the presence or absence of monensin. At the times indicated 2-mL aliquots were withdrawn and intracellular degradation of pulse-labeled collagen was determined as the percentage of total hydroxy[^{14}C]proline synthesized that was ethanol-soluble.

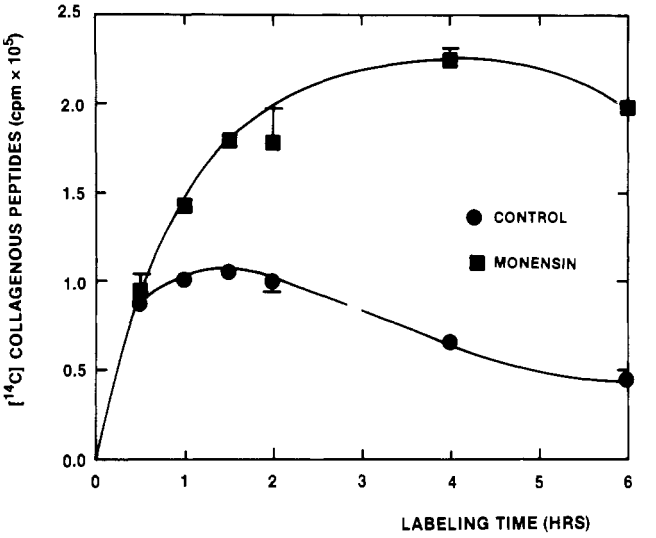

FIGURE 7: Continuous labeling of cellular collagenous polypeptides in monensin-treated cultures. Two-milliliter suspension cultures containing 7.5 × 10^6 cells/mL were continuously labeled in the presence of 2 μCi/mL [^{14}C]proline. The cellular content of newly synthesized collagen was determined as the collagenase-digestable radioactivity associated with cells that had been separated from the growth medium by centrifugation.

the curves for control cultures and monensin-treated cultures, the intracellular degradation was shown to achieve plateau levels and proceed at reduced rates of degradation or stop altogether. The time point in the control culture occurred at approximately 90 min of chase, while in the monensin-chased cultures the rate of intracellular degradation did not level off until at least 4 h of chase.

Since it was shown that the inhibition of collagen secretion was accompanied by the retention of 3–4 times as much collagenous polypeptides in monensin-treated cells (Figure 2), the filling of cellular pools was analyzed during constant labeling in the presence or absence of 0.1 μM monensin. It was shown in these experiments (Figure 7) that the rate at which cellular pools of collagenous polypeptides filled in the presence of monensin was not significantly different from the rate displayed by the control cultures. In agreement with the data presented in Figure 4, the monensin-treated cells accumulated about 3–4 times as much collagenous polypeptides as did the control cultures. These data indicate that the filling of the cellular pool of collagenous polypeptides has occurred with equal rates in both the control and monensin-treated cultures and that the increased level of collagenous polypeptides in the monensin-treated cells is consistent with the impaired exit of newly synthesized collagen from one or more of those pools. The buildup of intracellular collagen in the monensin-treated cells was eventually halted by a combination of increased intracellular degradation and by a currently unknown process that caused the inhibition of collagen synthesis (Figure 2).

Discussion

By use of the carboxylic ionophore monensin to block the secretion of collagen from the cells, it was demonstrated that the intracellular degradation of newly synthesized collagen was allowed to continue in the presence of complete inhibition of procollagen secretion (Figures 1, 2, and 4). The ability of monensin to inhibit the secretion of newly synthesized collagen (Uchida et al., 1979, 1980; Nishimoto et al., 1982a,b) and other secretory proteins (Tartakoff & Vassali, 1977, 1978; Nagase et al., 1983; Ledger & Tanzer, 1984; Tartakoff, 1983) has now been well established. Ultrastructural (Tartakoff & Vassali, 1977; Nagase et al., 1983; Ledger et al., 1980) and subcellular fractionation (Uchida et al., 1980) evidence has indicated that the monensin-induced secretory block occurs in the Golgi apparatus between the cis and trans elements, causing the accumulation of the secretory protein in the cis face of the Golgi or the rough endoplasmic reticulum. The inhibition of collagen secretion in the chick tendon cells used in these studies did not decrease the level of basal intracellular degradation, indicating that basal intracellular degradation occurs at or before the level of the secretory block.

Unexpectedly, the synthesis of collagen was decreased and eventually shut off in the tendon cells incubated with monensin. This observed inhibition of collagen synthesis is in contrast to the effects of monensin described for other fibroblasts (Uchida et al., 1979). Since noncollagen proteins are also not secreted, it remains to be determined if the inhibition of protein synthesis is confined to collagen and other secretory proteins or also includes cytoplasmic proteins.

Two pools of kinetically distinguishable collagenous polypeptides have been demonstrated in the isolated tendon cells used in this study (Kao et al., 1977, 1979) and in cultured human fibroblasts (Uchida et al., 1980). These have been identified by their respective rates of secretion from pulse-labeled cells and have been shown to include one pool that is very rapidly secreted and constitutes the majority of the collagen that is labeled during short to medium-length pulses; a second pool requires longer periods of time to be partially secreted and may be present in the cells for several hours. Interestingly, the monensin-induced secretory block is mediated in cultured human fibroblasts by causing the shift from mixed-rate secretion to the slower rate (Uchida et al., 1980).

The varied biological effects of monensin such as the inhibition of protein secretion (Uchida et al., 1979, 1980; Nishimoto et al., 1982a,b; Tartakoff & Vassalli, 1977, 1978; Nagase et al., 1983; Ledger & Tanzer, 1984; Tartakoff, 1983), the inhibition of proteoglycan core protein glycosylation and sulfation (Nishimoto et al., 1982a,b; Yanagishita & Hascall,

1985), and the inhibition of high-mannose glycoprotein trimming and conversion to complex forms (Ledger et al., 1983) suggest that the monensin secretory block occurs at the level of or prior to the cis face of the Golgi. The lack of effect of monensin on intracellular degradation suggests that the endoplasmic reticulum or Golgi apparatus is the location of either the degradation mechanism per se or the sorting process in the collagen secretory pathway where collagen polypeptides are diverted from the secretory pathway and degraded (Bienkowski, 1985).

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