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Osmotically Induced Microinjection of Ricin Bypasses a Ricin Internalization Defect in a Chinese Hamster Ovary Mutant Cell Line

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By osmotic lysis of pinocytic vesicles we were able to inject ricin or ricin A chain directly into the cytosol of Chinese hamster ovary cells. The lag time of 1 to 2 h before the onset of the inhibition of protein synthesis by ricin in intact cells was reduced to 15 to 30 min by this method. Preincubation of cells with a low concentration of nigericin, which was shown earlier to enhance the cytotoxicity of ricin, had no effect under this condition. Direct transfer of either intact ricin or the ricin A subunit by osmotic lysis of pinocytic vesicles into the cytosol of the ricin-resistant CHO mutant cell line 4-10 rendered the mutant 4-10 cells as sensitive to ricin as the CHO *pro* wild-type cells. Both the lag time and the rate of inhibition of protein synthesis in the wild-type and mutant cell lines after the introduction of ricin by osmotic lysis of pinocytic vesicles were the same. These results indicate that injection of ricin into the cytosol by osmotic lysis of pinosomes bypasses the internalization defect in the mutant cell line.

Ricin inhibits protein synthesis in eucaryotic cells. The mechanism of action of ricin involves three steps: (i) binding of the toxin to the cell surfaces through D-galactose- or *N*-acetylgalactosamine-terminal membrane oligosaccharide residues, (ii) delivery of the toxin moiety into the cell cytoplasm, and (iii) inactivation of the 60S subunit of the ribosome, resulting in the inhibition of protein synthesis (11). Very little is known about how ricin is internalized into the cytosol. Earlier we showed that nigericin pretreatment at low ionophore concentration enhances the internalization of ricin into Chinese hamster ovary cells (14, 15). To understand the molecular events involved in ricin internalization, several somatic cell mutants have been isolated that are resistant to the toxic action of ricin (3, 5, 6, 17, 18). Some of these mutant cells are toxin resistant due to reduced binding of ricin, whereas other mutants are resistant due to defects in steps subsequent to ricin binding. Earlier we reported the isolation of the CHO mutant strain 4-10, which is resistant to both ricin and *Pseudomonas* toxin (16). Interestingly, this mutant can bind similar amounts of both toxins as compared with the wild-type CHO *pro* cells, suggesting that the resistance phenotype is due to a failure to deliver surface-bound toxin to the cytosol, where it exerts its biological effect (16). Direct evidence for this possibility has come from biochemical and electron microscopic autoradiographic studies of [^{125}I]ricin internalization (16).

Recently, Okada et al. have shown that macromolecules can be injected into the cytosol of mammalian cells by osmotic lysis of pinocytic vesicles (10). In this paper, we report that this method can be used to introduce ricin and ricin A chain directly into the cytoplasm of CHO cells. In addition, we compared the kinetics and efficiency of the introduction of ricin into the cytosol by osmotic lysis of pinocytic vesicles with those of the normal, receptor-mediated process in both the wild-type cell line and the ricin-resistant mutant 4-10 cell line. Our results suggest that the introduction of ricin into the cytosol of CHO cells by osmotic lysis of pinosomes bypasses the normal receptor-mediated pathway of ricin uptake.

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MATERIALS AND METHODS

Materials. Sucrose was purchased from Bio-Rad Laboratories. Polyethylene glycol (PEG)-1000 was obtained from J. T. Baker Chemical Co. Galactose was obtained from Sigma Chemical Company. Ricin was a product of Miles Laboratories, Inc. Ricin A and ricin B chains were obtained from E-Y Laboratories, Inc. [^{3}H]Leucine (110 Ci/mmol) was obtained from ICN Chemical and Radioisotope Division. Fetal bovine serum was obtained from Dutchland, Inc. Powdered α-minimal essential medium (MEM) and α-MEM suspension culture media were obtained from Flow Laboratories, Inc.

Cell culture. A CHO cell line auxotrophic for proline (CHO *pro*) was obtained from L. Siminovitch, Toronto, Canada. The mutant 4-10 was isolated from CHO *pro* by selection for simultaneous resistance to ricin and *Pseudomonas* toxin (16). Cells were grown either on plates containing α-MEM supplemented with 10% fetal bovine serum and 10 μg each of adenosine, guanosine, cytidine, and thymidine per ml or in suspension culture in α-MEM supplemented with 10% fetal bovine serum, 40 μg of proline per ml, and 10 μg of each of the above nucleosides per ml. Penicillin (100 μg/ml) and streptomycin (100 μg/ml) were routinely used in cell culture media. The cells were incubated in a humidified 5% CO_2 atmosphere at 37°C.

Preparation of osmotic lysis medium. PEG-1000 was dissolved in α-MEM containing 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 0.1 M galactose, and 0.5 M sucrose to a final concentration of 10%. The pH was adjusted to 6.8, and the mixture was filter sterilized.

Osmotic lysis of pinocytic vesicles. This procedure has been previously described (10). Cells (5×10^5) were seeded in 35-mm plastic dishes. Twenty-four hours later cells were washed twice with phosphate-buffered saline (Ca^{++} and Mg^{++} free) and incubated in osmotic lysis medium or in HEPES-buffered α-MEM containing ricin or ricin A chain for 10 min at 37°C. Ten minutes later cells were exposed to hypotonic HEPES-buffered α-MEM (six parts α-MEM to four parts water) and incubated in the same medium for 2 min at room temperature. The cells were then rinsed with normal culture medium three times.

Cytotoxicity assay. The cytotoxicity of ricin was measured by the determination of plating efficiency and by in vivo protein synthesis of cells after treatment with various concentrations of ricin in osmotic lysis medium or in HEPES-buffered α-MEM. For the experiment concerning the enhancement of cytotoxicity by pretreatment with nigericin, cells were pretreated with 10^{-8} M nigericin for 72 h before the cytotoxicity assay.

(i) Plating efficiency. Four hours after a 10-min exposure of cells to ricin either in osmotic lysis medium or in HEPES-buffered α-MEM, cells were removed from the plate by trypsinization and replated on 100-mm dishes. After incubation at 37°C, distinct and visible colonies were counted after staining with 0.2% methylene blue in 50% methanol.

(ii) In vivo protein synthesis. After treatment with various concentrations of ricin, ricin A, or ricin B chains, either in osmotic lysis medium or in HEPES-buffered α-MEM, cells were incubated in α-MEM containing 1% fetal calf serum for 20 h at 37°C. After the incubation the cells were washed and incubated in serum-free, leucine-free medium with [^{3}H]leucine (0.5 μCi/ml) for 2 h at 37°C. The monolayers were fixed with two washes of 10% (wt/vol) perchloric acid–2% (wt/vol) phosphotungstic acid. The fixed monolayer was washed with phosphate-buffered saline and dissolved in 0.5 N NaOH. An 0.1-ml sample of the dissolved cells was added to the scintillation vial, neutralized with 0.1 N HCl, mixed with 4 ml of Ready-Solv EP scintillation solution (Beckman Instruments, Inc.), and counted in a Beckman LS7500 liquid scintillation counter.

RESULTS

Cytotoxicity of ricin in CHO *pro* cells. The cytotoxicity of ricin was assessed by the determination of plating efficiencies of cells treated with various concentrations of ricin in osmotic lysis medium or in HEPES-buffered medium, with or without 0.1 M galactose. When the CHO *pro* cells were incubated in the presence of various concentrations of ricin in HEPES-buffered medium without galactose (receptor-mediated pathway), the plating efficiency of CHO *pro* cells was reduced to half that of the control (50% lethal dose [LD_{50}]) at a ricin concentration of 275 ng/ml; under the same conditions, nigericin pretreatment enhanced the cytotoxicity of ricin slightly more than fivefold by reducing the LD_{50} to 50 ng/ml (Fig. 1A). When 0.1 M galactose was added to block the receptor-mediated uptake, the LD_{50} of ricin was increased 24-fold to 7 μg/ml (Fig. 1A). When CHO *pro* cells were incubated in the presence of different concentrations of ricin in osmotic lysis medium before a 2-min hypotonic shock, the LD_{50} was reduced to 100 ng/ml (Fig. 1A). Under this condition, the enhancement of cytotoxicity by pretreatment with nigericin was not observed (Fig. 1A). These results indicate that nigericin pretreatment enhances the rate-limiting step in the normal receptor-mediated endocytosis of ricin but does not change the rate of uptake by the nonspecific fluid phase endocytic pathway. The entry of ricin via osmotically induced lysis of pinocytic vesicles was more efficient (2.75-fold) than that by the normal receptor-mediated pathway. These results suggest that in osmotic lysis medium, the normal transport mechanism of ricin by receptor-mediated endocytosis is bypassed.

Further evidence that osmotic shock treatment bypassed the normal receptor-mediated transport mechanism was obtained by comparing the efficiencies of inhibition of cellular protein synthesis by ricin that was introduced by these different pathways. Figure 2 shows that the 50% inhibitory dose for ricin via the receptor-mediated pathway was 325 ng/ml, and nigericin pretreatment enhanced this pathway, as judged by the reduction in the 50% inhibitory dose to 80 ng/ml (Fig. 2A). Ricin that was internalized by osmotic lysis showed a more efficient inhibition of protein synthesis as compared with the receptor-mediated pathway in the presence of 0.1 M galactose (Fig. 2B). Under this condition nigericin pretreatment had no effect in enhancing the inhibition of protein synthesis by ricin. The efficiency of this method depended on the presence of both sucrose and PEG-

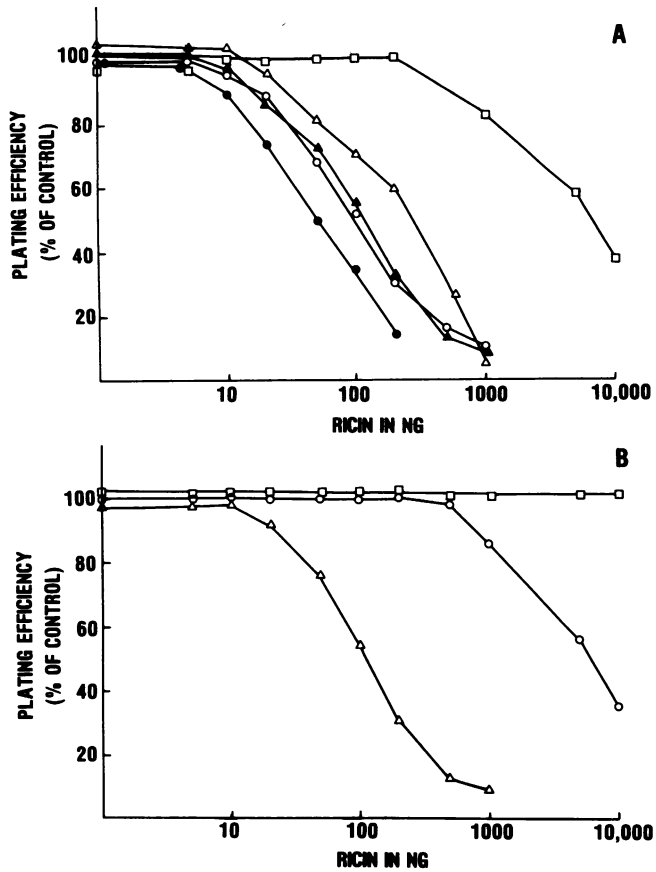

FIG. 1. Cytotoxicity of ricin in CHO *pro* and 4-10 cells based on a plating efficiency assay. Cells (5×10^5) were seeded into 35-mm dishes 24 h before the experiment. The CHO *pro* and 4-10 cells were exposed to HEPES-buffered medium with or without galactose or osmotic lysis medium containing different concentrations of ricin for 10 min. Cells that were exposed to osmotic lysis medium were then incubated in hypotonic medium for 2 min and washed with normal medium three times. After 4 h, cells were collected from plate by trypsinization and plated into 100-mm dishes; after incubation at 37°C, distinct and visible colonies were counted after staining with 0.2% methylene blue in 50% methanol. (A) CHO *pro* cells. Symbols: △, control cells plus ricin in HEPES-buffered medium; ●, cells that were pretreated with nigericin (10^{-8} M) for 72 h and washed, with cytotoxicity of ricin then measured in the absence of nigericin in HEPES-buffered medium; ○, control cells plus ricin in osmotic lysis medium; ▲, cells that were pretreated with nigericin (10^{-8} M) for 72 h and washed, with cytotoxicity of ricin then measured in the absence of nigericin in osmotic lysis medium; □, control cells plus ricin in HEPES-buffered medium + 0.1 M galactose. (B) 4-10 cells. Symbols: △, cells plus ricin in osmotic lysis medium; ○, cells plus ricin in HEPES-buffered medium; □, cells plus ricin in HEPES-buffered medium plus 0.1 M galactose. Values for 100% of control were 175 colonies for CHO *pro* cells and 140 colonies for 4-10 cells in normal medium and 168 colonies for CHO *pro* cells and 131 colonies for 4-10 cells in osmotic lysis medium.

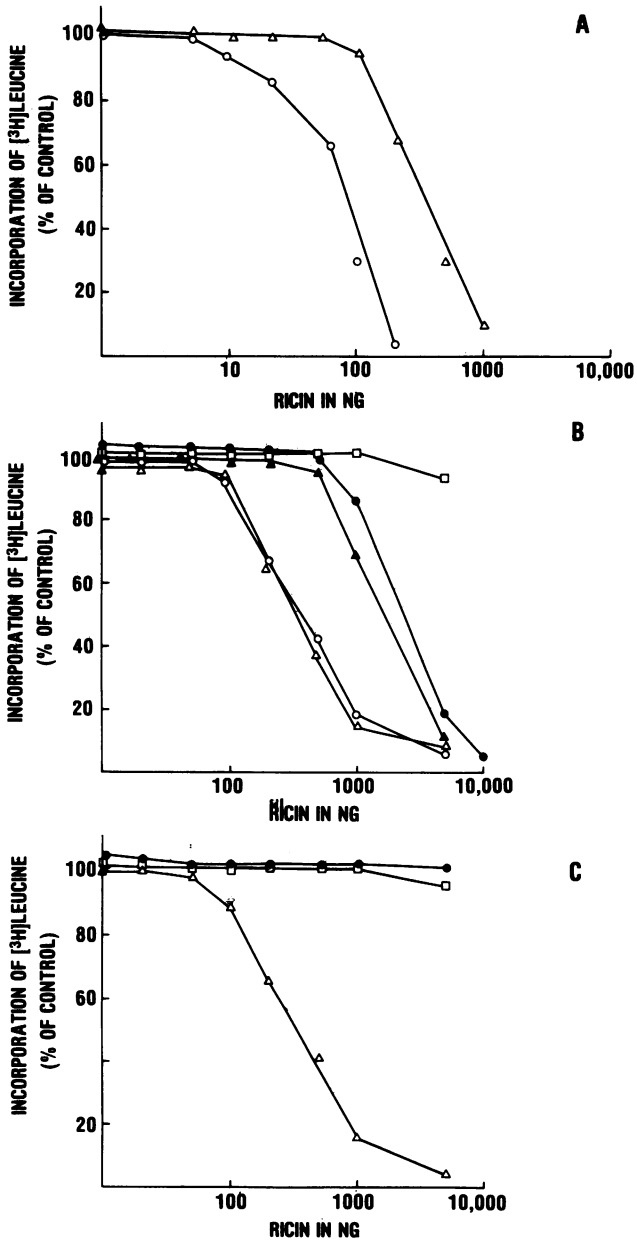

FIG. 2. Effect of ricin on cellular protein synthesis in CHO *pro* and 4-10 cells. The cells were exposed to different concentrations of ricin for 10 min as described in the legend to Fig. 1. After 20 h, the cells were washed and incubated in serum-free, leucine-free α-MEM and labeled with [^{3}H]leucine (0.5 μCi/ml) for 2 h at 37°C. After 2 h, the cell monolayers were fixed with two washes of 10% perchloric acid–2% phosphotungstic acid and washed twice with phosphate-buffered saline. The monolayers were dissolved in 0.5 N NaOH, and samples of 0.1 ml were taken for ^{3}H-radioactivity determinations. (A) In HEPES-buffered medium. Symbols: △, control CHO *pro* cells plus ricin; ○, CHO *pro* cells that were pretreated with nigericin (10^{-8} M) for 72 h and washed plus ricin in the absence of nigericin. (B) In osmotic lysis medium containing both PEG-1000 and sucrose. Symbols: ○, control CHO *pro* cells plus ricin; △, CHO *pro* cells that were pretreated with nigericin (10^{-8} M) for 72 h and washed plus ricin in the absence of nigericin; ●, control CHO *pro* cells plus ricin in osmotic lysis medium lacking PEG-1000; ▲, control CHO *pro* cells plus ricin in osmotic lysis medium lacking sucrose; □, control CHO *pro* cells plus ricin in osmotic lysis medium lacking PEG-1000 and sucrose. (C) 4-10 cells. Symbols: □, 4-10 cells plus ricin in HEPES-buffered medium; ●, 4-10 cells plus ricin in HEPES-buffered medium plus 0.1 M galactose; △, 4-10 cells plus ricin in osmotic lysis medium. Values for 100% of control were 45,243 dpm for CHO *pro* cells and 42,257 dpm for 4-10 cells.

1000. When either of these compounds was absent, the cytotoxicity of ricin was significantly reduced (Fig. 2B).

Kinetics of inhibition of protein synthesis. When the kinetics of inhibition of cellular protein synthesis by ricin were studied in wild-type CHO *pro* cells, 10 μg of ricin per ml was found to inhibit 90% of the cellular protein synthesis after an initial lag of ca. 1 to 2 h (Fig. 3A). However, incubation of CHO *pro* cells with ricin in osmotic lysis medium reduced the initial lag period to 15 to 30 min (Fig. 3A). This rapid inhibition of protein synthesis by toxin that was introduced by osmotic lysis suggests that toxin introduced by osmotic lysis reaches the cytosol (the site of ricin action) more rapidly than the toxin internalized through a normal sequence of events involved in the receptor-mediated endocytosis.

Effect of introduction of ricin into 4-10 cells by osmotic lysis of pinocytic vesicles. We previously reported the isolation and characterization of a CHO *pro* mutant 4-10 cell line defective in ricin internalization. Figure 1B shows the effect of ricin internalized by either the receptor-mediated pathway or osmotic lysis of pinocytic vesicles. A comparison of Fig. 1A and B shows that the mutant 4-10 cell line was 24 times more resistant to receptor-mediated ricin cytotoxicity (LD_{50}, 6 μg/ml) than the wild-type strain (275 ng/ml). Figure 1B also shows that 0.1 M galactose greatly reduced ricin cytotoxicity under these conditions. On the other hand, the LD_{50} of ricin that was internalized via the osmotic lysis mechanism into the mutant cells was indistinguishable from that for the wild-type cells (Fig. 1B). These results indicate that the introduction of ricin into the mutant cells by osmotic lysis bypasses the internalization defect in the CHO *pro* 4-10 mutant.

Evidence that ricin internalized by osmotic lysis bypasses the internalization defect in 4-10 cells was also obtained by comparing the efficiencies of inhibition of protein synthesis by ricin when the toxin was introduced by either pathway. When the 4-10 cells were exposed to different concentrations of ricin in HEPES-buffered medium with or without galactose, there was no inhibition of protein synthesis up to a ricin concentration of 5 μg/ml (Fig. 2C). When the ricin was introduced into 4-10 cells by osmotic lysis, however, the ricin concentration required to reduce the protein synthesis of 4-10 cells to half that of the control (50% inhibitory dose) was found to be the same as that of the CHO *pro* cells (Fig. 2B and C). These results show that, as in the case of ricin cytotoxicities determined by plating efficiencies, the mutant and wild-type cells were equally sensitive to ricin introduced by osmotic lysis. These results also indicate that the resistance of 4-10 cells does not reside in the protein synthesis machinery.

Kinetics of inhibition of protein synthesis in 4-10 cells. Figure 3B shows the kinetics of inhibition of protein synthesis in 4-10 cells by ricin at 10 μg/ml. No inhibition of cellular protein synthesis occurred in 4-10 cells when ricin was introduced via the receptor-mediated pathway. Introduction of ricin by osmotic lysis, however, resulted in a rapid inhibition of protein synthesis in the 4-10 cell line. More importantly, the lag period of 15 to 30 min before the onset of inhibition of protein synthesis in 4-10 cells was indistinguishable from that in CHO *pro* wild-type cells under the same conditions (Fig. 3A).

Increasing ricin concentrations reduced the initial lag

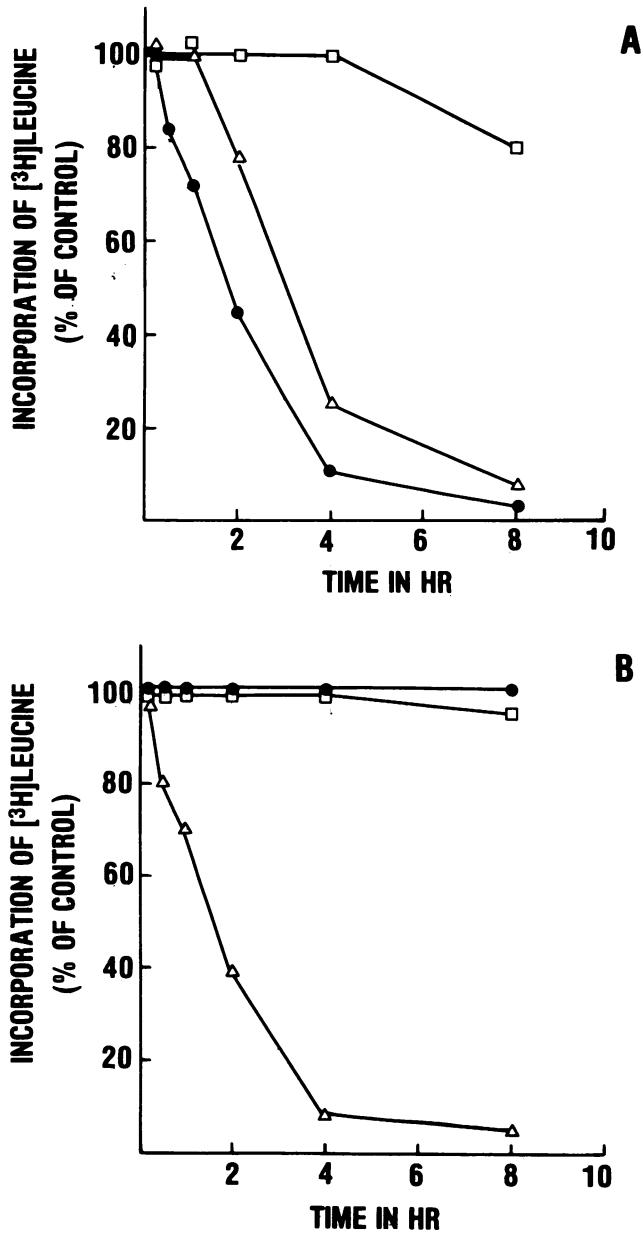

FIG. 3. Kinetics of inhibition of protein synthesis in CHO *pro* and 4-10 cells by ricin (10 μg/ml). The CHO *pro* and 4-10 cells in 35-mm dishes were exposed to 10 μg of ricin per ml in HEPES-buffered medium or osmotic lysis medium for 10 min as described in the legend to Fig. 1. After 10 min, cells were labeled with [^{3}H]leucine, and at various times the cell monolayers were fixed with two washes of 10% perchloric acid–2% phosphotungstic acid, and ^{3}H-radioactivity was determined as described in the legend to Fig. 2. (A) Symbols: △, CHO *pro* cells plus ricin in HEPES-buffered medium; ●, CHO *pro* cells plus ricin in osmotic lysis medium; □, CHO *pro* cells plus ricin in HEPES-buffered medium + 0.1 M galactose. (B) Symbols: □, 4-10 cells plus ricin in HEPES-buffered medium; ●, 4-10 cells plus ricin in HEPES-buffered medium plus 0.1 M galactose; △, 4-10 cells plus ricin in osmotic lysis medium. Values for 100% of control for CHO *pro* cells were 3,844 dpm (15 min), 8,608 dpm (30 min), 15,100 dpm (60 min), 28,470 dpm (2 h), and 31,084 dpm (4 h) for 4-10 cells; the 100% control values were 4,345 dpm (15 min), 8,465 dpm (30 min), 15,268 dpm (60 min), 31,035 dpm (2 h), and 31,084 dpm (4 h).

before the onset of inhibition of cellular protein synthesis in mutant 4-10 cells. This same effect was not observed in wild-type CHO *pro* cells (Fig. 4A and B). The longer lag in the inhibition of cellular protein synthesis by ricin in 4-10 cells probably reflects a slower delivery of ricin into the cytosol of the mutant cell line as compared with that occurring in the wild-type cells.

Introduction of ricin A and B chains into CHO cells by osmotically induced microinjection. Ricin is composed of two subunits, the A chain (or effectomer) and the B chain (or haptomer) (11). Although only the intact ricin is a potent inhibitor of protein synthesis in intact cells, the isolated A chain is an enzyme which inhibits protein synthesis in vitro (11). The function of the B chain is to mediate specific binding of ricin to cell surface receptors. As expected, when CHO *pro* and resistant 4-10 cells were exposed to either the A chain or the B chain in HEPES-buffered medium (the receptor-mediated pathway), no toxicity was observed even at 5 μg/ml (Fig. 5). However, when either cell line was subjected to microinjection of the ricin A chain by osmotic lysis, significant cytotoxicity was observed (Fig. 5). Microinjection of the B chain alone under the same conditions had no effect (Fig. 5). These results indicate that the ricin A chain introduced into the cytosol by osmotic lysis of pinocytic vesicles bypasses the need for receptor-mediated uptake, which normally involves binding of the intact toxin to the cell via the B chain.

To ascertain whether introduction of free B chain into the cytosol could have a synergistic effect on the cytotoxicity of the A chain, CHO *pro* cells and resistant 4-10 cells were subjected to microinjection of a mixture of free A and B chains by osmotic lysis. The addition of the ricin B chain under these conditions did not affect the efficiency of inhibition of cellular protein synthesis that was produced by the ricin A chain alone (Fig. 5). These results indicate that there is no synergistic effect when both chains are directly introduced into the cytosol.

DISCUSSION

Microinjection of ricin into cultured mammalian cells by osmotic lysis of pinocytic vesicles offers a new and simple method for inducing toxin uptake in a manner which bypasses the normal, receptor-mediated pathway. A comparison of these methods of cellular intoxication by ricin provides valuable information about the normal uptake pathway of ricin. The microinjection technique used here was developed by Okada and Rechsteiner (10) as a method of introducing macromolecules directly into cytosol of mammalian cells under conditions in which cell viability is retained. The cellular viability after the osmotic lysis treatment, as measured by plating efficiency and by [^{3}H]leucine incorporation, was found to be 85 to 90% of that of the control cells for both CHO *pro* and 4-10 cells. Based on the original description by Okada and Rechsteiner of this technique, the microinjection of ricin into CHO cells may be accomplished by the uptake and hypotonic rupture of ricin-containing pinocytic vesicles, releasing the toxin directly into the cytosol. The receptor-mediated uptake of ricin is blocked by the presence of galactose in the incubation medium, and ricin uptake thus occurs by the fluid phase pinocytosis. The pinocytic vesicles, formed in the presence of 0.5 M sucrose and 10% PEG-1000, have a high internal osmotic pressure and break when the cells are placed in a hypotonic medium. The efficiency of cellular intoxication by this method requires the presence of both sucrose and PEG-1000; an omission of either sucrose or PEG-1000 greatly reduced the cytotoxicity of ricin. The

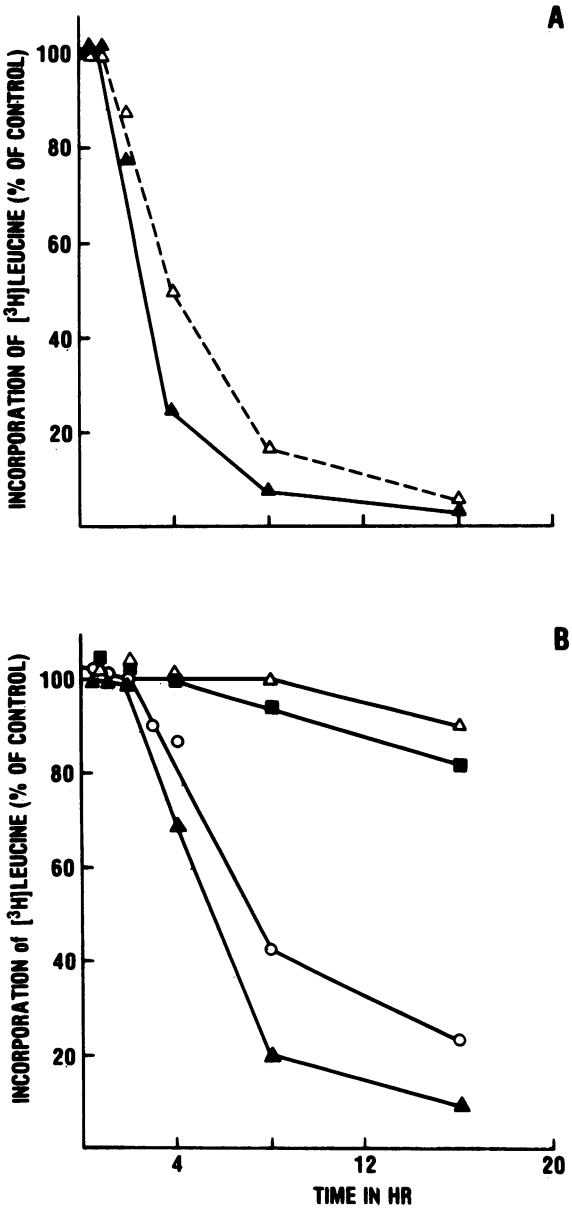

FIG. 4. Effect of varying ricin concentration on the kinetics of inhibition of cellular protein synthesis in HEPES-buffered medium. The CHO *pro* and 4-10 cells in 35-mm dishes were exposed to different ricin concentrations in HEPES-buffered medium without galactose for 10 min. After 10 min, cells were labeled with [^{3}H]leucine, and at various times the cells monolayers were fixed with two washes of 10% percholoric acid–2% phosphotungstic acid, and ^{3}H-radioactivity was determined as described in the legend to Fig. 2. (A) CHO *pro* cells. Symbols: △, 2 μg; ▲, 10 μg. (B) 4-10 cells. Symbols: △, 2 μg; ■, 10 μg; ○, 50 μg; ▲, 100 μg.

function of PEG-1000 is not clear, but it has been reported that treatment of cells with PEG-1000 for a short period of time enhances the fluidity of the membrane, which may affect the susceptibility of pinosomes to ruptures by hypotonic treatment (9). The introduction of ricin into the cytosol of ricin-resistant cells has also been accomplished by fusing cells with ricin-containing liposomes (1, 2, 8). One of the disadvantages of using liposomes as carriers is the fact that there is no defined lipid composition which favors fusion rather than endocytosis. Consequently, results obtained with liposome transfer experiments are ambiguous, due to the possibility of endocytosis of the small liposomes (12).

Direct access of ricin, introduced by the osmotic lysis of pinocytic vesicles, to the protein synthesis machinery in the cytosol is suggested by the following findings: rapid onset of inhibition of cellular protein synthesis by ricin and the inhibition of protein synthesis in intact cells by the ricin A chain, which is capable of inhibiting protein synthesis only in

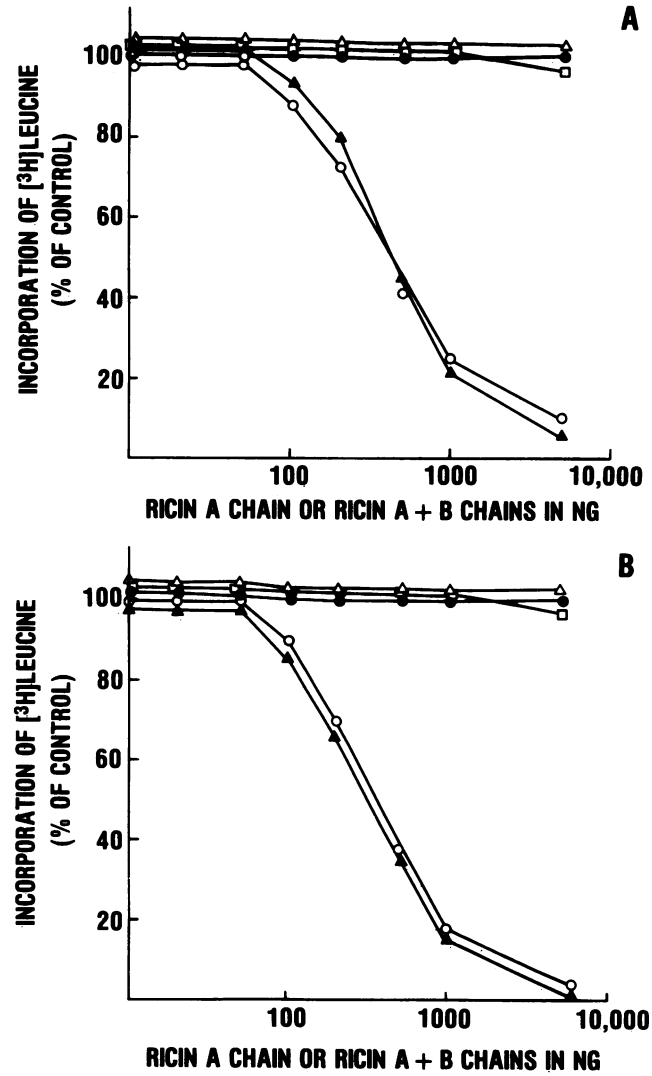

FIG. 5. Effect of ricin A chain or A and B chains jointly on the inhibition of cellular protein synthesis. The CHO *pro* and 4-10 cells were grown in 35-mm dishes and were exposed to different concentrations of ricin A chain or ricin A and B chains jointly in HEPES-buffered medium or in osmotic lysis medium as described in the legend to Figure 1. After 20 h, the inhibition of protein synthesis was measured as described in the legend to Fig. 2. (A) CHO *pro* cells. Symbols: △, ricin A chain in HEPES-buffered medium plus 0.1 M galactose; ○, ricin A chain in osmotic lysis medium; ▲, ricin A and B chains in osmotic lysis medium; ●, ricin A and B chains in HEPES-buffered medium plus 0.1 M galactose; □, ricin B chain in osmotic lysis medium. (B) 4-10 cells. Symbols: △, ricin A and B chains in HEPES-buffered medium plus 0.1 M galactose; ○, ricin A chain in osmotic lysis medium; ▲, ricin A and B chains in osmotic lysis medium; ●, ricin A chain in HEPES-buffered medium plus 0.1 M galactose; □, ricin B chain in osmotic lysis medium. Values for 100% control were 72,837 dpm and 42,257 dpm for CHO *pro* and 4-10 cells, respectively.

cell-free systems (11). The evidence provided by these studies indicates that the normal uptake mechanism of ricin in mammalian cells can be bypassed by the osmotic lysis method.

We previously reported the isolation of the CHO *pro* mutant cell line 4-10, which is resistant to ricin and *Pseudomonas* toxin (16). Based on biochemical and electron microscopic autoradiographic studies, this mutant binds normal amount of both toxins but is defective in the transport of surface-bound ricin and, presumably, *Pseudomonas* toxin to the cytosol, where they exert their cytotoxic effects. Kinetic studies reported here show that a lag period occurring before the onset of inhibition of protein synthesis is longer in the mutant cell line than in the wild-type cell line. This defect in the mutant cell line was not seen when ricin was introduced by the microinjection technique, however, since both the mutant and the wild-type cell lines showed the same kinetics of inhibition of cellular protein synthesis by ricin and since both cell lines were equally sensitive to the toxin. These results show that the microinjection technique bypasses the internalization defect in the normal, receptor-mediated pathway of the mutant cells. Furthermore, these results confirm that the ricin resistance in mutant 4-10 is not due to an alteration in the protein-synthesizing machinery, the target of ricin action.

Our results also show that both intact ricin molecules and ricin A chains introduced by osmotic lysis of pinocytic vesicles cause rapid and marked inhibition of protein synthesis. This observation is consistent with the report (8) that intact ricin molecules encapsulated in lipid vesicles cause rapid inhibition of protein synthesis and does not support the proposal that intoxication of cultured cells by ricin requires the separation of cell surface-bound toxin into its constituent subunits before the transport of effectomer into the cell (13). Earlier it was reported that the ricin A chain and B chain act synergistically to inhibit protein synthesis in intact cells (4, 7, 19). The precise molecular events underlying the synergy are not clear. Our results show that introduction of ricin A and B chains directly into the cytosol of both CHO *pro* wild-type and resistant 4-10 cells by osmotic lysis of pinocytic vesicles does not cause any synergistic effect. This finding is not surprising since it has been postulated that the B chain may facilitate the translocation of the A chain across the endocytic membrane into the cytoplasm (4, 7, 19).

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