Biochimica et Biophysica Acta, 471 (1977) 466—476

BBA 77865

CALCIUM TRANSPORT IN DISPERSED ACINAR CELLS FROM RAT PANCREAS

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(Received May 16th, 1977)

Summary

The present studies were performed to attempt to elucidate the basis for the discrepancy between results of Kondo and Schulz (1976, Biochim. Biophys Acta 419, 76—92), who found that cholecystokinin and cholinergic agents increase uptake of ^{45}Ca by dispersed acinar cells from rat pancreas, and results of others (Matthews, E.K., Petersen, O.H. and Williams, J.A. (1973) J. Physiol. 234, 689—701; Chandler, D.E. and Williams, J.A. (1974) J. Physiol. 243, 831—846; Case, R.M. and Clausen, T. (1973) J. Physiol. 235, 75—102; Gardner, J.D., Conlon, T.P., Klaeveman, H.L., Adams, T.D. and Ondetti, M.A. (1975) J. Clin. Invest. 56, 366—375; Christophe, J.P., Frandsen, E.K., Conlon, T.P., Krishna, G. and Gardner, J.D. (1976) J. Biol. Chem. 251, 4640—4645; Shelby, H.T., Gross, L.P., Lichty, P. and Gardner, J.D. (1976) J. Clin. Invest. 58, 1482—1493 and Deschodt-Lanckman, M., Robberecht, P., de Neef, P., Lammens, M. and Christophe, J. (1976) J. Clin. Invest. 58, 891—898). They have reported that cholecystokinin and cholinergic agents do not alter or cause a slight decrease in uptake of ^{45}Ca by pancreatic acinar cells. Our present results indicate that increased uptake of ^{45}Ca by acinar cells incubated with cholecystokinin occurs only in cells washed with iced, 160 mM choline chloride and reflects increased cellular uptake of radioactivity from the wash solution but not from the incubation medium. We detected no effect of cholecystokinin on uptake of ^{45}Ca by cells washed with 160 mM choline chloride containing 5 mM ethylenediaminetetraacetate or by cells washed with Krebs-Ringer bicarbonate. Furthermore, cells washed with 160 mM choline chloride accumulated a substantial amount of ^{45}Ca from the wash solution and this accumulation was increased in cells that had been preincubated with cholecystokinin. Cells washed with Krebs-Ringer bicarbonate did not take up ^{45}Ca from the wash solution.

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Introduction

The present studies were performed to attempt to resolve a discrepancy. Kondo and Schulz [1] reported that cholinergic agents and cholecystokinin increase uptake of ^{45}Ca by dispersed acinar cells prepared from rat pancreas. The authors' kinetic analysis of the uptake data indicated that the secretagogues caused a 60—77% increase in ^{45}Ca influx and a 32—50% decrease in the coefficient for fractional outflux of ^{45}Ca (Table II, ref. 1). In contrast, others have reported that in mouse [2,3] or rat [4] pancreas as well as in dispersed pancreatic acinar cells from guinea pig [5—7] or rat [8] cholecystokinin and cholinergic agents cause no change or a slight decrease in ^{45}Ca uptake and a significant increase in outflux of ^{45}Ca. After their original report [1] Kondo and Schulz subsequently reported that pancreatic secretagogues increased outflux of ^{45}Ca from dispersed rat pancreatic acinar cells [9]. The basis for this difference was not established. Two technical differences between the studies of Kondo and Schulz [1] and those of others who used dispersed acinar cells are the calcium concentration in the incubation medium and the composition of the solution used to wash the cells. In their initial study [1], Kondo and Schulz used 1.25 mM calcium in the incubation medium while we [5—7] and Deschodt-Lanckman et al. [8] used a calcium concentration of 0.5 mM. Kondo and Schulz washed the cells with isotonic choline chloride (precise composition not given) while we and Deschodt-Lanckman et al. used Krebs-Ringer bicarbonate buffer (pH 7.4) as the wash solution. One finding which suggests that the discrepancy between the two different reports from Kondo and Schulz may be related to the composition of the wash solution is that secretagogues increased fractional outflux of ^{45}Ca when the wash solution was Krebs-Ringer bicarbonate [9] but decreased fractional outflux when the wash solution was choline chloride [1].

In the present study we have used the technique reported previously by Kondo and Schulz [1] to measure uptake as well as release of ^{45}Ca by dispersed pancreatic acinar cells from rats and guinea pigs. We have examined the effects of synthetic C-terminal octapeptide of porcine cholecystokinin (cholecystokinin octapeptide) on calcium transport in cells incubated with 0.5 or 1.25 mM calcium and have investigated the effects of different wash solutions on values for cellular ^{45}Ca. In addition to examining the effect of cholecystokinin octapeptide on uptake of ^{45}Ca by pancreatic acinar cells, two other types of experiments appeared to be important. We [5—7] and Deschodt-Lanckman et al. [8] found that adding cholecystokinin octapeptide to cells which had been preloaded with ^{45}Ca caused a substantial decrease in cellular radioactivity during the initial few minutes of incubation. Although Kondo and Schulz have not published results for this type of experiment, the values for the parameters of the model which they have reported [1] predict that under these conditions, cellular ^{45}Ca would be increased by cholecystokinin octapeptide (Fig. 1, left). We have tested this prediction in the present study. The model originally published by Kondo and Schulz [1] also predicted that in acinar cells loaded with ^{45}Ca and then incubated with an agent which chelates extracellular calcium, adding cholecystokinin octapeptide would slow the loss of ^{45}Ca from the cells (Fig. 1, right). Although others [2—8] as well as Kondo and Schulz, themselves

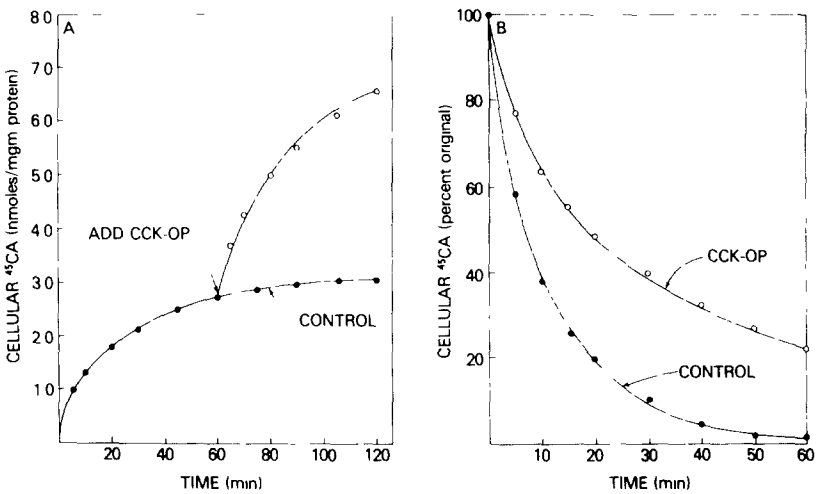

Fig. 1. Effects of cholecystokinin octapeptide (CCK-OP) on cellular ^{45}Ca in dispersed acinar cells from rat pancreas as predicted from the model of Kondo and Schulz. Values shown were computed using the mathematical model and parameter values given in Table II of ref. 1. The left panel (A) gives predicted values for cellular ^{45}Ca in acinar cells which were incubated for 60 min with ^{45}Ca and then for an additional 60 min with or without cholecystokinin octapeptide. The right panel (B) gives predicted values for the effect of cholecystokinin octapeptide on cellular ^{45}Ca in acinar cells which had been preloaded with ^{45}Ca for 60 min, washed to remove extracellular ^{45}Ca (assuming no loss of cellular radioactivity) and then incubated for 60 min.

[9] have reported that cholecystokinin octapeptide increased outflux of ^{45}Ca, we have repeated this experiment.

Materials and Methods

Materials. Male rats (150—250 g) or male guinea pigs (150—350 g) were obtained from the Small Animals Section, Veterinary Resources Branch, National Institutes of Health, Bethesda, Md. ^{45}Ca (12.5 mCi/mg) was purchased from New England Nuclear Corp., Boston, Mass.; crude collagenase (clostridium histolyticum), crude hyaluronidase (bovine testis) and chromatographically purified soybean trypsin inhibitor from Sigma Chemical Co., St. Louis, Mo.; bovine plasma albumin fraction V from Armour Pharmaceutical Co. Phoenix, Ariz. Zynthetic C-terminal octapeptide of porcine cholecystokinin (cholecystokinin octapeptide) was a gift from Dr. Miguel A. Ondetti, Squibb Institute for Medical Research, Princeton, N.J. All other reagents were of the highest grade commercially obtainable.

Methods. After an overnight fast the animal was killed by a blow to the head. The pancreas was dissected free of fat and mesentery and dispersed acinar cells were prepared as described previously [5—7] using minor modifications of the technique originally described by Amsterdam and Jamieson [10—12]. Unless otherwise specified cells (5—20 · 10^6 cells/ml) were suspended in standard incubation solution composed of Krebs-Ringer bicarbonate (pH 7.4) containing L-amino acid supplement [12], 0.1 mg/ml soybean trypsin inhibitor, 15 mM glucose, 1% (w/v) albumin, 1.25 mM calcium and 1.2 mM magnesium and were

gassed with 95% O_2: 5% CO_2. Cell concentrations were determined by counting a properly diluted suspension in a standard hemocytometer.

Uptake of ^{45}Ca by dispersed pancreatic acinar cells was determined using a technique similar to that described by Kondo and Schulz [1]. Cells were incubated at 37°C in standard incubation solution (gassed with 95% O_2: 5% CO_2) containing ^{45}Ca. At appropriate times 250 μl of cell suspension was added to a disposable conical centrifuge tube containing 12.5 ml of wash solution at 4°C and centrifuged at 1100 × *g* for 45 s. The supernatant was decanted and the tube was inverted. After the tube had drained, 1 ml of distilled water was added and the cells were disrupted by vigorus agitation. Aliquots were removed for determination of protein using the method of Lowry et al. [14] and bovine plasma albumin as a standard. Aliquots were also analyzed for ^{45}Ca by adding 100 μl to 17 ml of liquid scintillation solution composed of 15 parts toluene (T. Baker Chemical Co., Phillipsburg, N.J.), 5 parts Triton X-100 (New England Nuclear) and 1 part liquifluor (New England Nuclear). Liquid scintillation counting was performed with a Packard model 3320 liquid scintillation counter (Packard Instrument Co. Inc., Downers Grove, Ill.) ^{45}Ca trapped between the cells or adhering to the centrifuge tube was determined by measuring ^{45}Ca uptake in cells incubated with 0.5 mM ^{45}Ca plus 5 mM ethylenediamenetetraacetate (EDTA). All results were corrected for this value.

Results

Dispersed acinar cells from rat pancreas were preincubated in the standard incubation solution containing 1.25 mM calcium for 30 min and then incubated with ^{45}Ca for 60 min. When calcium uptake was determined using choline chloride (160 mM, pH 7.4) as the wash solution, values for cellular radioactivity increased rapidly during the initial 10 min of incubation and then at a slower, but steady, rate during the subsequent 50 min (Fig. 2). In agreement with the finding of Kondo and Schulz [1], adding cholecystokinin octapeptide (10^{-7} M) at the beginning of the preincubation caused a significant increase in measured uptake of ^{45}Ca (Fig. 2). When calcium uptake was measured using Krebs-Ringer buffer (i.e., a solution which was identical to the incubation solution except that it contained no radioactivity) as the wash solution values for uptake of ^{45}Ca were 20—50% less than corresponding values obtained using choline chloride as the wash solution (Fig. 2). Furthermore, adding cholecystokinin octapeptide (10^{-7} M) at the beginning of the preincubation did not alter values for calcium uptake in cells washed with Krebs-Ringer (Fig. 2). Effects virtually identical to those illustrated in Fig. 2 were obtained using standard incubation solution containing 0.5 mM calcium instead of 1.25 mM calcium (not shown). Results obtained using guinea pig pancreatic acinar cells (not shown) were similar to those obtained with acinar cells from rat pancreas (Fig. 2).

Adding 5 mM EDTA to the choline wash solution reduced the value for ^{45}Ca uptake in rat pancreatic acinar cells by 50% and abolished the increase measured in cells preincubated and incubated with cholecystokinin octapeptide (Table I). In contrast, adding 5 mM EDTA to Krebs-Ringer wash solution did not alter values for cellular uptake of ^{45}Ca in control cells or in cells preincu-

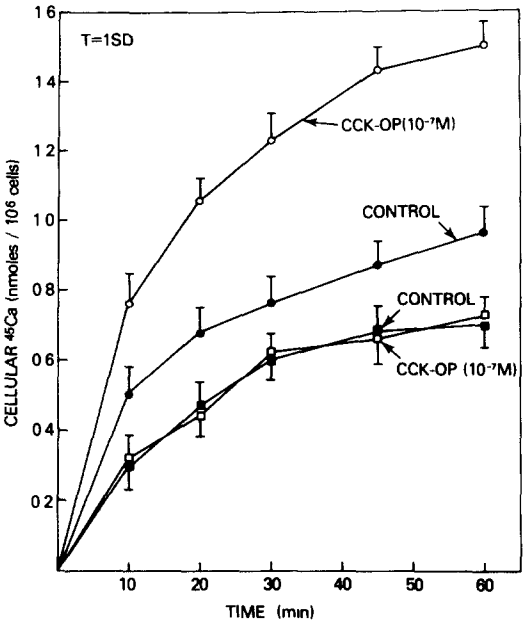

Fig. 2. Effect of the composition of the wash solution on values for uptake of ^{45}Ca by rat pancreatic acinar cells incubated with or without cholecystokinin octapeptide (CCK-OP). Dispersed acinar cells from rat pancreas were preincubated in standard incubation solution containing 1.25 mM calcium with or without cholecystokinin octapeptide (10^{-7} M) for 30 min at 37°C. ^{45}Ca was added and the incubation was continued for 60 min at 37°C. Circles represent values from cells washed with iced (4°C) 160 mM choline chloride (pH 7.4). Squares represent values from cells washed with iced (4°C) Krebs-Ringer buffer (i.e., a solution which was identical to the incubation solution except that it contained no radioactivity). Results shown are means from 7 separate experiments.

bated and incubated with cholecystokinin octapeptide (Table I).

To see whether cells take up ^{45}Ca during the wash procedure, rat acinar cells were incubated at 37°C for 60 min in standard incubation solution containing 1.25 mM calcium. An aliquot of cell suspension was added to a tube containing

TABLE I

EFFECT OF COMPOSITION OF WASH SOLUTION ON VALUE FOR CELLULAR UPTAKE OF ^{45}Ca BY DISPERSED RAT PANCREATIC ACINAR CELLS

Acinar cells from rat pancreas were resuspended in standard Krebs buffer containing 1.25 mM calcium and 1% (w/v) albumin. Cells were incubated for 30 min at 37°C with or without cholecystakinin octapeptide (10^{-7}) and then for an additional 30 min with ^{45}Ca. Samples from each incubation were washed with the indicated wash solution at 4°C. Choline wash solution contained 160 mM choline chloride (pH 7.4). Krebs wash solution was identical to the incubation solution. EDTA, when present, was 5 mM. Results given are means ±1 S.D. of seven separate experiments.

Wash solution	Cellular ^{45}Ca (nmol/10^6 cells)	
	Control	Cholecystokinin octapeptide (10^{-7} M)
Choline	1.42 ± 0.13	2.34 ± 0.29
Choline plus EDTA	0.74 ± 0.20	0.78 ± 0.15
Krebs	1.04 ± 0.12	1.06 ± 0.11
Krebs plus EDTA	1.01 ± 0.13	1.03 ± 0.14

TABLE II

UPTAKE OF ^{45}Ca FROM WASH SOLUTION BY DISPERSED RAT PANCREATIC ACINAR CELLS

Acinar cells from rat pancreas were resuspended in standard Krebs buffer containing 1.25 mM calcium and 1% (w/v) albumin and incubated for 60 min at 37°C with or without cholecystokinin octapeptide (10^{-7} M). Samples from each incubation were washed with the indicated wash solution (at 4°C) containing ^{45}Ca. Choline wash solution contained 160 mM choline chloride (pH 7.4). Krebs wash solution was identical to the incubation solution. ^{45}Ca adhering to the test tube or trapped between cells was determined using wash solution containing 5 mM EDTA and all results were corrected for this value. Results given are means ±1 S D. of 4 separate experiments.

Wash solution	Cellular ^{45}Ca (nmol/10^6 cells)	
	Control	Cholecystokinin octapeptide (10^{-7} M)
Choline	0.71 ± 0.16	1 60 ± 0.21
Krebs	0.04 ± 0.08	0.03 ± 0 09

iced wash solution plus an amount of ^{45}Ca identical to that added when ^{45}Ca was present in the incubation solution and was processed as usual (Table II). When the wash solution was choline chloride control cells accumulated 0.71 nmol $^{45}Ca/10^6$ cells during the wash procedure and cells incubated with 10^{-7} M cholecystokinin octapeptide accumulated approximately twice as much radioactivity (1.60 nmol/10^6 cells) (Table II). In contrast, acinar cells incubated with or without cholecystokinin octapeptide failed to accumulate ^{45}Ca from Krebs-Ringer wash solution (Table II).

To examine whether values for cellular ^{45}Ca depended on the period of time that cells were exposed to the wash solution, acinar cells were loaded with ^{45}Ca, added to wash solution and incubated at 4°C for up to 6 min. With each of the 4 wash solutions tested (choline, choline plus EDTA, Krebs-Ringer or Krebs-Ringer plus EDTA) values for cells processed immediately were the same as those for cells incubated in the wash solution for 2, 4 or 6 min (not shown).

Rat pancreatic acinar cells were preincubated in standard incubation solution containing 1.25 mM calcium for 30 min and then for an additional 60 min with ^{45}Ca. At the end of the 90 min preincubation appropriate agents were added and cellular ^{45}Ca was determined at intervals during a 60 min incubation. When cells were washed with choline chloride, values for cellular ^{45}Ca tended to increase slightly during the 60 min incubation (Fig. 3, left). Adding cholecystokinin octapeptide (10^{-7} M) caused an 18% decrease in the value for cellular ^{45}Ca after 5 min of incubation but values obtained during the subsequent 55 min were not significantly different from those in control cells (Fig. 3, left). After adding 5 mM EDTA, values for cellular ^{45}Ca decreased by 50% during the initial 5 min of incubation and then by another 15% during subsequent 55 min (Fig. 3, left). After adding EDTA plus cholecystokinin octapeptide, values for cellular ^{45}Ca decreased by approximately 85% during the initial 5 min of incubation and then during the subsequent 55 min decreased in parallel with those for cells incubated with EDTA alone (Fig. 3, left).

When rat pancreatic acinar cells were washed with Krebs-Ringer, values for cellular ^{45}Ca increased progressively by approximately 50% during the 60 min incubation (Fig. 3, right). Adding cholecystokinin octapeptide (10^{-7} M) caused a 50% decrease in the value for cellular ^{45}Ca by 5 min of incubation, after

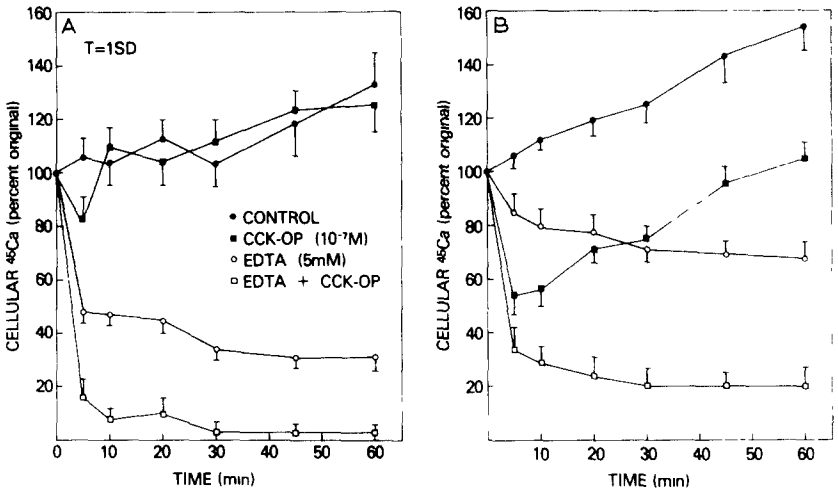

Fig. 3. Effect of composition of wash solution on values for cellular ^{45}Ca in rat pancreatic acinar cells. Dispersed acinar cells from rat pancreas were preincubated in standard incubation solution containing 1.25 mM calcium for 30 min at 37°C and then for an additional 60 min with ^{45}Ca. At the end of the 90 min preincubation appropriate agents were added and cellular ^{45}Ca was determined at intervals during a 60 min incubation at 37°C. The left panel (A) illustrates results obtained using iced (4°C) 160 mM choline chloride (pH 7.4) as the wash solution. The right panel (B) illustrates results obtained using iced (4°C) Krebs-Ringer buffer as the wash solution. Results shown are means from 6 separate experiments. CCK-OP, cholecystokinin octapeptide.

which values increased in parallel with those from control cells (Fig. 3, right). After adding 5 mM EDTA, values for cellular ^{45}Ca decreased by 15% during the first 5 min of incubation and by another 15% during the subsequent 55 min of incubation (Fig. 3, right). After adding EDTA plus cholecystokinin octapeptide, values for cellular ^{45}Ca decreased by about 70% during the initial 5 min of incubation and then during the remainder of the incubation decreased in parallel with values from cells incubated with EDTA alone (Fig. 3, right). Results similar to those illustrated in Fig. 3 were obtained using rat pancreatic acinar cells incubated in standard incubation solution containing 0.5 mM calcium (not shown) or using dispersed acinar cells prepared from guinea-pig pancreas (not shown).

To evaluate further the effect of varying the composition of the wash solution on values for cellular ^{45}Ca, rat pancreatic acinar cells were preincubated for 30 min with or without cholecystokinin octapeptide (10^{-7} M) and then for 60 min with ^{45}Ca. At the end of the preincubation, cells were incubated for 5 min with different agents and cellular ^{45}Ca was determined. With each of the different wash solutions (choline, choline plus EDTA or Krebs-Ringer), preincubation with or without cholecystokinin octapeptide and then incubation for 5 min with EDTA caused a decrease in values for cellular ^{45}Ca (Table III). Adding cholecystokinin octapeptide to the incubation medium with or without EDTA decreased values for cellular ^{45}Ca in cells preincubated without cholecystokinin octapeptide but not in cells preincubated with cholecystokinin octapeptide. When the wash solution was choline, plus EDTA or Krebs-Ringer, values for loss of ^{45}Ca from rat acinar cells preincubated with cholecystokinin octapeptide and then incubated with EDTA for 5 min were the same as those

TABLE III

EFFECT OF COMPOSITION OF WASH SOLUTION ON VALUES FOR CELLULAR ^{45}Ca IN DISPERSED RAT PANCREATIC ACINAR CELLS

Acinar cells from rat pancreas were resuspended in standard Krebs buffer containing 1.25 mM calcium and 1% (w/v) albumin Cells were preincubated for 30 min at 37°C with or without cholecystokinin octapeptide (10^{-7} M) and then for an additional 60 min with ^{45}Ca 0.5 ml aliquots were then incubated for 5 min at 37°C with the indicated agents Samples from each incubation were washed with the indicated wash solution at 4°C Choline wash solution contained 160 ml choline chloride (pH 7.4). Krebs wash solution was identical to standard incubation solution. EDTA, when present in the wash solution, was 5 mM Results given are means ± 1 S.D. of four separate experiments.

Incubation	Cellular ^{45}Ca (nmol/10^6 cells)					
	Preincubation		Preincubation		Preincubation	
	Buffer	Cholecystokinin octapeptide (10^{-7} M)	Buffer	Cholecystokinin octapeptide (10^{-7} M)	Buffer	Cholecystokinin octapeptide (10^{-7} M)
	Choline wash		Choline plus EDTA wash		Krebs-Ringer wash	
Buffer	1.58 ± 0.23	2.60 ± 0 31	0.80 ± 0.09	0.84 ± 0.08	1.09 ± 0.12	1.19 ± 0.14
EDTA (5 mM)	0.84 ± 0.08	0.81 ± 0.09	0.65 ± 0.07	0 69 ± 0.06	0 92 ± 0.11	0.96 ± 0.08
Cholecystokinin octapeptide (10^{-7} M)	1.29 ± 0.14	2.71 ± 0.28	0 39 ± 0 05	0.78 ± 0 07	0.66 ± 0 07	1 16 ± 0 13
EDTA plus cholecystokinin ictaoeotide	0.23 + 0.04	0.76 ± 0.09	0.20 ± 0.04	0.67 ± 0.07	0.39 ± 0.05	0.94 ± 0.10

for cells preincubated without cholecystokinin octapeptide *. When the wash solution was choline, cells preincubated with cholecystokinin octapeptide appeared to lose 2.5 times more ^{45}Ca during a 5 min incubation with EDTA than did control cells. This apparent increase was attributable solely to cells preincubated with cholecystokinin octapeptide and incubated with buffer having values for cellular ^{45}Ca which were significantly higher than those for cells preincubated without cholecystokinin octapeptide. After a 5 min incubation with EDTA, values for ^{45}Ca in cells preincubated with cholecystokinin octapeptide were the same as those for control cells. Furthermore, values for ^{45}Ca in cells incubated with EDTA and washed with choline were the same as those in cells incubated with buffer and washed with choline plus EDTA.

To explore the effect of cholecystokinin octapeptide on protein content of acinar cells from rat pancreas, cells were incubated with or without cholecystokinin octapeptide (10^{-7} M) for 4 h at 37°C. Protein content of acinar cells incubated for 4 h with cholecystokinin octapeptide (245 ± 21 $\mu g/10^6$ cells; mean ±1 S.D., 4 experiments) were 31% lower than that of control cells (353 ± 17 $\mu g/10^6$ cells). Our value for protein content of control cells agreed closely with the value reported by Kondo and Schulz (390 $\mu g/10^6$ cells [1]).

* Loss of ^{45}Ca was calculated by subtracting the value obtained after a 5 min incubation with 5 mM EDTA from the corresponding value obtained after a 5 min incubation without EDTA.

Discussion

In agreement with previous studies [1,5—9], we found that in dispersed pancreatic acinar cells, cholecystokinin octapeptide appeared to increase uptake of ^{45}Ca when the cells were washed with choline chloride, but not when the cells were washed with Krebs-Ringer. The increased uptake of ^{45}Ca by cells incubated with cholecystokinin octapeptide and washed with iced choline chloride does not reflect increased uptake of radioactivity by the cells while they are in the incubation solution, but instead, results from increased cellular uptake of ^{45}Ca from the wash solution. Adding 5 mM EDTA to the choline wash solution reduced values for cellular ^{45}Ca in control cells by 50% and abolished the apparent increased uptake caused by cholecystokinin octapeptide. Furthermore, acinar cells accumulated substantial amounts of ^{45}Ca (0.71 nmol/10^6 cells, Table II) from the choline wash solution and this uptake was increased significantly in cells that had been incubated with cholecystokinin octapeptide. Moreover, in cells incubated with cholecystokinin octapeptide the increased uptake of ^{45}Ca from the wash solution (0.89 nmol/10^6 cells, Table II) was the same as the increase observed when ^{45}Ca was present during the incubation (0.92 nmol/10^6 cells, Table I). Adding 5 mM EDTA to Krebs-Ringer wash solution did not alter values for cellular ^{45}Ca and there was no detectable cellular uptake of ^{45}Ca from Krebs-Ringer wash solution.

In studying the effect of secretagogues on outflux of ^{45}Ca from dispersed pancreatic acinar cells, Kondo and Schulz [9] reported that cells preincubated with cholecystokinin octapeptide plus ^{45}Ca contained 59% more ^{45}Ca than did cells preincubated without cholecystokinin octapeptide *. Since, in these experiments, the cells were washed with Krebs-Ringer bicarbonate instead of choline chloride, this finding cannot be attributed to cholecystokinin octapeptide-induced uptake of ^{45}Ca from the choline wash solution and disagree with our present findings as well as other reports [2—8] that cholecystokinin octapeptide does not alter uptake of ^{45}Ca in cells preincubated with cholecystokinin octapeptide. This finding is probably attributable to cholecystokinin octapeptide causing a decrease in cellular protein content. In these experiments [9], Kondo and Schulz calculated ^{45}Ca present in the cells at the end of the preincubation period from the cumulative radioactivity released into the incubation medium plus the cellular radioactivity measured at the end of the 2 h incubation. Values were expressed as nmol ^{45}Ca/mg cell protein. Since acinar cells preincubated and incubated with cholecystokinin octapeptide exactly as reported by Kondo and Schulz had 31% less protein than did control cells, this cholecystokinin octapeptide-induced decrease in cellular protein would cause

* It should be noted that the magnitude of the effect of cholecystokinin octapeptide on total exchangeable cellular calcium determined by measuring cumulative outflux of ^{45}Ca (59% increase, Table II, ref. 9) was significantly less than that obtained previously by these same authors using the choline wash technique (136% increase, Table II, ref. 1). The authors attributed this difference to loss of ^{45}Ca during the wash procedure in the cumulative outflux technique; however, assuming that the loss during the wash procedure can be calculated from the results in their original paper (Table II, ref. 1), this loss of cellular ^{45}Ca should have magnified the apparent increase in total exchangeable calcium caused by cholecystokinin octapeptide (i.e , Kondo and Schulz should have observed an increase of at least 160%)

an apparent 45% increase in cellular ^{45}Ca/mg protein when, in fact, none was present.

We found that in rat pancreatic acinar cells that had been preloaded with ^{45}Ca, cholecystokinin octapeptide caused a decrease in cellular ^{45}Ca when the cells were washed with Krebs-Ringer but not when the cells were washed with choline. This difference can be explained by our finding that cholecystokinin octapeptide increased outflux of ^{45}Ca from acinar cells washed with choline or with Krebs-Ringer and that the octapeptide increased cellular uptake of ^{45}Ca from choline wash solution but not from Krebs-Ringer wash solution. Thus, in cells washed with choline the cholecystokinin octapeptide-induced loss of cellular ^{45}Ca in the incubation solution is obscured by the cholecystokinin octapeptide-induced increase in the uptake of ^{45}Ca from the wash solution.

Kondo and Schulz [9] have also reported that outflux of ^{45}Ca from rat pancreatic acinar cells that had been preincubated with cholecystokinin octapeptide for 30 min and then with cholecystokinin octapeptide plus ^{45}Ca for 90 min was significantly greater than that from corresponding control cells. The magnitude by which outflux was increased cannot be determined since the authors did not provide values for the amount of ^{45}Ca remaining in the cells as a function of time, but instead provided derived values for only two of the three exponential processes which they used to describe ^{45}Ca transport (Table I, ref. 9). As mentioned previously, these results do not agree with an earlier study from the same authors [1]. These results are also at variance with the findings by Deschodt-Lanckman et al., using rats [8], and by us, using guinea pigs [5—7], that values for outflux of ^{45}Ca from acinar cells preincubated with cholecystokinin octapeptide were the same as outflux from control cells. In the present studies we found that outflux of ^{45}Ca from cells preincubated with cholecystokinin octapeptide plus ^{45}Ca and washed with Krebs-Ringer or choline plus EDTA was the same as in cells preincubated without the octapeptide. In cells washed with choline, outflux of ^{45}Ca from cells preincubated with cholecystokinin octapeptide appeared to be 2.5-times greater than outflux from control cells. However, this apparent increase does not reflect increased ^{45}Ca outflux. Instead, it is due to cells preincubated with cholecystokinin octapeptide taking up more ^{45}Ca from the wash solution than cells preincubated without cholecystokinin octapeptide.

Finally, the model proposed by Kondo and Schulz [1] to describe effects of cholecystokinin octapeptide on ^{45}Ca transport in rat pancreatic acinar cells fails to predict the effect of cholecystokinin octapeptide on cellular ^{45}Ca in cells preloaded with the tracer (compare Fig. 1A with Fig. 3A) as well as an outflux of ^{45}Ca (compare Fig. 1B with Fig. 3 and Table III). This failure is attributable primarily to changes in cellular ^{45}Ca which occur during the wash procedure with choline chloride, but may also result from the original model having been based on invalid assumptions (e.g. that calcium transport could be described by a 2-compartment, parallel model and that the system reached a steady-state within 30 min [1]).

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