ORIGINALS

Calcium Induced Glucagon Release in Monolayer Culture of the Endocrine Pancreas. Studies with Ionophore A23187

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Summary. The possible role of Ca^{2+} in glucagon release has been investigated by the use of ionophore A23187. This ionophore permits Ca^{2+} entry down a suitable concentration gradient by complexing and releasing Ca^{2+} , thereby acting as a carrier in plasma membranes. Cultured cells obtained by enzymatic digestion of pancreases from newborn rats were studied on the third day of culture. As expected the effects of the ionophore were dependent upon the presence of Ca^{2+} in the medium. However, either stimulation or inhibition of glucagon release resulted when different concentrations of ionophore and Ca^{2+} were used. With 1.0 mM Ca^{2+} in the medium, glucagon release was stimulated in the presence of 0.01 and 0.1 $\mu\text{g}/\text{ml}$ ionophore, but inhibited in the presence of 3.0 and 10.0 $\mu\text{g}/\text{ml}$. With 0.1 $\mu\text{g}/\text{ml}$ ionophore, glucagon release was stimulated by 0.3 and 1.0 mM Ca^{2+} but not by 2.5 mM Ca^{2+} . With 10 $\mu\text{g}/\text{ml}$ ionophore glucagon release was stimulated by 0.03, 0.1 and 0.3 mM Ca^{2+} , whereas at 1.0 mM, glucagon release was depressed. These findings suggest that by increasing Ca^{2+} , glucagon is released from the A-cells, whereas too large an increase in Ca^{2+} is inhibitory. The effect to stimulate release was not completely specific for Ca^{2+} in that while the ionophore did not stimulate release in the presence of either Mg^{2+} or Sr^{2+} in the absence of Ca^{2+} , it did stimulate release when Ba^{2+} was tested. Furthermore Ba^{2+} at 0.3 mM was stimulatory even in the absence of ionophore. Glucagon release in the absence of ionophore was also enhanced by addition of 30 mM Ca^{2+} or by omission of Ca^{2+} from the medium. It is concluded that Ca^{2+} , which plays an essential role in the stimulus-secretion coupling in several different cell types, may be involved in the stimulation of glucagon release from the A-cells of the pancreas.

Key words: Glucagon release, monolayer culture, ionophore A23187, calcium, endocrine pancreas.

Calcium plays an essential role in the secretion of hormones from endocrine glands and in the control of numerous biological mechanisms. Studies on the effects of Ca^{2+} have been facilitated recently by the use of divalent cation ionophores, such as A23187, a carboxylic acid antibiotic [1] with the ability to transport divalent cations across biological membranes [2–5]. By the use of this agent and a favourable concentration gradient for Ca^{2+} across cell membranes it is possible to introduce Ca^{2+} into the cell interior in a concentration dependent manner. Thus the ionophore has been used to study the effects of Ca^{2+} on a variety of functions, including histamine release from mast cells [6–7], fluid secretion from fly salivary gland [8], K^{+} release from the parotid gland [9], amylase secretion from the exocrine pancreas [10, 11], dopamine release from synaptosomes [12], ATP release from platelets [13], vasopressin release from neurohypophysis [14], and hormone release from islets of Langerhans [15–18]. In all these studies it was shown that physiological secretion processes could be stimulated by increasing the amount of cell Ca^{2+} in the absence of other known physiological stimulators for these responses. In the case of insulin release, Ca^{2+} entry facilitated by the ionophore A23187 was shown to stimulate and sustain insulin release [15]. The release occurred at non-stimulatory glucose concentrations and was dependent upon the Ca^{2+} concentration available for entry.

Thus investigations using the ionophore were performed to study the role of Ca^{2+} in the glucagon release system. As in our previous study with the ionophore [15] the monolayer culture of newborn rat pancreas, which provides near maximal exposure of cell surfaces and responds in a physiologic manner to modulators of glucagon release [19], was used as the test preparation.

Material and Methods

The culture procedure for endocrine pancreas has been described previously [19, 20]. In brief (for one culture preparation) 100–120 pancreases from 1–3 day old Wistar rats were excised using sterile technique. The pooled pancreases were cut into pieces 1–2 mm in diameter. After one washing in calcium and magnesium-free phosphate buffered saline (PBS) the pieces of pancreas were submitted to approximately ten consecutive treatments with a mixture of trypsin and collagenase in calcium and magnesium-free PBS, containing 2.8 mM glucose at 37° C. The 3–4 initial supernatants were discarded. The subsequent supernatants containing isolated cells were kept and diluted with cold (+ 4° C) culture medium 199 containing 10% calf-serum, 14 mM sodium bicarbonate, 16.7 mM glucose and Na-penicillin G 400 U/ml. The pooled supernatants were centrifuged at $150 \times G$ and the packed cells resuspended in medium 199. The final suspension was diluted to contain about 500 000 cells/ml and plated in plastic Petri dishes. By this procedure 40–50 culture dishes were obtained. The cells were cultured at 37° C in the culture medium 199 in an incubator with automatic pH regulation. About 14 h after plating, at a time when a major portion of fibroblastoid cells, but only a minor portion of the epithelioid cells, had attached to the bottom of the culture dishes, the supernatants were decanted into new Petri dishes and the residual dishes discarded. As reported previously [19, 20] this decantation yielded cultures which were rich in epithelioid cells. 44 hours after plating, when the epithelioid cells had attached to the bottom of the dish and rearranged in clusters of varying size, the cells were washed and fresh culture medium containing 5.6 mM glucose was added. On the third day of culture the cells were studied under conditions of short incubation in Krebs-Ringer bicarbonate buffer (KRB). By this time amylase activity could no longer be detected in the culture medium and exocrine cells were devoid of zymogen granules when examined by electron microscopy [21].

The KRB buffer contained 0.5% dialyzed bovine serum albumin, 250 Kallikrein inhibitory units/ml

Trasylol and 2.8 mM glucose. The ionic composition of the standard KRB-buffer was the following: Na^+ 142.9 mM, K^+ 5.8 mM, Ca^{2+} 2.5 mM, Mg^{2+} 1.2 mM, Cl^- 128.4 mM, SO_4^{2-} 1.2 mM, H_2PO_4^- 1.2 mM and HCO_3^- 24.4 mM. Calcium depleted media were obtained by omission of CaCl_2 from the standard buffer. To the calcium depleted buffer varying concentrations of CaCl_2 , MgSO_4 , SrCl_2 or BaCl_2 were added where appropriate. A23187 was dissolved in dimethyl sulfoxide before addition to the test media; dimethyl sulfoxide at a final concentration of 0.33% had no effect alone, but was added to control media in all cases where the ionophore was being tested. In the experiments in which Ca^{2+} concentrations up to 30 mM were used, the phosphate concentration was reduced to 0.1 mM to avoid precipitation of calcium phosphate.

Incubations were carried out over 30 min or 1 h at 37° C and pH was maintained at 7.40 ± 0.05 . Samples taken at the end of the incubation were centrifuged 15 min at 3500xG, decanted and then frozen and stored at -20° C until assay. Immunoreactive glucagon (IRG) was measured by radioimmunoassay using rabbit anti-porcine glucagon serum, and pork glucagon as standard. Antibody-bound hormone was separated from free hormone using dextran-coated charcoal as described by Unger et al. [22]. At the doses used, the ionophore did not interfere with the assay. Glucagon content of the cultured cells was determined after incubation by washing the cells twice with PBS, then extracting with 0.15 N HCl in 75% ethanol and assaying after dilution.

Results are expressed as means $\pm$ SEM. Petri dishes were paired before the experiments into test and control dishes. Statistical analysis was by the paired Student's t-test.

The chemicals employed and their sources were as follows: trypsin (Difco Laboratories, Detroit, Mich.), collagenase (Worthington Biochemical Corp., Freehold, N. J.), medium 199 (Grand Island Biological Co., Grand Island, N. Y.), sodium-penicillin G (Chas. Pfizer and Co., Inc., New York, N. Y.), Petri dishes (Falcon Plastics, Oxnard, Calif.), bovine serum albumin (Behringwerke A.G., Marburg, FRG), Trasylol (kindly provided by Dr. H. Ruff, Bayer Pharma A. G., Zurich, Switzerland), A23187 (kindly provided by Dr. O. Behrens, Eli Lilly, Indianapolis, Ind.), dimethyl sulfoxide (Merck A. G., Darmstadt, FRG), glucagon antiserum 30K (generously provided by Dr. R. H. Unger, University of Texas, Southwestern Medical School, Dallas, Texas. Glucagon was a gift from Dr. L. Heding, Novo Research Institute, Copenhagen, Denmark.

Fig. 1. Effect of different concentrations of A23187 on the glucagon release in the presence of 1 mM Ca^{2+} . The results are expressed as the increment or decrement in glucagon release relative to control dishes i. e. Δ ng IRG released/dish/30 min. Vertical lines represent $\pm$ SEM. The number of experiments is indicated in each bar. IRG, immunoreactive glucagon

Table 1. Effect of different concentrations of ionophore A23187 on glucagon release in the presence of 1 mM Ca^{2+} . Release expressed as ng IRG/dish/30 min

A 23187 (μg/ml)	Control	Test (A23187)	Δ	p	n
0.003	0.61 $\pm$ 0.05	0.69 $\pm$ 0.04	0.08 $\pm$ 0.07	<0.3	6
0.01	0.59 $\pm$ 0.07	0.81 $\pm$ 0.05	0.22 $\pm$ 0.09	<0.05	11
0.1	0.50 $\pm$ 0.04	0.71 $\pm$ 0.04	0.21 $\pm$ 0.04	<0.001	27
0.3	0.46 $\pm$ 0.10	0.51 $\pm$ 0.13	0.05 $\pm$ 0.13	<0.8	6
1.0	0.58 $\pm$ 0.05	0.47 $\pm$ 0.05	-0.01 $\pm$ 0.06	<0.9	30
3.0	0.52 $\pm$ 0.05	0.37 $\pm$ 0.06	-0.15 $\pm$ 0.06	<0.05	10
10.0	0.51 $\pm$ 0.04	0.30 $\pm$ 0.03	-0.21 $\pm$ 0.05	<0.001	25

Results

Dose-Response to the Ionophore

Initial experiments were performed in the presence of 1.0 mM Ca^{2+} in the medium and different concentrations of the ionophore. As in all experiments reported here, except when high glucose concentrations are specifically mentioned, 2.8 mM glucose was included in the incubation medium. The results are shown in Table 1. Under these conditions no significant alteration of glucagon release was achieved with an ionophore concentration of 0.003 μg/ml. The minimal effective concentration of ionophore was 0.01 μg/ml and resulted in stimulation of glucagon release as did 0.1 μg/ml. As the ionophore concentration was increased further the stimulatory effect on glucagon release was lost and at concentrations of 3 and 10 μg/ml the release of glucagon was significantly depressed relative to paired control cultures. These results are best seen in Fig. 1 in which the changes in glucagon release in response to the different concentrations of ionophore are shown.

Dose-Response to Ca^{2+}

The effect of ionophore A23187 at a concentration of 0.1 μg/ml was next tested in the presence of different concentrations of medium Ca^{2+} . These results are shown in Fig. 2. In the absence of Ca^{2+} in the medium the ionophore had no effect on glucagon release. With 0.3 mM Ca^{2+} in the medium, a significant stimulation of glucagon release occurred. A larger stimulation of release was achieved with 1.0 mM Ca^{2+} whereas, at 2.5 mM Ca^{2+} , no effect on glucagon release was observed.

Further studies were then performed in the presence of a high concentration of ionophore, 10 μg/ml (see Fig. 3). The response to different Ca^{2+} concentrations was again tested. With the high ionophore concentration a significant stimulation of glucagon release was present at a medium Ca^{2+} of only 0.03 mM. Similarly, release was stimulated in the presence of 0.1 and 0.3 mM Ca^{2+} . When the concentration of Ca^{2+} was raised to 1.0 mM the release of glucagon was significantly depressed, in agreement with the results shown in the right hand bar of Fig. 1.

Fig. 2. Effect of different concentrations of Ca^{2+} on glucagon release in the presence and absence of 0.1 $\mu\text{g/ml}$ A23187. Results expressed as ng IRG released/dish/30 min. Vertical lines represent $\pm$ SEM. The number of experiments is indicated in each bar. IRG, immunoreactive glucagon

Fig. 3. Effect of different concentrations of Ca^{2+} on glucagon release in the presence and absence of 10 $\mu\text{g/ml}$ A23187. Results are expressed as ng IRG released/dish/30 min. Vertical lines represent $\pm$ SEM. The number of experiments is indicated in each bar. IRG, immunoreactive glucagon

Table 2. Effect of different concentrations of Ca^{2+} on fractional glucagon release in the presence of either 0.1 or 10 $\mu\text{g/ml}$ A23187. The IRG release is expressed as the % of initial IRG content

Ca^{2+} (mM)	Control	A23187 0.1 $\mu\text{g/ml}$	Δ	p	n
0	9.3 $\pm$ 0.9	7.8 $\pm$ 1.1	-1.5 $\pm$ 0.9	< 0.2	17
0.3	7.9 $\pm$ 1.4	9.7 $\pm$ 1.0	1.8 $\pm$ 1.1	< 0.2	16
1.0	5.5 $\pm$ 1.2	9.4 $\pm$ 2.0	3.8 $\pm$ 1.8	= 0.05	15
2.5	9.8 $\pm$ 1.7	9.6 $\pm$ 1.7	-0.2 $\pm$ 1.5	< 0.95	17
		A23187 10 $\mu\text{g/ml}$			
0.03	8.9 $\pm$ 2.2	11.5 $\pm$ 2.0	2.5 $\pm$ 2.0	< 0.3	11
0.1	5.9 $\pm$ 1.3	10.0 $\pm$ 2.0	4.1 $\pm$ 1.9	< 0.1	12
0.3	4.0 $\pm$ 0.7	7.9 $\pm$ 2.0	3.9 $\pm$ 1.6	< 0.05	11
1.0	5.8 $\pm$ 0.7	5.1 $\pm$ 0.9	-0.7 $\pm$ 0.7	< 0.4	12

Glucagon Release Expressed as the Fraction of the Cellular Glucagon Content

At the end of all the experiments shown in Fig. 2 and 3, the content of glucagon remaining in the cultures was measured by acid ethanol extraction, dilution and immunoassay. Thus the amount of glucagon released could be expressed in terms of the original content of the cultures (i. e. glucagon release relative to glucagon content remaining at the end of the experiments plus

the glucagon released). The results are shown in Table 2. The largest release of glucagon was 11.5% of the initial hormone content.

Cation Specificity

In order to assess the specificity of the response to Ca^{2+} and to see whether other cations could replace Ca^{2+} in modulating glucagon release, experiments were performed in the presence of different concentrations of Mg^{2+} , Ba^{2+} and Sr^{2+} . The results from

Table 3. Effects of Mg^{2+} , Ba^{2+} and Sr^{2+} on glucagon release in the presence and absence of A23187, expressed as ng IRG/dish/30 min

a)			Test A23187				
	Ca ²⁺	Mg ²⁺	Control	0.1 µg/ml	Δ	p	n
	mM						
	1.0	1.2	0.27±0.02	0.61±0.07	0.34±0.03	<0.005	10
	0	1.2	0.31±0.02	0.26±0.03	-0.05±0.04	<0.3	10
	0	12	0.25±0.02	0.21±0.02	-0.04±0.04	<0.4	10
	0	24	0.21±0.02	0.28±0.02	0.07±0.03	<0.1	10
b)			Test A 23187				
	Ca ²⁺	Ba ²⁺	Control	0.1 µg/ml	Δ	p	n
	mM						
	1.0	0	0.46±0.05	0.70±0.01	0.23±0.08	<0.05	7
	0	0	0.58±0.06	0.77±0.04	0.19±0.08	<0.1	5
	0	0.03	0.65±0.10	0.84±0.18	0.20±0.23	<0.5	5
	0	0.1	0.70±0.27	0.95±0.16	0.25±0.15	<0.2	6
	0	0.3	1.24±0.09	1.11±0.08	-0.13±0.10	<0.3	7
c)			Test A23187				
	Ca ²⁺	Ba ²⁺	Control	10 µg/ml	Δ	p	n
	mM						
	0	0	0.26±0.07	0.34±0.04	0.08±0.08	<0.5	5
	0	0.1	0.33±0.02	0.70±0.10	0.37±0.10	<0.025	5
d)			Test A23187				
	Ca ²⁺	Sr ²⁺	Control	0.1 µg/ml	Δ	p	n
	mM						
	1	0	0.28±0.04	0.35±0.05	0.07±0.01	<0.001	11
	0	0	0.44±0.07	0.42±0.06	-0.02±0.04	<0.6	11
	0	1	0.44±0.06	0.46±0.06	0.02±0.04	<0.6	12
	0	3	0.48±0.06	0.36±0.05	-0.12±0.05	<0.05	12

experiments which were performed using 0.1 $\mu\text{g/ml}$ ionophore are presented in Table 3, where, in part (a), it can be seen that 1.0 mM Ca^{2+} in the presence of the standard 1.2 mM Mg^{2+} produced the expected ionophore induced stimulation of glucagon release. Removal of Ca^{2+} , however, prevented the stimulatory effect of ionophore on release. As the Mg^{2+} concentration was increased to 12 or 24 mM, in the absence of both ionophore and Ca^{2+} , the release of glucagon was diminished ($p < 0.005$). In the presence of ionophore increasing Mg^{2+} failed to stimulate glucagon release.

In the studies with Ba^{2+} , results presented in Table 3 (b), Ca^{2+} produced its expected stimulation of glucagon release in the presence of the ionophore. In the presence of all three concentrations of Ba^{2+} tested (0.03, 0.1 and 0.3 mM) and in the absence of Ca^{2+} the ionophore failed to induce glucagon release. It should be pointed out, however, that 0.3 mM Ba^{2+} alone stimulated glucagon release relative to the zero Ca^{2+} control ($p < 0.005$). For this reason Ba^{2+} was not tested at higher concentrations, but was tested using a higher ionophore concentration. Thus, using 10 $\mu\text{g/ml}$ ionophore and 0.1 mM Ba^{2+} in the absence of Ca^{2+} , a significant stimulation of glucagon release occurred

(Table 3 (c)). In the absence of Ca^{2+} and Ba^{2+} this high concentration of ionophore failed to stimulate release. Finally, the results of experiments with Sr^{2+} are shown in Table 3 (d). Sr^{2+} failed to mimic the effects of Ca^{2+} . 1 mM Sr^{2+} had no effect upon glucagon release in the presence of ionophore while 3.0 mM Sr^{2+} caused a significant decrease of glucagon release in the presence of the ionophore.

Effect of Different Ca^{2+} Concentrations in the Absence of Ionophore

It was also possible to assess the effect of Ca^{2+} depletion on glucagon release under control conditions. Summing up the results of the experiments in Table 3 (a, b and d), glucagon release at 1 mM Ca^{2+} was 0.32 ± 0.03 ng/dish/30 min. Under Ca^{2+} free conditions it was 0.41 ± 0.04 ng/dish/30 min. The difference, 0.08 ± 0.03 was significant ($p < 0.01$, $n = 25$). A similar stimulation by Ca^{2+} depletion was also detected in the experiments shown in Fig. 2.

In view of this effect of Ca^{2+} depletion, we examined the effect of high Ca^{2+} concentrations on glucagon release. Under control conditions, that is in the absence of ionophore, glucagon release was mea-

Fig. 4. Effect of different concentrations of Ca^{2+} , in the absence of ionophore, on glucagon release. Results expressed as ng IRG released/dish/60 min. Vertical lines represent $\pm$ SEM. The number of experiments is indicated in parenthesis. IRG, immunoreactive glucagon

Table 4. Reversible effect of high Ca^{2+} concentration on glucagon release. Release was measured over two 60 min periods, the first in presence of either Ca^{2+} 3 or 30 mM, the second in presence of Ca^{2+} 3 mM. Between the two incubations the dishes were washed three times before fresh medium was added. Release expressed as ng IRG/dish/60 min. $n = 6$

Ca^{2+} (mM)		IRG Release		
1st hour	2nd hour	1st hour	2nd hour	p
3	3	0.14 ± 0.02	0.14 ± 0.01	—
30	3	0.30 ± 0.01	0.14 ± 0.01	<0.001
p		<0.001		—

sured in the presence of 0.3, 1.0, 3.0 10.0 and 30.0 mM Ca^{2+} . From the results shown in Fig. 4 it can be seen that a large stimulation of glucagon release occurred in the presence of 30 mM Ca^{2+} ($p < 0.01$). Furthermore, as is shown in Table 4, the stimulatory effect of 30 mM Ca^{2+} is completely reversible.

Effect of Ionophore on Glucose-Suppressed Glucagon Release

Experiments were performed to see if the ionophore in the presence of Ca^{2+} in the medium could stimulate glucagon release in the face of a suppressive concentration of glucose. A comparison was made under control conditions (1.0 mM Ca^{2+}) of the glucagon released in the presence of 2.8 mM and 16.7 mM glucose. Release in 2.8 mM glucose was 0.31 ± 0.04 ng/dish/30 min and in 16.7 mM glucose it was 0.21 ± 0.04 , $\Delta = -0.10 \pm 0.02$, $p < 0.01$, $n = 5$. Thus high

glucose suppressed glucagon release. In 16.7 mM glucose 0.1 $\mu\text{g/ml}$ ionophore almost doubled the glucagon release. Under these conditions, the release was 0.39 ± 0.04 ng/dish/30 min, $\Delta = 0.18 \pm 0.06$, $p < 0.05$, $n = 5$, demonstrating that stimulation of glucagon release is possible even during glucose suppression.

Discussion

It is clear from these data and those of Ashby and Speake [18] that Ca^{2+} , in the presence of ionophore A23187, is able to stimulate glucagon release. This occurs when glucagon release is studied in the presence of low glucose, or inhibited in the presence of high glucose in the medium. Thus, as in previous studies in other release processes, the ionophore, by acting as a membrane carrier and permitting Ca^{2+} entry into cells, can mimic the effects of physiological stimulation. This has been shown for histamine release, fluid secretion, potassium efflux, amylase secretion, dopamine release, secretion by platelets and vasopressin and insulin release [6–18]. That the effect is not due to destruction of cells and liberation of cell contents seems apparent from experiments in which high ionophore concentrations were used. Under these conditions with normal Ca^{2+} (1 mM) the release of glucagon was inhibited. The ionophore is inactive in the absence of Ca^{2+} and stimulation is the result of a complex combination of ionophore and Ca^{2+} concentrations. With 1.0 mM Ca^{2+} in the medium, low concentrations of ionophore 0.01 and 0.1 $\mu\text{g/ml}$ will stimulate release (Table 1); as the ionophore concentration is increased the stimulatory effect is lost and with further increase of ionophore to 3 and 10 $\mu\text{g/ml}$, glucagon release is depressed. As the ionophore concentration is increased, so Ca^{2+} entry is able to reach excessively high levels and inhibition of release occurs, perhaps by inhibition of key enzyme systems involved in the release process, or in cell metabolism. In this respect these results are similar to those obtained on insulin release [15], when the ionophore in the presence of high medium Ca^{2+} gave less stimulation of release than in the presence of lower Ca^{2+} concentrations. Inhibition of glucose-induced insulin release by high concentrations of Ca^{2+} and A23187 has also been reported by Hellman [23]. This concept, that an increase in the cell cytosol Ca^{2+} concentration causes glucagon release and that excess Ca^{2+} is inhibitory, is further borne out by consideration of the results obtained at different Ca^{2+} concentrations. With a low ionophore concentration (0.1 $\mu\text{g/ml}$), stimulation of release occurs at 0.3 and 1.0 mM Ca^{2+} but is lost at 2.5 mM. With 10 $\mu\text{g/ml}$ A23187, low

Ca^{2+} concentrations of 0.03 mM are effective, whereas 1.0 mM is now inhibitory. This points to the requirement for careful control of intracellular Ca^{2+} in the intact cells. Under our experimental conditions, we presume that 0.1 $\mu\text{g}/\text{ml}$ ionophore is rate limiting, whereas 10 $\mu\text{g}/\text{ml}$ is not. Thus, at the latter concentration 0.03 mM Ca^{2+} is stimulatory. Assuming that 0.03 mM is the maximal concentration that can be achieved in the cell cytosol under these conditions, the resting concentration of free Ca^{2+} in the cell cytosol must obviously be less than this.

Replacement of Ca^{2+} by Mg^{2+} or Sr^{2+} failed to stimulate glucagon release in the presence of the ionophore at concentrations of 24 mM for Mg^{2+} and 3 mM for Sr^{2+} . Thus with respect to Mg^{2+} and Sr^{2+} the effect of Ca^{2+} is clearly specific. No effect of Mg^{2+} was detected and the effect of Sr^{2+} was inhibitory rather than stimulatory to glucagon release.

The situation regarding Ba^{2+} is more complicated. At 0.3 mM Ba^{2+} stimulated glucagon release even in the absence of ionophore, and Ba^{2+} has been shown to directly stimulate release processes in other cells. For instance insulin release [15, 24] and growth hormone release [25, 26] are both stimulated by the presence of Ba^{2+} in the medium. With the ionophore at 0.1 $\mu\text{g}/\text{ml}$ no effect of Ba^{2+} on release was demonstrated and it was suspected that this might be due to the low transport ability of A23187 for this cation. It is known that the transport ability of A23187 for different cations is in the order $\text{Mn}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+} > \text{Sr}^{2+} > \text{Ba}^{2+}$ [27]. Thus with Ba^{2+} at 0.1 mM, a concentration that does not stimulate alone, the ionophore at 10 $\mu\text{g}/\text{ml}$ elicited a stimulation of glucagon release. The mechanism of Ba^{2+} induced release is unknown but it could be by mimicry of Ca^{2+} at its stimulatory site.

The demonstration of calcium induced glucagon release does not conflict with the reported calcium dependency of arginine stimulated glucagon release from the perfused rat pancreas [28]. It might, however, seem to be at variance with reports that Ca^{2+} depletion causes glucagon release in the perfused rat pancreas [29] and the confirmation of this effect reported here. Previous studies on the role of divalent cations in glucagon release in pieces of pancreas from duct-ligated rats have shown that omission of either Ca^{2+} or Mg^{2+} or both ions from the medium caused stimulation of glucagon release, whereas elevation of Ca^{2+} to 4 mM had no effect and elevation of Mg^{2+} to 10 mM was inhibitory [30]. Similarly, injection of CaCl_2 into the pancreatic artery of dogs produced no effect upon glucagon release [31]. In our results a complex pattern of glucagon release in response to different Ca^{2+} concentrations was seen. Thus, in the absence of Ca^{2+} in the medium, release was greater than in the presence of 1.0 mM Ca^{2+} . However, when

high Ca^{2+} concentrations were studied, it was found that 30 mM Ca^{2+} caused a large release of glucagon. Thus the effects of different concentrations of Ca^{2+} are biphasic, both depletion and loading of the cells by Ca^{2+} being stimulatory. If we assume that the ionophore induced secretion process indicates a role for Ca^{2+} in the stimulus-secretion coupling mechanism, as it does in several cell types, it might be concluded that 30 mM Ca^{2+} (in the absence of ionophore) so loads the cell Ca^{2+} stores that saturation occurs and cell cytosol concentrations rise to stimulatory levels. We are currently unable to suggest a mechanism for the increased glucagon secretion observed under the conditions of Ca^{2+} depleted media.

Resolution of the role of Ca^{2+} in the physiological stimulation of glucagon release awaits precise measurements of cytosol Ca^{2+} in the A-cell. This is hampered by the lack of a pure A-cell preparation and even when this is achieved by the current techniques for the measurement of low concentrations of Ca^{2+} ions in cells.

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