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# THE ACTIONS OF ARGININE AND GLUCOSE ON GLUCAGON SECRETION ARE MEDIATED BY OPPOSITE EFFECTS ON CYTOPLASMIC $Ca^{2+}$

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Cytoplasmic $Ca^{2+}$ ($Ca^{2+}_i$) was monitored in single guinea-pig pancreatic $\alpha_2$-cells exposed to modulators of glucagon release. The stimulatory amino acid arginine raised $Ca^{2+}_i$ from 62 to 160 nM, whereas the inhibitor glucose reduced both the latter concentration and basal $Ca^{2+}_i$ by 30 %. Epinephrine which potentiates arginine-stimulated secretion by increasing cAMP, does so without affecting $Ca^{2+}_i$. The results indicate that glucagon secretion is positively modulated by $Ca^{2+}_i$. It is suggested that glucose-induced lowering of $Ca^{2+}_i$ is a fundamental effect in cells where the sugar is readily metabolized. 

Both nutrients and hormones are important physiological regulators of glucagon secretion (1). Whereas amino acids are the major stimuli, the actions of which are potentiated by epinephrine, glucose is an inhibitor. Although there have been divergent opinions about the role of $Ca^{2+}$ in glucagon release (2), recent electro-physiological patch-clamp studies have clearly established that stimulation of secretion is associated with an increased frequency of $Ca^{2+}$-dependent action potentials (3). Moreover, glucagon release can be readily stimulated by the $Ca^{2+}$ ionophore A-23187 (4) and in permeabilized $\alpha_2$-cells by $Ca^{2+}$ itself (5). With the latter approach it was also found that protein kinases dependent on cAMP and phospholipids modulate the secretory activity at a given $Ca^{2+}$ concentration. However, so far nothing is known about the $Ca^{2+}_i$ supposed to control glucagon release. We present here the first measurements of $Ca^{2+}_i$ in the $\alpha_2$-cells. It was found that the stimulatory amino acid arginine raised $Ca^{2+}_i$, the subsequent addition of the potentiator epinephrine had no effect and the inhibitor glucose actually lowered cytoplasmic $Ca^{2+}$.

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**Abbreviations:** $Ca^{2+}_i$, cytoplasmic $Ca^{2+}$ concentration.

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## MATERIALS AND METHODS

Pancreatic islets were isolated by collagenase digestion from the splenic part of the pancreas of 2-4 months old pigmented guinea-pigs. The islets were dispersed into single cells by vigorous shaking in a $Ca^{2+}$-deficient medium (6). The cells were then allowed to become attached to circular 25 mm cover glasses during culture overnight in RPMI 1640 medium supplemented with 10 % NU-SERUM (Collaborative Research Inc., Lexington, MA), 100 IU/ml penicillin, 100 µg/ml streptomycin and 60 µg/ml gentamycin. After rinsing in the medium subsequently used, which was physiologically balanced in cations with $Cl^-$ as the sole anion (7), the attached cells were loaded with fura-2 by incubation for 40 min at 37° C in the presence of 0.5 µM fura-2 acetoxymethylester. The cover glasses were rinsed further and used as the bottom of an open chamber suitable for microscopic work (8). For perifusion two injection needles were inserted on opposite sides of the silicon rubber wall of the chamber. A two-channel peristaltic pump was connected both to the chamber inlet and outlet and the flow (1.2 ml/min) was approximately five times the chamber volume per min. The chamber was placed on the stage of an inverted microscope (Nikon Diaphot) within a climate box and maintained at 37° C by an air-stream incubator. The microscope was equipped for epifluorescence microfluorometry with a 100 W Hg light source, quartz illumination optics and a 100 x UV-fluorite objective. The excitation wavelength was altered manually by changing between 340 and 360 nm interference filters (half-bandwith 1 nm). Excitation intensity was adjusted with neutral density filters. Emitted light was collected through a 500 nm interference filter (half-bandwith 15 nm) and measured with a Nikon DC photometer P1. Single $\alpha_2$-cells were centered within the cell-sized measuring field of the microfluorometer. The $Ca^{2+}$-dependent fluorescence excited at 340 nm, was monitored continuously with frequent checks of the $Ca^{2+}$-independent fluorescence excited at 360 nm. The 340/360 nm fluorescence ratio was used to calculate $Ca^{2+}_i$ as described previously (9), using a $K_D$ for the $Ca^{2+}$-fura-2 complex of 231 nM (10).

Statistical significances were calculated from the differences between paired test and control data using Student's distribution. Each observation represents the mean $Ca^{2+}_i$ data from 3 cells in one animal. The results are given as mean values ± SEM for 5-6 animals.

## RESULTS

When pancreatic $\alpha_2$-cells were exposed to 10 mM arginine there was a rapid rise of $Ca^{2+}_i$ (Fig. 1) from 61.8 ± 6.4 nM to 160.2 ± 14.6 nM within 1 min ($P<0.001$; n=6). The subsequent addition of 20 mM glucose resulted in a slightly slower reduction to 111.7 ± 11.6 nM ($P<0.002$; n=6), and after removal of the test substances there was a return of $Ca^{2+}_i$ to 64.1 ± 6.8 nM ($P<0.001$; n=6). Addition of glucose alone reduced the basal $Ca^{2+}_i$ (Fig. 2) from 75.2 ± 4.9 to 52.4 ± 5.9 nM ($P<0.001$; n=5). 3-O-Methylglucose used as an osmotic control for glucose did not affect $Ca^{2+}_i$ in 4 experiments (data not shown). In the presence of arginine, epinephrine had no effect on $Ca^{2+}_i$ (Fig. 3).

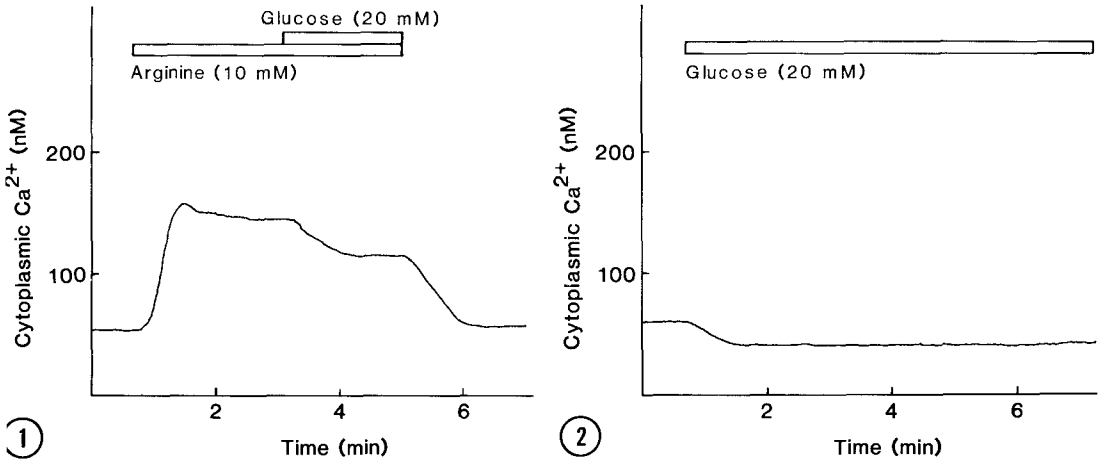


Fig. 1. Effects of arginine and glucose on the cytoplasmic $Ca^{2+}$ concentration of a single guinea-pig $\alpha_2$-cell. The test substances were present during the periods indicated by the horizontal bars. One typical experiment of 18 (3 cells per animal, 6 animals).

Fig. 2. Effect of glucose on the basal cytoplasmic $Ca^{2+}$ concentration of a single guinea-pig $\alpha_2$-cell. The sugar was present during the period indicated by the horizontal bar. One typical experiment of 15 (3 cells per animal, 5 animals).

## DISCUSSION

With the introduction of membrane-permeable esters of fluorescent indicators like quin-2 and fura-2, measurements of $Ca^{2+}_i$ became possible in suspensions of small cells (9). However, such studies were still difficult in pancreatic islet cells because of

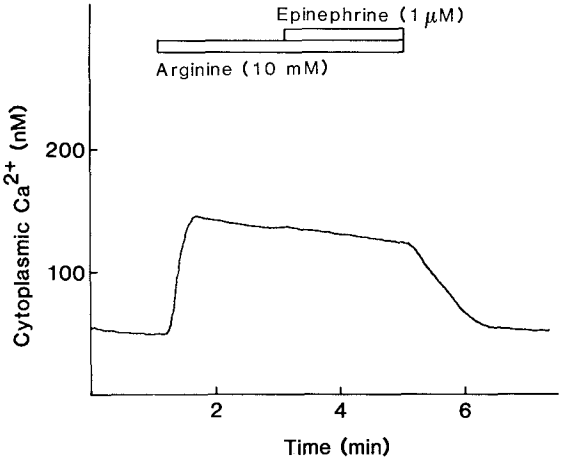


Fig. 3. Effects of arginine and epinephrine on the cytoplasmic $Ca^{2+}$ concentration of a single guinea-pig $\alpha_2$-cell. The test substances were present during the periods indicated by the horizontal bars. One typical experiment of 15 (3 cells per animal, 5 animals).

the limited number of cells available. Taking advantage of the numerous and unusually large ob/ob-mouse islets, which contain more than 90 % ß-cells, studies of how insulin release is related to $Ca^{2+}_i$ became feasible (10-14). The problems associated with similar analyses of glucagon release are considerably greater, since no rich source of $\alpha_2$-cells is known. Pancreatic islets contain only 15-35 % $\alpha_2$-cells (15,16), and even after selective destruction of most of the ß-cells the contribution has been reported to be still below 60 % (15). We have now addressed the problem of measuring $Ca^{2+}_i$ in $\alpha_2$-cells by taking advantage of a microfluorometric system originally developed for analyses of single parathyroid cells (10). Using pancreatic islet cells from the guinea-pig, the $\alpha_2$-cells could be easily identified on the basis of their large size (17). These cells have also a bright appearance in darkfield microscopy and exhibit the same electrophysiological characteristics as rat $\alpha_2$-cells enriched by autofluorescence-activated cell sorting (3,18).

Stimulation of insulin secretion by glucose, sulfonylureas and $K^+$ is associated with a rise of cytoplasmic $Ca^{2+}$ in the pancreatic ß-cells (11,12,14). These actions are mediated by depolarization and influx of $Ca^{2+}$ through voltage-dependent channels. In addition, cAMP appears to sensitize the secretory machinery to the $Ca^{2+}$ signal, since potentiation of glucose-stimulated secretion occurs without an increase of $Ca^{2+}_i$ (13). The present data indicate a similar regulation of glucagon secretion with a rise of $Ca^{2+}_i$ after exposure to the depolarizing stimulator arginine. Moreover, there was no effect of subsequent addition of epinephrine, which potentiates secretion after raising cAMP by a ß-adrenergic mechanism (19).

In the ß-cells the glucose-induced rise of $Ca^{2+}_i$ is preceded by an initial lowering attributed to intracellular sequestration and outward transport (12,14). It was evident from a number of experimental situations that glucose-induced reduction of $Ca^{2+}_i$ in the ß-cell results in inhibition of insulin release (20-23). The glucose-induced lowering of $Ca^{2+}_i$ was even suggested to be a general phenomenon representing a more primitive action of the sugar than its depolarization of the ß-cells (14). With the discovery that glucose lacks effect on membrane potential and electrical activity of the $\alpha_2$-cells, it was attractive to postulate that glucose inhibits glucagon release by lowering $Ca^{2+}_i$ (3). The present data clearly demonstrate that the sugar has such an effect. Whereas

both the pancreatic $\alpha_2$- and ß-cells respond to glucose with initial decrease of $Ca^{2+}_i$, only the ß-cells will be depolarized, resulting in a subsequent rise of cytoplasmic $Ca^{2+}$. Further studies should clarify whether there is a functionally important reduction of $Ca^{2+}_i$ also in other cells rapidly metabolizing glucose or other nutrients. The somatostatin-producing $\alpha_1$-cell is another type of islet cell which may belong to this category. In stimulating the release of somatostatin (24), glucose can even be expected to have dual effects on $Ca^{2+}_i$ as observed in ß-cells.

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**REFERENCES**

1. Pipeleers, D.G., Schuit, F.C., Van Schravendijk, F.H., and Van De Winkel, M. (1985) Endocrinology **117**, 817-823.
2. Leclercq-Meyer, V., and Malaisse, W.J. (1983) In Handbook of Experimental Pharmacology (P.J. Lefebvre, Ed.) Vol 66/II pp. 59-74. Springer Verlag, Berlin.
3. Rorsman, P., and Hellman, B. (1987) Biophys. J. (in press).
4. Hii, C.S.T., and Howell, S.L. (1987) Mol. Cell. Endocrionol. **50**, 37-44.
5. Niki, I., Tamagawa, T., Niki, A., Niki, H., Koide, T., and Sakamoto, N. (1986) Biomed. Res. **7**, 291-294.
6. Lernmark, Å. (1974) Diabetologia **10**, 431-438.
7. Hellman, B. (1975) Endocrinology **97**, 392-398.
8. Sykes, J.A., and Moore, E.B. (1959) Proc. Soc. Exp. Biol. Med. **100**, 125-127.
9. Grynkiewicz, G., Poenie, M., and Tsien, R.Y. (1985) J. Biol. Chem. **260**, 3440-3450.
10. Johansson, H., Larsson, R., Nygren, P., Lindh, E., Rastad, J., Åkerström, G., and Gylfe, E. (1987) submitted.
11. Rorsman, P., Abrahamsson, H., Gylfe, E., and Hellman, B. (1984) FEBS Lett. **170**, 196-200.
12. Abrahamsson, H., Berggren, P.-O., and Rorsman, P. (1985) FEBS Lett. **190**,21-24.
13. Rorsman, P., and Abrahamsson, H. (1985) Acta Physiol. Scand. **125**, 639-647.
14. Hellman, B., Gylfe, E., Bergsten, P., Johansson, H., and Wesslén, N. (1987) In Pathogenesis of Non-Insulin Dependent Diabetes Mellitus (V. Grill and S. Efendic, Eds.) Raven Press, New York, (in press).
15. Petersson, B., Hellerström, C., and Gunnarsson, R. (1970) Horm. Metab. Res. **2**, 313-317.
16. Alm, G., and Hellman, B. (1964) Acta Endocrinol. **46**, 307-316.
17. Petersson, B. (1966) Acta Endocrinol. **53**, 480-488.
18. Wesslén, N., Pipeleers, D., Van De Winkel, M., Rorsman, P., and Hellman, B. (1987) Acta Physiol. Scand. (in press).

19. Schuit, F.C., and Pipeleers, D.G. (1986) Science **232**, 875-877.
20. Hellman, B., Honkanen, T., and Gylfe, E. (1982) FEBS Lett. **148**, 289-292.
21. Hellman, B., and Gylfe, E. (1984) Quart. J. Exp. Physiol. **69**, 867-874.
22. Bergsten, P., and Hellman, B. (1986) Biochem. Biophys. Res. Commun. **125**, 875-881.
23. Bergsten, P., and Hellman, B. (1986) Biochem. Biophys. Res. Commun. **139**, 557-563.
24. Efendic, S., Enzmann, F., Nylén, A., Uvnäs-Wallensten, K., and Luft, R. (1979) Proc. Natl. Acad. Sci. USA **76**, 5901-5904.