Endocrine, *vol. 10, no. 1, 1–6, February 1999* 0969–711X/99/10:1–6/$11.50 © 1999 by Humana Press Inc. All rights of any nature whatsoever reserved.

Review

Abnormal Cell Calcium Homeostasis in Type 2 Diabetes Mellitus

A New Look on Old Disease

Joseph Levy

Department of Medicine, Diabetes Section, Wayne State University School of Medicine, Detroit, MI

Cumulative evidence reveals that diabetes is a condition in which cell Ca^{2+} homeostasis is impaired. Defects in cell Ca^{2+} regulation were found in erythrocytes, cardiac muscle, platelets, skeletal muscle, kidney, aorta, adipocytes, liver, osteoblasts, arteries, lens, peripheral nerves, brain synaptosomes, retinal tissue, and pancreatic β cells, confirming that this defect in cell Ca^{2+} metabolism is a basic pathology associated with the diabetic state. Though different defects in a variety of functions that regulate cell Ca^{2+} homeostasis were described in diabetes, the most common finding is an increase in $[Ca^{2+}]_i$ levels. However, it is not clear whether the defect in cell Ca^{2+} metabolism in diabetes precedes or succeeds the overt diabetic condition. It is also not clear which of the multiple functions involved in cell Ca^{2+} regulation has the primary defect.

Defects in cell Ca^{2+} metabolism may be significant for the observed pathologies in insulin secretion and insulin action in diabetes. They may also play an important role in the vascular complications seen in this condition, such as hypertension, atherosclerosis, and microangiopathy. Therefore, better understanding of the impairment in cell Ca^{2+} metabolism in diabetes may markedly enhance our understanding of this condition.

Key Words: Type 2 diabetes; cellular calcium; insulin secretion; insulin action; complications.

Introduction

Type 2 diabetes mellitus is a systemic disease characterized by a significant level of insulin resistance, varying degrees of insulin deficiency, and a wide spectrum of micro- and macrovascular, neurologic, ocular, and cardiac complications *(1)*. It is because of this wide diversity in the manifestations of the diabetes syndrome that it has been considered a phenotypic heterogeneous condition. However, cumulative evidence reveals that abnormal-cell Ca^{2+} ($[Ca^{2+}]_i$) homeostasis is a ubiquitous finding common to all tissues of animal and human diabetes. Consequently, abnormalities in cell Ca^{2+} metabolism may be a basic pathology in type 2 diabetes and may be of significance for many of the manifestations of the disease *(2)*. These defects in cell Ca^{2+} regulation may be of significance for the impaired insulin secretion, and the impaired insulin action seen in type 2 diabetes, and may play an important role in the associated vascular complications, such as atherosclerosis and hypertension, as well as in the pathogenesis of some of the microvascular, ocular, and neurologic complications. Therefore, changes in cell Ca^{2+} homeostasis could be a common pathology that could link between most of the diverse features of the type 2 diabetes syndrome *(2–5)*.

Received September 28, 1998; Accepted October 5, 1998.

Author to whom all correspondence and reprint requests should be addressed: Dr. Joseph Levy, Division of Endocrinology and Metabolism, 4201 St. Antoine, University Health Center-4H, Detroit, MI 48201; E-mail: jlevy@oncgate.roc.wayne.edu

Cell Ca^{2+} Regulation

Most tissues share some common mechanisms for the maintenance of $[Ca^{2+}]_i$ homeostasis, although there is also a tissue specificity *(2,6–8)*. $[Ca^{2+}]_i$ is maintained at a level that is 10^4 times lower than the extracellular Ca^{2+} levels by a complex, well-orchestrated interplay among Ca^{2+} transporters responsible for Ca^{2+} extrusion out of the cell, the Na^+/Ca^{2+} exchanger, the calmodulin- (CaM) dependent Ca^{2+}-ATPase, and the different Ca^{2+} channels that regulate Ca^{2+} entry into the cell *(9–13)*. In the cell, Ca^{2+} is stored mainly in organelles, the endoplasmic sarcoplasmic reticulum (ER/SR), and the mitochondria *(13)*. The ER is more active than the mitochondria in $[Ca^{2+}]_i$ regulation *(10)*. Ca^{2+} release from the ER can be mediated by inositol triphosphate (Ip_3), by Ca^{2+}-mediated Ca^{2+} release via ryanodine receptors *(9–13)*, and by cyclic adenosine triphosphate-ribose (cADP-ribose) *(14)*. Entry of Ca^{2+} into the ER is

mediated by a CaM-independent Ca^{2+}-ATPase *(9–13)*. Ca^{2+} flooding into the cytoplasm, secondary to its release from cellular stores, is a mediator in a panoply of cellular responses *(12)*. Then this excess Ca^{2+} is pumped out of the cell and back into the ER by the Ca^{2+}-ATPase pumps. Because repeated such cycles may deplete the Ca^{2+} stores to replenish the ER, Ca^{2+}-specific Ca^{2+} channels in the plasma membrane, open to allow a small amount of Ca^{2+} to enter the cell and to be pumped into the Ca^{2+} stores. A Ca^{2+} influx factor and the cytoplasmic Ca^{2+} levels determine the rate of this Ca^{2+} entry *(10,12)*. Additional mechanisms that regulate $[Ca^{2+}]_i$ include those that affect $[Na^+]_i$ such as the $(Na^+ + K^+)$-ATPase and the Na^+/H^+ exchanger *(15)*. Increase or decrease in $[Na^+]_i$ will inhibit or stimulate the Na^+/Ca^{2+} exchanger and increase or decrease $[Ca^{2+}]_i$ levels. Consequently, abnormalities in any of the above mechanisms could disrupt $[Ca^{2+}]_i$ homeostasis *(2)* and may impair Ca^{2+}-regulated ligand functions. It is important to emphasize, however, that the calcium signal is very specific to the particular stimuli involved. This specificity is achieved since two factors determine the "calcium language": (1) the amplitude of the change in $[Ca^{2+}]_i$ level, and (2) the distance that this change spreads. These two determinants create a specific pattern of change in the level and distribution of the cation in the cell, and make each Ca^{2+} signal unique to the specific stimuli *(10,13,16)*.

Abnormal-Cell Ca^{2+} Regulation in Diabetes

Although $[Ca^{2+}]_i$ is important for vital functions in all cells, some of its effects are tissue-specific, such as contraction in muscle and secretion in glands *(2–5)*. Consequently, defects in $[Ca^{2+}]_i$ homeostasis might be expressed differently in the different tissues involved. Further, since the mechanisms that regulate $[Ca^{2+}]_i$ are tightly interrelated, a defect in one mechanism may impair the function of the other mechanisms, making the identification of the primary defect a difficult task. Indeed, various abnormalities in $[Ca^{2+}]_i$ regulation were described in animal models of diabetes and obesity and in obese and diabetic patients *(2)*. These include pathologies in function of the plasma membrane and the ER/SR Ca^{2+}-ATPase, $(Na^+ + K^+)$-ATPase, Na^+-Ca^{2+} exchanger, Ca^{2+}-induced Ca^{2+} release from the ER, the tissue total calcium content, and the actual $[Ca^{2+}]_i$ levels *(2,17)*. Defects in cell Ca^{2+} homeostasis were described in erythrocytes, cardiac muscle, platelets, skeletal muscle, kidney, aorta, adipocytes, liver, osteoblasts, arteries, lens, peripheral nerves, brain synaptosomes, retinal tissue, and in pancreatic β cells *(2,17–21)*. However, there is marked variability with regard to the described specific defect in each of the $[Ca^{2+}]_i$ regulatory functions. For example, basal $[Ca^{2+}]_i$ levels have been described to be high, low, or normal in cardiomyocytes in diabetes *(22–25)*. Similarly, basal activity of the plasma membrane Ca^{2+}-ATPase was described to be high, low, or normal *(26–28)*. However, even when basal functions were normal, dynamic studies that tested responses of the $[Ca^{2+}]_i$ regulatory function to an agonist were abnormal *(22,26–28)*, suggesting that dynamic studies might be more sensitive in identifying early defects in cell Ca^{2+} homeostasis in diabetes and obesity. Since the functional state of the $[Ca^{2+}]_i$ regulatory mechanisms, such as the ATPases, depends on many factors, such as the membrane phospholipid content, level of Mg^{2+}, free fatty acids, cholesterol, and glucose *(29–33)*, and these conditions were not standardized in the different studies, it is not surprising that what sometimes seem to be conflicting results were obtained in the different reports. However, the most frequently described defect in cell $[Ca^{2+}]_i$ homeostasis in diabetes and obesity is an increase in the level of $[Ca^{2+}]_i$ *(2)*.

Impaired-Cell Ca^{2+} Homeostasis and Insulin Secretion

Thus far, defects in β-cell $[Ca^{2+}]_i$ homeostasis were described only in animal models of diabetes and in islet cells exposed to high glucose for a long time, but it is likely that similar defects occur also in human diabetes. Islets from db/db mice lack the initial reduction of $[Ca^{2+}]_i$ and the subsequent Ca^{2+} oscillations in response to glucose *(17)*, and islets from neonatal induced type 2 diabetic rats who lack the insulin response to glucose, but respond to amino acid stimulation *(34)* have selective defect in the increase of $[Ca^{2+}]_i$ in response to glucose *(35)*. Also the L-type voltage-dependent Ca^{2+} channel activities are increased in β cells from these type 2 diabetic rats *(36)*, and their Ca^{2+}-ATPase activity is decreased *(21)*. Since oscillations of $[Ca^{2+}]_i$ are important for the pulsatile insulin secretion, loss of these oscillations may play a significant role in the loss of pulsatile insulin secretion seen early in type 2 diabetes *(37–39)*. Finally, there is evidence that the "glucose toxicity," which selectively impairs insulin secretion in response to glucose (and may play a significant role in the β-cell dysfunction in diabetes) may be mediated in part by a glucose-induced impairment in the β cells' $[Ca^{2+}]_i$ homeostasis *(6,40)*. Culture of rat islet cells in media that contain high-glucose concentration cause a progressive, dose- and time-dependent decrease in the membrane Ca^{2+}-ATPase activity of the islet cells *(21)*. In turn, the significance of the Ca^{2+}-ATPase for normal insulin secretion has recently been reviewed *(41)*.

Impaired-Cell Ca^{2+} Regulation and Glucose Homeostasis

Glucose homeostasis is determined mainly by the rate of glycolysis, gluconeogenesis, glycogen synthesis, and glycogenolysis. All these pathways are Ca^{2+}-regulated *(9,42)* and may be affected by abnormal $[Ca^{2+}]_i$. The key enzyme in glycolysis, phosphofructokinase (PFK), becomes activated when it is bound to the cytoskeleton. This is a Ca^{2+}- and

CaM-regulated process, and high $[Ca^{2+}]_i$ inhibits the PFK binding and decreases glycolysis *(42)*. Abnormal $[Ca^{2+}]_i$ may also affect gluconeogenesis, since an initial transient rise in $[Ca^{2+}]_i$ initiates hepatic gluconeogenesis from glutamine *(43)*. Finally, glycogen synthase, a key enzyme for glycogen synthesis, is inactivated in its phosphorylated form, and high $[Ca^{2+}]_i$ inhibits the dephosphorylation of the enzyme *(44)*. In contrast, the glycogen phosphorylase, a key enzyme for glycogenolysis, is activated in its phosphorylated state. Binding of Ca^{2+} to the calmodulin subunit of phosphorylase kinase activates the kinase, leads to the phosphorylation of glycogen synthase and glycogen phosphorylase, and thus leads to a simultaneous decrease in glycogen synthesis and an increase in glycogenolysis *(9,44)*. In diabetes in which increased $[Ca^{2+}]_i$ is the most common defect in $[Ca^{2+}]_i$ homeostasis, glycogen synthase is inhibited, and neither insulin nor glucose stimulates the enzyme in a physiologic manner, causing glucose resistance in addition to the insulin resistance *(2)*. In turn, glycogenolysis is facilitated *(9,44)*. Finally, high $[Ca^{2+}]_i$ may affect also glucose transport by decreasing the intrinsic activity of the Glut 4 transporter *(45)*.

Impaired-Cell Ca^{2+} Homeostasis and Insulin Action

Several lines of evidence suggest that insulin action is mediated in part by $[Ca^{2+}]_i$. Insulin may increase or decrease $[Ca^{2+}]_i$ in its target cells, and there is an optimal range of $[Ca^{2+}]_i$ levels for insulin action, whereas chelation of $[Ca^{2+}]_i$ inhibits insulin bioeffects *(5,46–48)*. Ca^{2+} is required for the internalization of the insulin receptor *(49)*, and high $[Ca^{2+}]_i$ inhibits dephosphorylation of the insulin receptor *(44)*. Insulin high-affinity binding and insulin sensitivity are also increased by Ca^{2+} binding to the insulin receptor *(50)*. Ca^{2+} also affects postbinding insulin actions *(50)*. Insulin phosphorylates and stimulates the membrane Ca^{2+}-ATPase in a dose-dependent manner and anti-Ca^{2+}-ATPase monoclonal antibodies (MAbs), which block insulin regulation of the ATPase, decrease insulin bioeffects *(46,51,52)*. Insulin also regulates CaM gene expression *(53)*.

In conditions of insulin resistance, such as diabetes, obesity, and aging, the insulin effect on $[Ca^{2+}]_i$ homeostasis is impaired *(2,54)*. Insulin loses its ability to increase $[Ca^{2+}]_i$ in fat cells from obese or aged rats *(5)*, and to regulate the Ca^{2+}-ATPase in tissues from type 2 diabetes and obesity animal models *(27,28)*. Also, improvement in insulin action is associated with regained ability of insulin to regulate the ATPase *(55)*. Thus, the abnormal $[Ca^{2+}]_i$ regulation seen in obesity and aging *(2,56)* may contribute to the increased incidence of type 2 diabetes in obesity and with age. Insulin was found to induce Ca^{2+} transport in vascular smooth muscle cells, and this effect is impaired in hypertension and diabetes *(57)*. Insulin also induces Ca^{2+} transport in liposomes *(58)*, and attenuates the Ca^{2+} and contractile response of vascular smooth muscle to a number of agonists, including endothelin *(59)*. Thus, insulin regulation of cell Ca^{2+} metabolism is of significance for vascular physiology.

Impaired-Cell Ca^{2+} Homeostasis and Cardiovascular Disease

Recent evidence suggests that cell Ca^{2+} overload may contribute to the pathogenesis of hypertension, atherosclerosis, and cardiomyopathy *(2,24,60–65)*. Therefore, the commonly observed increased $[Ca^{2+}]_i$ in diabetes may explain, at least in part, the increased incidence of these complications in the diabetes syndrome *(2)*. Diabetes is characterized by increased vascular reactivity *(66)*. Though the exact mechanisms of this pathology are not clear, abnormal $[Ca^{2+}]_i$ in the smooth muscle cells was suggested as an explanation by several investigators *(67–70)*. It has been shown that changes in Ca^{2+}-ATPase activity and in L-type Ca^{2+} channels occur in cardiac tissue in diabetes *(70–72)*. These changes were considered to be of significance for the development of cardiomyopathy in this condition *(72,73)*.

Atherosclerosis is characterized by increased endothelial permeability, monocyte infiltration, intimal smooth muscle cell proliferation, platelet aggregation, and accumulation of lipids, calcium, and extracellular matrix components in the vessel wall *(74–76)*. Although the precise mechanisms for these pathologies have yet to be elucidated, it was suggested that these changes depend heavily on abnormal cell calcium metabolism *(74–77)*. Evidence also exists for a significant interrelationship between the effects of cholesterol and cell Ca^{2+} metabolism to accelerate the atherosclerosis process *(32,78,79)*.

Impaired-Cell Ca^{2+} Homeostasis and Microvascular Disease

Diabetic microangiopathy, a generalized pathology in diabetes that can affect all tissues *(80)*, is characterized by basement membrane thickening and increased capillary permeability *(81–85)*. Abnormal-cell Ca^{2+} homeostasis may contribute to both components of this pathology.

The basement membrane is composed of laminin, fibronectin, entactin, and proteoglycans *(2,86–89)*, which are synthesized and secreted by the endothelial cells *(90,91)*. Since both these processes, the synthesis and the secretion, are Ca^{2+}-regulated *(92,93)*, increased $[Ca^{2+}]_i$, as seen in diabetes, can affect the production and secretion of these proteins, leading to the observed changes in basement membrane composition and width *(2,85,94)*. Also, the increased Ca^{2+} binding to the basement membrane, as is seen in diabetes, can increase laminin aggregation and the formation of laminin–entactin complexes, and thus, change the basement membrane structure *(95)*. These changes in the laminin metabolism can also impair the proteolitic digestion of the protein and may aggravate the effect of

glucose to impede the degradation of the basal membrane proteins, further contributing to its increased width *(2,96)*.

Changes in $[Ca^{2+}]_i$ may also increase the porosity of the capillaries *(2)* by enhancing the contraction of the endothelial cells *(96–98)*. Adjacent endothelial cells contracting away from each other increase the porosity of the capillaries *(97,99)*.

The Cause for the Abnormal-Cell Ca^{2+} Regulation in Diabetes

There are no available data to determine whether the impairment in $[Ca^{2+}]_i$ regulation is a primary defect in diabetes. This issue becomes further complicated by observations that high glucose *per se* can impair $[Ca^{2+}]_i$ regulation. It inhibits Ca^{2+}-ATPase activity in erythrocytes and pancreatic islet cells, decreases Ca^{2+} uptake by vascular smooth muscle cells, and inhibits the Ca^{2+} signal in kidney tissue *(21,40–42)*. Other metabolites, such as free fatty acids, triglycerides, and cholesterol, can also affect Ca^{2+}-ATPase activity *(2,46)*. Thus, a vicious cycle may be created in which abnormal $[Ca^{2+}]_i$ may contribute to the impaired carbohydrate tolerance, whereas the impaired carbohydrate tolerance can further impair $[Ca^{2+}]_i$ homeostasis. One approach to resolving the question of which defect is the primary one is to conduct studies that will evaluate cell Ca^{2+} homeostasis in populations that are genetically at increased risk to develop diabetes-like siblings of diabetic parents before the appearance of overt diabetes. Another approach is to conduct studies on cultured cells obtained from tissues of type 2 diabetic patients and, thus, to eliminate the potential effect of the diabetic metabolic milieu on cell Ca^{2+} metabolism. Such studies might help to answer the question of whether the defects in cell Ca^{2+} metabolism precede or succeed the diabetes state. It will also help to determine whether the abnormalities in cell Ca^{2+} in diabetes are genetically determined. However, there are still several questions that must be addressed first.

1. Which tissue may be the best representative for such studies?
2. Which function in cell Ca^{2+} homeostasis should be studied first?
3. Should these studies be performed by investigating abnormalities in functional mechanisms of cell Ca^{2+} homeostasis (such as enzyme activities or Ca^{2+} transport) or by evaluating specific genes with a key role in maintaining cell Ca^{2+} homeostasis, like ATPase genes.

However, these kinds of investigations are further complicated by the fact that abnormal-cell Ca^{2+} homeostasis is observed in a variety of other common conditions, such as aging *(5,56)*, hypertension *(70)*, and obesity *(28)*, which are also associated with impaired insulin action/insulin resistance even in the absence of diabetes. However, what is unique to the diabetes condition and differentiates this condition from obesity or hypertension is the decrease in function of the pancreatic β cells. Therefore, the specific defect in cell Ca^{2+} homeostasis in this tissue may better represent the defect in cell Ca^{2+} homeostasis in diabetes, which might be different from that seen in obesity or hypertension. Understanding this defect may enable us to understand the key pathology in cell Ca^{2+} homeostasis in diabetes. This defect might be different from the initial defect in cell Ca^{2+} homeostasis seen in the other conditions (obesity, hypertension, aging), even though in all of them the end result is an impairment in cell Ca^{2+} homeostasis and increased $[Ca^{2+}]_i$ levels.

Since it is almost impossible to carry out studies on pancreatic β cells in large populations, we first have to determine which of the more easily accessible cells, like adipocytes, fibroblasts, or blood elements, can best represent the pancreatic β cells. This task is not easy, since so far different pathologies in different functions of cell Ca^{2+} homeostasis were described in diabetes, and tissue specificity exists.

Thus, it seems that although it is clear that cell Ca^{2+} homeostasis is impaired in diabetes, our current knowledge of the nature of the abnormalities in cell Ca^{2+} homeostasis is in its infancy. However, the significance of better understanding of this pathology might prove to be of extreme significance.

Summary

Evidence reveals that impaired cell Ca^{2+} homeostasis is a ubiquitous finding in the diabetes syndrome. It may cause or contribute to the known defects in insulin secretion, insulin action, and most of the vascular and other complications seen in the diabetes syndrome. It is further aggravated by the hyperglycemia and other metabolic abnormalities seen in diabetes. However, the nature of the primary defect in cell Ca^{2+} homeostasis and whether it is acquired or genetically determined is still unknown.

Acknowledgments

This work was supported by the National American Diabetes Association Research Grant. The excellent secretarial help of Eleanor May is appreciated.

References

1. Weir, G. C. (1982). *Am. J. Med.* **73,** 461–464.
2. Levy, J., Gavin, J. R., III, and Sowers, J. R. (1994). *Am. J. Med.* **96,** 260–273.
3. Levy, J., Zemel, M. B., and Sowers, J. R. (1989). *Am. J. Med.* **87(6A Suppl.),** 7–15.
4. Levy, J. and Sowers, J. R. (1994). In: Piero, P. F. and Walsh, M. F. (eds.). *Endocrinology and Metabolism*, vol 6, Springer-Verlag: New York, NY, pp. 116–136.
5. Draznin, B. (1988). *Am. J. Med.* **85(Suppl. 5A),** 44–58.
6. Okamoto, Y., Ishida, H., Taminato, T., Tsugi, K., Kurose, T., Tsuura, Y., et al. (1992). *Diabetes* **41,** 1555–1561.
7. Rasmussen, H. (1989). *Bone Miner.* **5,** 233–248.
8. Galione, A. (1993). *Science* **229,** 325–326.
9. Rasmussen, H. (1986). *N. Engl. J. Med.* **314,** 1094–1011, 1164–1170.

10. Petersen, O. H., Petersen, C. C. H., and Kasai, H. (1994) *Annu. Rev. Physiol.* **56,** 297–319.
11. Berridge, M. J. (1993). *Nature* **361,** 315–325.
12. Clapham, D. E. (1993). *Nature* **364,** 763,764.
13. Logan, A. (1993). *Lancet* **341,** 1248.
14. Takasawa, S., Naja, K., Annexure, H., and Oxamide, H. (1993). *Science* **259,** 370–373.
15. Aviv, A. (1996). *Curr. Opinion Cardiol.* **11,** 477–482.
16. Allbritton, N. L., Meyer, T., and Stryer, L. (1992). *Science* **258,** 1812–1815.
17. Roe, M. W., Philipson, L. H., Frangakis, C. J., Kuznestov, A., Mertz, R. J., Lancaster, M. E., et al. (1994). *J. Biol. Chem.* **269,** 18,279–18,282.
18. Janicki, P. K., Horn, J. L., Singh, G., Franks, W. T., and Franks, J. J. (1994). *Life Sci.* **55,** 359–364.
19. Tomizawa, H., Yamazaki, M., Kunika, K., Itakura, M., and Yamashita, K. (1993). *Diab. Res. Clin. Pract.* **19,** 1–8.
20. Kern, T. S., Kowluru, R. A., and Engerman, R. L. (1994). *Invest. Ophthalmol. Vis. Sci.* **35,** 2962–2967.
21. Levy, J., Zhu, Z., and Dunbar, J. (1998). *Metabolism* **47,** 185–189.
22. Yu, Z., Quamme, G. A., and McNeill, J. H. (1994). *Am. J. Physiol.* **266,** H2334–H2342.
23. Yu, J. Z., Rodrigues, B., and McNeil, J. H. (1997). *Cardiovasc. Res.* **34,** 91–98.
24. Schaffer, S. W. and Mozaffari, M. (1996) *Coronary Artery Dis.* **7,** 109–115.
25. Hayashi, H. and Noda, N. (1997). *Cardiovasc. Res.* **34,** 99–103.
26. Levy, J., Rempinski, D., and Kuo, T. H. (1994). *Metabolism* **43,** 604–613.
27. Levy, J. and Rempinski, D. (1994). *Metabolism* **43,** 1055–1061.
28. Levy, J. and Gavin, J. R., II (1995). *Int. J. Obesity* **19,** 97–102.
29. Gonzalez Flecha, F. L., Bermudez, M. C., Cedola, N. V., Gag Liardio, J. J., and Rossi, J. P. F. C. (1990). *Diabetes* **39,** 704–711.
30. Williams, R. and Schrier, R. W. (1993). *Kidney Int.* **44,** 344–351.
31. Mene, P., Pugliese, G., Pricci, F., DiMario, U., Cinotti, G. A., and Pugliese, F. (1993). *Kidney Int.* **43,** 585–591.
32. Bialecki, R. A., Tulenko, T. N., and Colucci, W. S. (1991). *J. Clin. Invest.* **86,** 1894–1900.
33. Levy, J., Gavin, J. R., III, Grunberger, G., and Abioli, L. V. (1988). *Diabetologia* **31,** 315–321.
34. Giroix, M. H., Portha, B., Kerogoat, M., Bailbe, D., and Picon, L. (1983). *Diabetes* **32,** 445–451.
35. Tsuji, K., Taminato, T., Ishida, H., Okamota, Y., Tsuura, Y., Kato, S., et al. (1993). *Metabolism* **42,** 1424–1428.
36. Kato, S., Ishida, H., Tsuura, Y., Okamoto, Y., Tsuji, K., Horie, M., et al. (1994). *Metabolism* **43,** 1395–1400.
37. Leahy, J. L. (1990). *Diabetes Care* **13,** 992–1010.
38. Juntti-Berggren, L., Larson, O., Rorsman, P., Ammala, C., Bokvist, K., Vahlander, K., et al. (1993) *Science* **261,** 86–90.
39. Hellman, B., Gylfe, E., Bergsten, P., Grapengiesser, E., Lund, P. E., Berts, A., et al. (1994). *Diabetologia* **37(2 Suppl.),** S11–S20.
40. Dunbar, J. C., Houser, F., and Levy, J. (1989). *Diab. Res. Clin. Pract.* **7,** 187–196.
41. Gagliardino, J. J. and Rossi, J. P. (1994). *Diab. Metab. Rev.* **10,** 1–17.
42. Reitner, R. (1993). *Int. J. Biochem.* **25,** 297–305.
43. Koike, M., Kashiwagura, T., and Takeguchi, N. (1992). *Biochem. J.* **283,** 265–272.
44. Begum, N., Sussman, K. E., and Draznin, B. (1991). *Cell Calcium* **12,** 423–430.
45. Begum, N., Leitner, W., Reusch, J. E. B., Sussman, K. E., and Draznin, B. (1993). *J. Biol. Chem.* **268,** 3352–3356.
46. Levy, J., Gavin, J. R., Morimoto, S., and Avioli, L. V. (1986). *Diabetes* **35,** 899–905.
47. Pershadsingh, H. A., Shade, D. L., Delfert, D. M., and McDonald, J. M. (1987). *Proc. Natl. Acad. Sci. USA* **84,** 1025–1029.
48. Draznin, B., Kao, M., and Sussman, K. E. (1986). *Diabetes* **36,** 174–178.
49. Carpentier, J. L., Gorden, P., and Lew, D. P. (1992). *Exp. Cell. Res.* **198,** 144–149.
50. Williams, P. F., Caterson, I. D., Cooney, G. J., Zilkens, R. R., and Turtle, J. R. (1990). *Calcium* **11,** 547–556.
51. Levy, J. and Rempinski, D. (1992). Inhibition of insulin bioeffects in adipocytes by monoclonal antibodies to the membrane (Ca^{2+}+Mg^{2+})-adenosine triphosphatase. Proceedings of the 74th Annual Meeting the Endocrine Society, San Antonio, TX, June 24–27:22 (Abstract).
52. Levy, J. and Rempinski, D. (1992). Insulin phosphorylates the membrane (Ca^{2+}+Mg^{2+})-adenosine triphosphatase. Proceedings of the 74th Annual Meeting the Endocrine Society, San Antonio TX, June 24–27:22 (Abstract).
53. Solomon, S. S., Palazzolo, M. R., Elam, M. B., Green, S., and Raghow, R. (1994). *J. Lab. Clin. Med.* **124,** 348–358.
54. Ferrannini, E., Buzzigoli, G., Bonadonna, R., Giorico, M. A., Oleggini, M., Graziade, L., et al. (1987). *N. Engl. J. Med.* **317,** 350–357.
55. Levy, J., Grunberger, G., Karl, I., and Gavin, J. R., III. (1990). *Metabolism* **39,** 25–33.
56. Draznin, B. (1993). *Am. J. Kidney Disease* **21(6 Suppl.),** 32–38.
57. Touyz, R. M. and Schiffrim, E. L. (1994). *Hypertension* **23,** 931–935.
58. Brimble, K. S. and Ananthanarayanan, V. S. (1992). *Biochim. Biophys. Aeta* **1105,** 319–327.
59. Dick, G. M. and Sturek, M. (1996). *Diabetes* **45,** 876–880.
60. Gonzalez, J. M. and Suki, W. N. (1995). *Semin. Nephrol.* **15,** 564–568.
61. Oshima, T. and Toung, E. W. (1995). *Semin. Nephrol.* **15,** 496–503.
62. Paoletti, R., Corsini, A., Soma, M. R., and Bernini, F. (1996). *Blood Pressure Suppl.* **4,** 12–15.
63. Tulenko, T. N., Laury-Kleintop, L., Walter, M. F., and Mason, R. P. (1997). *Int. J. Cardiol.* **(62 Suppl.) 2,** S55–S66.
64. Demer, L. L. (1995). *Circulation* **92,** 2029–2032.
65. Chang, K. C., Screur, H., Weiner, M. W., and Camacho, S. A. (1996). *Am. J. Physiol.* **271,** H228–H234.
66. Christlieb, A. R., Janka, H. U., Kraus, B., Gleason, R. E., Icasas-Cabral, E. A., Aiello, L. M., et al. (1976). *Diabetes* **25,** 268–274.
67. Dandona, P., James, I. M., and Beckett, A. G. (1981). *Lancet* **2,** 694.
68. Rhie, F. H., Christlieb, A. R., Sandor, T., Gleason, R. E., Rand, L. I., Shah S. T., et al. (1982). *Diabetes* **31,** 1056–1060.
69. Sowers, J. R. Khoury, S., Standley, P., Zemel, P., and Zemel, M. (1991). *Am. J. Hypertens.* **4,** 177–182.
70. Orlov, S. N., Li, J. M., Tremblay, J., and Hanet, P. (1995). *Semin. Nephrol.* **15,** 569–592.
71. Borda, E. Pascual, J., Wald, M., and Sterin-Borda, L. (1988). *Can. J. Cardiol.* **4,** 57–101.
72. Nobe, S., Aomine, M., Artia, M., Ito, S., and Takaki, R. (1990). *Cardiovasc. Res.* **24,** 381–389.
73. Schaffer, S. W. (1991). *Mol. Cell. Biochem.* **107,** 1–20.
74. Strickberger, S. A., Russek, L. N., and Phair, R. D. (1988). *Circ. Res.* **62,** 75–80.
75. Ross, R. and Glomset, J. A. (1976). *N. Engl. J. Med.* **295,** 420–425.
76. Orimo, H. and Ouchi, Y. (1990). *Ann. NY Acad. Sci.* **598,** 444–457.
77. Shi, H., Severs, N. J., and Robenek, H. (1996). *FASEB J.* **10,** 491–501.
78. Wells, K. E., Miguel, R., and Alexander, J. J. (1997). *J. Surg. Res.* **67,** 126–131.
79. Jerome, D. S., Clifford, L. D., Fromm, B. S., Marsh, J. D., and Levy, J. (1996). *J. Bone Min. Res.* **II,** 260 (Abstract).
80. Johnson, P. C., Bendel, K., and Meezan, E. (1982). *Arch. Pathol. Lab. Med.* **106,** 214–217.

81. Deckret, T., Feldt-Rasmussen, B. F., Borch-Johnson, T., and Kofoed-Enevoldsen, A. (1989). *Diabetologia* **32,** 219–226.
82. Boshell, B. R. and Austria, M. J. (1989). *Diabetic Complications* **3,** 183–186.
83. Williamson, J. R., Tilton, R. G., Chang, K., and Kilo, C. (1982). *Arch. Pathol. Lab. Med.* **106,** 214–217.
84. Siperstein, M. D. (1972). *Adv. Intern. Med.* **18,** 325–344.
85. Barnett, A. H. (1991). *Am. J. Med.* **90(6A Suppl.),** 67S–73S.
86. Yurchenco, P. D. and Schittny, J. C. (1990). *FASEB J.* **4,** 1577–1590.
87. Roy, S., Sala, R., Cagliero, E., and Lorenzi, M. (1990). *Proc. Natl. Acad. Sci. USA* **87,** 404–408.
88. Couchman, J. R. (1987). *J. Cell. Biol.* **105,** 1901–1916.
89. Fujiwara, S., Wiedemann, H., Timple, R., Lustig, A., and Engel, J. (1984). *Eur. J. Biochem.* **143,** 145–157.
90. Papadimitriou, E., Manolopoulos, V. G., Hayman, G. T., Maragoudakis, M. E., Unsworth, B. R., Frenton, J. W., II, et al. (1997). *Am. J. Physiol.* **272,** C1112–C1122.
91. Kamihagi, K., Katayama, M., Ouchi, R., and Kato, I. (1994). *Biochem. Biophys. Res. Commun.* **200,** 423–428.
92. Lodish, H. F. and Kong, N. (1990). *J. Biol. Chem.* **265,** 10,893–10,899.
93. Lodish, H. F. (1988). *J. Biol. Chem.* **263,** 2107–2110.
94. Williamson, J. R., Tilton, R. G., Chang, K., and Kilo, C. (1988). *Metab. Rev.* **4,** 339–370.
95. Paulsson, M. (1988). *Biol. Chem.* **263,** 5425–5430.
96. Paulsson, M., Saladin, K., and Landwehr, R. (1988). *Eur. J. Biochem.* **177,** 477–481.
97. Michel, C. C. and Philips, M. E. (1989). *Quat. J. Exp. Phys.* **74,** 7–18.
98. Majno, G., Shea, S. M., and Leventhal, M. (1969). *J. Cell. Biol.* **42,** 647–672.
99. Fox, J., Galey, F., and Wayland, H. (1980). *Microvas. Res.* **19,** 108–126.