Biochemical and Biophysical Research Communications **267,** 179–183 (2000)
doi:10.1006/bbrc.1999.1921, available online at http://www.idealibrary.com on IDEAL®

Parallel Oscillations of Intracellular Calcium Activity and Mitochondrial Membrane Potential in Mouse Pancreatic B-Cells

Peter Krippeit-Drews,[1] Martina Düfer, and Gisela Drews
Institute of Pharmacy, Department of Pharmacology, University of Tübingen, Auf der Morgenstelle 8, D-72076 Tübingen, Germany

Received November 8, 1999

Insulin secretion in normal B-cells is pulsatile, a consequence of oscillations in the cell membrane potential (MP) and cytosolic calcium activity ($[Ca^{2+}]_c$). We simultaneously monitored glucose-induced changes in $[Ca^{2+}]_c$ and in the mitochondrial membrane potential $\Delta\Psi$, as a measure for ATP generation. Increasing the glucose concentration from 0.5 to 15 mM led to the well-known hyperpolarization of $\Delta\Psi$ and ATP-dependent lowering of $[Ca^{2+}]_c$. However, as soon as $[Ca^{2+}]_c$ rose due to the opening of voltage-dependent Ca^{2+} channels, $\Delta\Psi$ depolarized and thereafter oscillations in $[Ca^{2+}]_c$ were parallel to oscillations in $\Delta\Psi$. A depolarization or oscillations of $\Delta\Psi$ cannot be evoked by a substimulatory glucose concentration, but Ca^{2+} influx provoked by 30 mM KCl was followed by a depolarization of $\Delta\Psi$. The following feedback loop is suggested: Glucose metabolism via mitochondrial ATP production and closure of K^+_{ATP} channels induces an increase in $[Ca^{2+}]_c$. The rise in $[Ca^{2+}]_c$ in turn decreases ATP synthesis by depolarizing $\Delta\Psi$, thus transiently terminating Ca^{2+} influx. © 2000 Academic Press

Oscillations in cell membrane potential (MP) and cytosolic Ca^{2+} activity ($[Ca^{2+}]_c$) of pancreatic B-cells are a prerequisite for normal pulsatile insulin secretion (1, 2). Accordingly, B-cell oscillatory activity is impaired in diabetes mellitus (3–6). It has been known for many years that mitochondria are an important link in the stimulus-secretion coupling of B-cells. Glucose metabolism involves mitochondrial ATP production (7, 8). The rise in cytosolic ATP concentration ($[ATP]_c$) causes closure of ATP-sensitive K^+ (K^+_{ATP}) channels, depolarization of the cell membrane and opening of voltage-dependent Ca^{2+} channels (9, 10) with bursts of action potentials (11). To date it is unclear whether or to what extend mitochondria are involved in the generation of B-cell oscillations. The important question in this context is whether a feedback of the glucose signaling cascade to the mitochondria exists. Since glucose increases not only $[Ca^{2+}]_c$, but also intramitochondrial Ca^{2+} activity ($[Ca^{2+}]_m$) (12, 13), there is indeed evidence for the involvement of $[Ca^{2+}]_m$ in B-cell stimulus–secretion coupling.

It has been shown for hepatocytes that an increase in $[Ca^{2+}]_m$ activates dehydrogenases, which, however, only resulted in a transient increase in NAD(P)H (14). Also for B-cells an increase in dehydrogenases (15) and in NAD(P)H as a response to a glucose-evoked elevation in $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ has been demonstrated (12). In a limited number of a subpopulation of B-cells it was even found that NAD(P)H fluorescence oscillated upon glucose stimulation and that it also increased in response to a KCl induced increase in $[Ca^{2+}]_c$ (16). However, such effects were not observed by others (17) and the mechanism leading to the NAD(P)H oscillations remained unclear (16).

Oscillations in oxygen consumption, $[Ca^{2+}]_c$ and insulin secretion coexist in rat pancreatic islets (18) and it could be demonstrated that oscillations in $[Ca^{2+}]_c$ are promoted by oscillations in K^+_{ATP} channel activity (19). Furthermore, it was found that changes in $[ATP]_c$ and in K^+_{ATP} current seem to be parallel (20). In addition, it has been shown that a rise in $[Ca^{2+}]_c$ diminishes the ATP/ADP ratio not only in hepatocytes (14) but also in mouse islets (21). Therefore, the initiation of interburst phases may be due to an activation of the K^+_{ATP} current by a reduction in $[ATP]_c$, but the origin of the ATP/ADP oscillations, thought to be responsible for the glucose-induced oscillations, is still unclear (21).

The aim of the present study was to investigate whether $[Ca^{2+}]_c$ and the mitochondrial membrane potential $\Delta\Psi$, taken as a measure for relevant oxidative ATP production, influence each other. In addition, we wanted to elucidate how this possible interaction between $[Ca^{2+}]_c$ and $\Delta\Psi$ could trigger oscillations. Parts

[1] To whom correspondence should be addressed. Fax: #49-7071-292476. E-mail: peter.krippeit-drews@uni-tuebingen.de.

0006-291X/00 $35.00

of this study have been published earlier in abstract form (22).

MATERIALS AND METHODS

The experiments were performed with islet cells of fed female NMRI mice (25–30 g), killed by cervical dislocation. Islets were isolated by collagenase digestion of the pancreas. Single cells or clusters of cells were dispersed in Ca^{2+}-free medium and cultured up to 4 days in RPMI 1640 medium. Intracellular Ca^{2+} activity ($[Ca^{2+}]_c$) was measured with fura-2 (23), and $\Delta\Psi$ with rhodamine 123 (Rh123) (24). Cells were loaded with fura-2/AM (5 μM) for 30 min and/or with Rh123 (10 μg/ml) for 10 min at 37°C. Bath solution was composed of (mM) 140 NaCl, 5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 0.5 glucose, 10 Hepes, pH 7.4, adjusted with NaOH. In the bath solution with 30 mM KCl, K^+ was substituted at the expense of Na^+. Fluorescence was measured on an Axiovert 100 microscope (Zeiss, Stuttgart, FRG) with equipment and software delivered by TILL photonics (Planegg, FRG). Excitation light wavelength was adjusted by means of a diffractive grating and then directed through the objective (PlanNeofluar40x, Zeiss) by means of a glass fibre light guide and a dichroic mirror. The emitted light was filtered (LP 515 nm) and measured by a CCD camera. Intracellular fura-2 was excited with light of 340, 360, or 380 nm wavelength. The ratio of the emitted light intensity at 340 nm/380 nm excitation wavelength was used to calculate $[Ca^{2+}]_c$ according to an *in vitro* calibration with fura-2 salt. Rh123 fluorescence was excited at 480 nm. An increase of Rh123 fluorescence corresponds to a decrease in $\Delta\Psi$. NAD(P)H autofluorescence was detected at 360 nm excitation wavelength in unloaded cells. Traces were corrected for the light-induced loss in NAD(P)H autofluorescence. Measurements are illustrated by recordings that are representative of the indicated number of experiments performed with different cells. Cells of at least three different cell preparations have been used for each series of experiments. Means ± SEM are given in the text for the indicated number of experiments. The statistical significance of differences between means was assessed by Student's *t* test for paired values when two samples were compared. Multiple comparisons were made by ANOVA followed by Student-Newman-Keuls test. $P \leq 0.05$ was considered as significantly different.

Fura-2/AM and rhodamine 123 were obtained from Molecular Probes (Eugene, Oregon), and fura-salt, FCCP, and NaN_3 from Sigma (Deisenhofen, FRG). All other chemicals were purchased from Merck (Darmstadt, FRG) in the purest form available.

RESULTS

Glucose-Induced Changes in $[Ca^{2+}]_c$ and $\Delta\Psi$ of Mouse Pancreatic B-Cells

Figure 1 shows the effect of a low and a high glucose concentration (0.5 and 15 mM) on $[Ca^{2+}]_c$ (solid line) and $\Delta\Psi$ (dotted line) recorded simultaneously with fura-2 and rhodamine 123 in a small cluster of B-cells. Identical results were achieved in series of control experiments in which the cells were loaded with one dye only. The registration of $[Ca^{2+}]_c$ showed the typical behavior (25). The increase in the glucose concentration first resulted in a small decrease in $[Ca^{2+}]_c$ from 71 ± 5 nM to 48 ± 3 nM (n = 16) owing to the activation of storage-linked Ca^{2+} pumps by the augmented ATP production (25). After sufficient time for depolarization of the cell membrane and opening of Ca^{2+} channels, $[Ca^{2+}]_c$ drastically increased up to

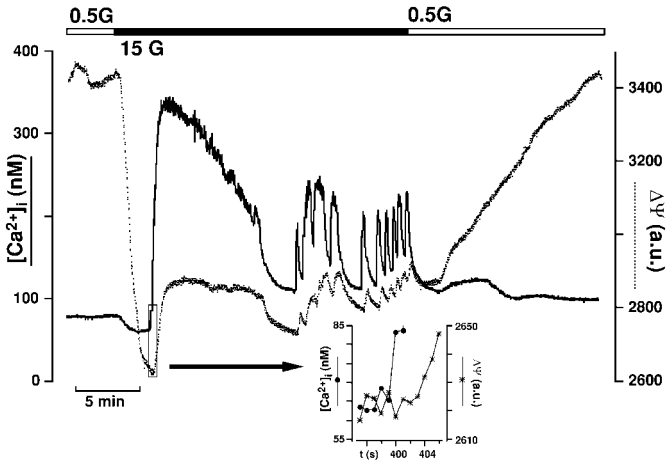

FIG. 1. Effect of 0.5 and 15 mM glucose on $[Ca^{2+}]_c$ (solid line) and $\Delta\Psi$ (dotted line). Solutions with different glucose concentrations were present for the time indicated by the horizontal bars. Cells were loaded with fura-2/AM and Rh123. Changes in $[Ca^{2+}]_c$ and $\Delta\Psi$ were recorded simultaneously. An increase in Rh123 fluorescence corresponds to a depolarization of $\Delta\Psi$. The inset shows scaled signals for Rh123 fluorescence and $[Ca^{2+}]_c$ at a higher time resolution for the period indicated by the rectangle. The experiment is representative of 16 with similar results.

355 ± 20 nM for a longer first period (presumably reflecting the first phase in insulin secretion) and then started to oscillate (n = 16). The same manoeuvre, i.e., the rise in glucose concentration, initially led to a hyperpolarization of $\Delta\Psi$ as indicated by the decrease in rhodamine 123 fluorescence by 647 ± 49 arbitrary units (a.u.) (n = 21). This reflected the increased activity of mitochondrial respiratory chains and the rise in ATP production (24). The important new observation is that every rise in $[Ca^{2+}]_c$ is followed by a depolarization of $\Delta\Psi$. The first rise in $[Ca^{2+}]_c$ immediately depolarized $\Delta\Psi$ by 192 ± 15 a.u. (n = 21) and subsequently oscillations in $\Delta\Psi$ paralleled those of the Ca^{2+} signal. The effect of glucose was reversible. The inset shows that the onset of the increase in $[Ca^{2+}]_c$ was several seconds (data points sampled in 1-s intervals) before $\Delta\Psi$ depolarized indicating that the latter is a consequence of the former. The crucial point for this conclusion is the difference in the onset of the two signals. The kinetics of fluorescence changes of both dyes may be very different, though.

The Increase in $[Ca^{2+}]_c$ and Not in Glucose Concentration Depolarized $\Delta\Psi$

Figure 2 shows that the depolarization of $\Delta\Psi$ was indeed brought about by an increase in $[Ca^{2+}]_c$ and not by oscillations in the metabolism of glucose. First, a nonstimulatory glucose concentration (5 mM) hyperpolarized $\Delta\Psi$ by 463 ± 59 a.u. (n = 4), but without the occurrence of oscillations or an increase in $[Ca^{2+}]_c$. Together with the initial small drop in $[Ca^{2+}]_c$ (−12 ± 3

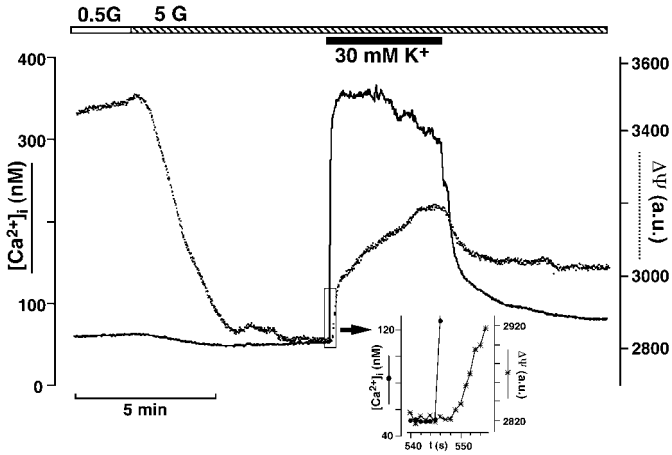

FIG. 2. Changes in $[Ca^{2+}]_c$ and $\Delta\Psi$ due to depolarization of the B-cell membrane by a high extracellular K^+ concentration. Glucose concentration was increased from 0.5 to 5 mM, as indicated by the horizontal bars. To provoke Ca^{2+} influx, the B-cells were depolarized by 30 mM K^+. The inset shows scaled signals for Rh123 fluorescence and $[Ca^{2+}]_c$ at a higher time resolution for the period indicated by the rectangle. The experiment is representative of 4 with similar results.

nM; $n = 3$; see above and (25)) this indicates that the intracellular ATP concentration rised, but obviously not enough to close so many K^+_{ATP} channels that the cells depolarized beyond the threshold potential at which voltage-dependent Ca^{2+} channels open. However, when this was provoked by further depolarizing the B-cells with KCl (30 mM), $[Ca^{2+}]_c$ immediately increased up to 367 ± 16 nM ($n = 3$) and $\Delta\Psi$ was depolarized by 230 ± 45 a.u. ($n = 4$). Again the inset shows that the rise in $[Ca^{2+}]_c$ started seconds before the rise in rhodamine 123 fluorescence. The effect of KCl was reversible as expected.

From these experiments one important question arose: Is the observed mean depolarization of 192 ± 15 a.u. sufficient to bring ATP production to a level low enough to interrupt B-cell activity? As mentioned above, the hyperpolarization of $\Delta\Psi$ was 463 ± 59 a.u. for a subthreshold glucose concentration (5 mM) and 647 ± 49 a.u. for a suprathreshold glucose concentration (15 mM). Thus, one may deduce that, as 184 a.u. of hyperpolarization of $\Delta\Psi$ seem to be sufficient to induce B-cell activity, a depolarization of 192 a.u. should be able to do the opposite.

Changes in NAD(P)H Fluorescence Can Be Independent of ATP Synthesis

Figure 3 shows a measurement of $\Delta\Psi$ in the upper trace and of NAD(P)H fluorescence in the lower trace. Increasing the glucose concentration from 0.5 to 15 mM led to a rise in glucose metabolism as indicated by the increase in NAD(P)H fluorescence by 9 ± 1 a.u. ($n = 6$). Correspondingly, $\Delta\Psi$ hyperpolarized by 648 ± 124 a.u. in this series of experiments ($n = 3$), indicating the rise in ATP production. After sufficient time for depolarization of the cell membrane and the increase in $[Ca^{2+}]_c$, $\Delta\Psi$ was partly depolarized by 187 ± 8 a.u. ($n = 3$) denoting the partial drop in ATP synthesis (see also above). Approximately at the point of time at which a rise in $[Ca^{2+}]_m$ is expected (12, 13), in some experiments slight increases in NAD(P)H fluorescence appeared, but this was not consistently observed. The mitochondrial inhibitors NaN_3 (5 mM) and FCCP (1 μM) both clearly and reversibly depolarized $\Delta\Psi$ by 1581 ± 38 a.u. ($n = 6$) and 1487 ± 214 a.u. ($n = 3$), respectively, indicating the energy depletion of the cells. However, NaN_3 increased NAD(P)H fluorescence by 20 ± 4 a.u. ($n = 6$), but FCCP led to a drop in NAD(P)H fluorescence by 8 ± 1 a.u. ($n = 6$). Apparently, the block of respiratory chains by the inhibition of cytochrome a_3 diminished the consumption of reduction equivalents (17). By contrast, short circuiting the H^+ gradient across the inner mitochondrial membrane with the mitochondrial uncoupler lowers NAD(P)H, presumably because of the stimulation of respiratory chain activity (26). Thus, it appears that a change in NAD(P)H is not appropriate to predict a corresponding change in ATP production in mitochondria.

DISCUSSION

Stimulus–secretion coupling in intact pancreatic B-cells proceeds with oscillations in ion currents, the cell membrane potential (MP), and $[Ca^{2+}]_c$ to ensure pulsatile insulin secretion even at stable glucose concentrations, and obviously, these oscillations are necessary for normally functioning cells (see (27) for a review).

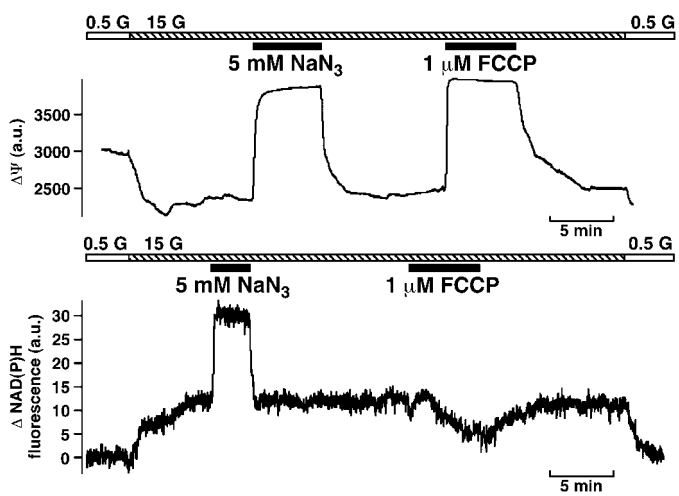

FIG. 3. Effects of NaN_3 (5 mM) and FCCP (1 μM) on glucose-induced changes in $\Delta\Psi$ (upper panel) and NAD(P)H autofluorescence (360 nm) (lower panel). The records are representative of 3 to 6 experiments for the individual manoeuvres for Rh123 fluorescence and of 6 experiments for NAD(P)H fluorescence, respectively.

In the present study we show for the first time in B-cells that a glucose-induced rise in $[Ca^{2+}]_c$ partly depolarized $\Delta\Psi$, and that the subsequent oscillations in $[Ca^{2+}]_c$ were also followed by oscillations in $\Delta\Psi$. Thus, our data support previous views of the mechanism underlying these oscillations (18, 19, 21) and provide new aspects which led us to propose the following feedback mechanism: Glucose metabolism increases NAD(P)H (Fig. 3) and activates mitochondrial respiratory chains. This hyperpolarizes the mitochondrial membrane potential $\Delta\Psi$ (Figs. 1–3), leading to an increased ATP production. Via closure of K^+_{ATP} channels, subsequent depolarization of the cell membrane, and opening of voltage-dependent Ca^{2+} channels, the rise in $[ATP]_c$ brings about an increase in $[Ca^{2+}]_c$. This in turn partly depolarizes $\Delta\Psi$ (Fig. 1), leading to a reduction in ATP synthesis and thus to an opening of K^+_{ATP} channels, repolarization of the cell membrane, and inhibition of Ca^{2+} influx. Ca^{2+} is sequestered into intracellular stores or to the extracellular space, $\Delta\Psi$ repolarizes, and a new loop may start.

Recently, a similar negative feedback loop between $[Ca^{2+}]_c$ and the ATP/ADP ratio has been proposed (21). In contrast to the present study, Detimary and coworkers (21) did not measure parameters showing dynamic fluctuations of the metabolism or other related signals, e.g., $\Delta\Psi$. They suggested that the Ca^{2+}-induced fall in the ATP/ADP ratio observed in their experiments was caused by an increased ATP consumption rather than by a decreased ATP production. However, in the same report it is shown that one of the most energy consuming processes under these conditions, namely, the insulin secretion, is not responsible for a drop in ATP that would reduce Ca^{2+} influx (21).

It is commonly accepted that an increase in $[Ca^{2+}]_c$ leads to an increase in $[Ca^{2+}]_m$. Furthermore, it has been put forward that a rise in $[Ca^{2+}]_m$ in turn increases the activity of mitochondrial dehydrogenases and as a consequence leads to a rise in NAD(P)H and in ATP synthesis (see (12, 26) for reviews). However, it has been demonstrated for isolated mitochondria from B-cells of ob/ob mice that ATP production is Ca^{2+}-dependent, but a primary activation at low $[Ca^{2+}]_m$ is followed by an inhibition at a high $[Ca^{2+}]_m$ (28, 29). Nevertheless, the immediate consequence of mitochondrial Ca^{2+} uptake is mitochondrial depolarization (Figs. 1 and 2 (26)). The reason for this may be either that Ca^{2+} influx into mitochondria occurs via the electrogenic Ca^{2+} uniporter (30, 26) or that an increase in $[Ca^{2+}]_m$ may lead to the opening of the low-conductance state of the permeability transition pore (PTP) (31) of the mitochondria, a large unspecific ion channel (32–34). In any case, a depolarization of $\Delta\Psi$ would reduce the electromotive force for H^+ and thus for ATP synthesis (26). Therefore, the current view in many working groups that an increase in NAD(P)H, via activation of respiratory chains, unquestionably leads to ATP production should be reconsidered: A rise in $[Ca^{2+}]_c$ is a start signal for cell activity in many cells, especially excitable cells. If this, via an increase in $[Ca^{2+}]_m$, would promote metabolism and thus ATP synthesis, some cells would indeed profit from this, because it would meet their increased energy demand, e.g., cardiac myocytes (35). However, this would not make sense for the B-cell. When B-cell activity starts with rising extracellular glucose concentrations, the fuel input and as a consequence $[ATP]_c$ is already very high. Therefore, B-cells can even easily use ATP as a link in stimulus-secretion coupling. Thus, a mechanism by which an increase in $[Ca^{2+}]_c$ and thereby in $[Ca^{2+}]_m$ would enhance ATP production would represent a positive feedback loop for the B-cell, because a rise in ATP would promote further Ca^{2+} influx. Such a positive feedback loop, however, cannot explain the occurrence of Ca^{2+} oscillations. Furthermore, it has been shown for B-cells (21) as well as for hepatocytes (14) that $[ATP]_c$ decreases with a rise in $[Ca^{2+}]_c$. For hepatocytes this was even found despite a sustained increase in the activity of dehydrogenases and a rise in NAD(P)H (14). In addition, increases in NAD(P)H or in respiratory chain activity are not necessarily coupled to ATP synthesis. Figure 3 shows that NAD(P)H increased even when respiratory chains and thereby ATP synthesis were blocked by NaN_3 [see also 17], and that it decreased when the H^+ gradient across the inner mitochondrial membrane was short circuited by FCCP [see also 26]. Thus, the periodic depolarizations in $\Delta\Psi$ brought about by oscillations in $[Ca^{2+}]_c$ (Fig. 1) may indeed denote oscillations in ATP production without obvious changes in NAD(P)H fluorescence (Fig. 3) (17). Nevertheless, the oscillations in NAD(P)H observed by Pralong *et al.* (16) may mirror periodic decreases in ATP synthesis instead of periodic increases.

It has been proposed before that oscillations in B-cell activity may be coupled to oscillations in metabolism (19, 20). However, Grapengiesser *et al.* (36) showed that B-cells exhibited oscillations elicited by tolbutamide in the absence of glucose. By contrast, it has been shown by Henquin (37) that B-cells need a minimum of fuel to respond properly even to sulfonylureas. In this context one should keep in mind that mitochondria also possess K^+_{ATP} channels (38) and that the $\Delta\Psi$ of B-cell mitochondria is sensitive to diazoxide and sulfonylureas (39, 40).

In conclusion, we show here that the mitochondrial membrane potential may be involved in a feedback loop which ensures oscillations of the MP, $[Ca^{2+}]_c$ and probably insulin secretion. These oscillations are thought to be essential for B-cells to function properly.

ACKNOWLEDGMENTS

This work was supported by grants of the Deutsche Forschungsgemeinschaft (Dr 225/4-1,2) and the Deutsche Diabetesgesellschaft.

REFERENCES

1. Santos, R. M., Rosario, L. M., Nadal, A., Garcia-Sancho, J., Soria, B., and Valdeolmillos, M. (1991) *Pflügers Arch. -Eur. J. Physiol.* **418,** 417–422.
2. Gilon, P., Shepherd, R. M., and Henquin, J.-C. (1993) *J. Biol. Chem.* **268,** 22265–22268.
3. Polonsky, K. S., Sturis, J., and Cauter, E. (1998) *Horm. Res.* **49,** 178–184.
4. Meissner, H. P., and Schmidt, H. (1976) *FEBS Letters* **67,** 371–374.
5. Kindmark, H., Köhler, M., Arkhammar, P., Efendic, S., Larsson, O., Linder, S., Nilsson, T., and Berggren, P.-O. (1994) *Diabetologia* **37,** 1121–1131.
6. Tornheim, K. (1997) *Diabetes* **46,** 1375–1380.
7. Dukes, I. D., McIntyre, M. S., Mertz, R. J., Philipson, L. H., Roe, M. W., Spencer, B., and Worley, J. F., III (1994) *J. Biol. Chem.* **269,** 10979–10982.
8. Misler, S., Barnett, D. W., and Falke, L. C. (1992) *Pflügers Arch. -Eur. J. Physiol.* **421,** 289–291.
9. Ashcroft, F. M., and Rorsman, P. (1989) *Prog. Biophys. Molec. Biol.* **54,** 87–143.
10. Zhou, Z., and Misler, S. (1996) *J. Biol. Chem.* **270,** 270–277.
11. Meissner, H. P., and Schmelz, H. (1974) *Pflügers Arch.* **351,** 195–206.
12. Kennedy, E. D., and Wollheim, C. B. (1998) *Diabetes Metab.* **24,** 15–24.
13. Rutter, G. A., Theler, J.-M., Murgia, M., Wollheim, C. B., Pozzan, T., and Rizzuto, R. (1993) *J. Biol. Chem.* **268,** 22385–22390.
14. Robb-Gaspers, L. D., Burnett, P., Rutter, G. A., Denton, R. M., Rizzuto, R., and Thomas, A. P. (1998) *EMBO J.* **17,** 4987–5000.
15. McCormack, J. G., Longo, E. A., and Corkey, B. E. (1990) *Biochem. J.* **267,** 527–530.
16. Pralong, W.-F., Spät, A., and Wollheim, C. B. (1994) *J. Biol. Chem.* **269,** 27310–27314.
17. Gilon, P., and Henquin, J.-C. (1992) *J. Biol. Chem.* **267,** 20713–20720.
18. Longo, E. A., Tornheim, K., Deeney, J. T., Varnum, B. A., Tillotson, D., Prentki, M., and Corkey, B. E. (1991) *J. Biol. Chem.* **266,** 9314–9319.
19. Larsson, O., Kindmark, H., Bränström, R., Fredholm, B., and Berggren, P.-O. (1996) *Proc. Natl. Acad. Sci.* **93,** 5161–5165.
20. Köhler, M., Norgren, S., Berggren, P.-O., Fredholm, B. B., Larsson, O., Rhodes, C. J., Herbert, T. P., and Luthman, H. (1998) *FEBS Letters* **441,** 97–102.
21. Detimary, P., Gilon, P., and Henquin, J.-C. (1998) *Biochem. J.* **333,** 269–274.
22. Düfer, M., Drews, G., and Krippeit-Drews, P. (1999) *Diabetologia* **42,** A140.
23. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985) *J. Biol. Chem.* **260,** 3440–3450.
24. Duchen, M. R., Smith, P. A., and Ashcroft, F. M. (1993) *Biochem. J.* **294,** 35–42.
25. Grapengiesser, E., Gylfe, E., and Hellman, B. (1988) *Biochem. Biophys. Res. Commun.* **150,** 419–425.
26. Duchen, M. R. (1999) *J. Physiol.* **516,** 1–17.
27. Henquin, J. C., Jonas, J. C., and Gilon, P. (1998) *Diabetes Metab.* **24,** 30–36.
28. Lembert, N., and Idahl, L.-Å. (1998) *Diabetes* **47,** 339–344.
29. Idahl, L.-Å., and Lembert, N. (1995) *Biochem. J.* **312,** 287–292.
30. Gunter, K. K., and Gunter, T. E. (1994) *J. Bioenerg. Biomembr.* **26,** 471–485.
31. Ichas, F., and Mazat, J.-P. (1998) *Biochim. Biophys. Acta* **1366,** 33–50.
32. Zoratti, M., and Szabò, I. (1995) *Biochim. Biophys. Acta* **1241,** 139–176.
33. Bernardi, P., and Petronilli, V. (1996) *J. Bioenerg. Biomembr.* **28,** 131–138.
34. Ichas, F., Jouaville, L. S., and Mazat, J-P. (1997) *Cell* **89,** 1145–1153.
35. Di Lisa, F., Fan, C. Z., Gambassi, G. Hogue, B. A., Kudryashova, I., and Hansford, R. G. (1993) *Am. J. Physiol.* **264,** H2188–H2197.
36. Grapengiesser, E., Gylfe, E., and Hellman, B. (1989) *Mol. Pharmacol.* **37,** 461–467.
37. Henquin, J.-C. (1998) *Endocrinology* **139,** 993–998.
38. Garlid, K. D. (1996) *Biochim. Biophys. Acta* **1275,** 123–126.
39. Grimmsmann, T., and Rustenbeck, I. (1998) *Br. J. Pharmacol.* **123,** 781–788.
40. Smith, P. A., Proks, P., and Moorhouse, A. (1999) *Pflügers Arch. -Eur. J. Physiol.* **437,** 577–588.