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^{99m}Tc-sesta-(2-methoxy-isobutyl-isonitrile) Uptake by Pancreatic Islets, Parotid Cells, and Mammary Carcinoma Cells

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^{99m}Tc-sesta-(2-methoxy-isobutyl-isonitrile) (Tc-MIBI) is currently used for imaging of several organs. In the present study, its uptake by rat pancreatic islets, rat parotid cells, and human breast adenocarcinoma cells (MCF-7 cells) was found to be grossly proportional to its concentration (up to 0.1 μ*M*), time-related (with a fractional turnover rate close to 2–3 $10^{-2} \cdot min^{-1}$), and stimulated by D-glucose. Comparable values for the fractional turnover rate were found in prelabeled islets and MCF-7 cells, D-glucose failing to affect Tc-MIBI efflux from prelabeled islets. In the islets, the uptake of Tc-MIBI was decreased at low temperature, in the presence of mitochondrial poisons and at high extracellular K^+ concentration, unaffected by the absence of extracellular Ca^{2+}, and increased by nutrient secretagogs, such as 2-ketoisocaproate and the association of L-leucine and L-glutamine. These findings are consistent with the view that Tc-MIBI uptake is ruled by its extracellular concentration, and the polarization of both plasma and mitochondrial membranes. It is proposed that this lipophilic cation may be useful to detect alteration of nutrient metabolism in pancreatic islets deprived of any exogenous fuel.

Key Words: ^{99m}Tc-MIBI; pancreatic islets; parotid cells; mammary carcinoma cells.

Introduction

^{99m}Tc-sesta-(2-methoxy-isobutyl-isonitrile) (Tc-MIBI) is a well-known radiopharmaceutical probe used for a decade in myocardial scintigraphy *(1–4)*. Other clinical applications emerged rapidly after its introduction in nuclear medicine, the first one being the imaging of abnormal parathyroid glands *(5–7)*. More recently, Tc-MIBI was found to be useful in oncological pathology mainly in breast cancer *(8–10)*, pulmonary cancer *(11–14)*, and brain tumor *(15–17)*. Some authors reported on possible applications in bone and bone marrow pathology *(18–21)*.

Mitochondrial trapping is the usually admitted modality of intracellular uptake of this cationic and lipophilic agent. The mechanism would involve a passive passage across plasma and mitochondrial membranes, as driven by the membrane's potentials. This was assessed by biochemical and subcellular distribution studies in cultured cells *(10,22–29)*. Moreover, Backus et al. *(28)* proposed that Tc-MIBI could be used for *in situ* measurements of the mitochondrial membrane potential. However, the intracellular distribution of MIBI is not easy to assess. Indeed, the homogenization technique used before separation of the subcellular fractions may cause potential membrane changes leading to the release of the tracer from the concerned organelles.

In the present study, we have investigated the uptake of Tc-MIBI in rat pancreatic cells, parotid cells, and a human breast cancer cell line (MCF-7 cells). The selection of these three cell types was motivated mainly by the following considerations. In pancreatic islets, an increase in extracellular D-glucose concentration leads to depolarization of the plasma membrane *(30)* and hyperpolarization of the B-cell mitochondrial membrane *(31)*. Salivary glands exhibit a high uptake of Tc-MIBI in clinical studies (Blocklet and Schoutens, unpublished observation). According to a recent report *(10)*, MCF-7 cells also demonstrate a high uptake of Tc-MIBI. Based on the comparison between these three cell types, the major aim of these investigations was to explore whether the uptake of Tc-MIBI may provide a new and valuable tool to assess overall changes in nutrient catabolism in pancreatic islets.

Results

Pancreatic Islets

Except if otherwise mentioned, all results refer to experiments conducted over 90-min incubation at 37°C in a salt-balanced medium containing 22 n*M* Tc-MIBI. Under these experimental conditions, the basal net uptake of Tc-MIBI averaged, in a series of 7 experiments, 2.42 ± 0.08 fmol/islet (n = 31).

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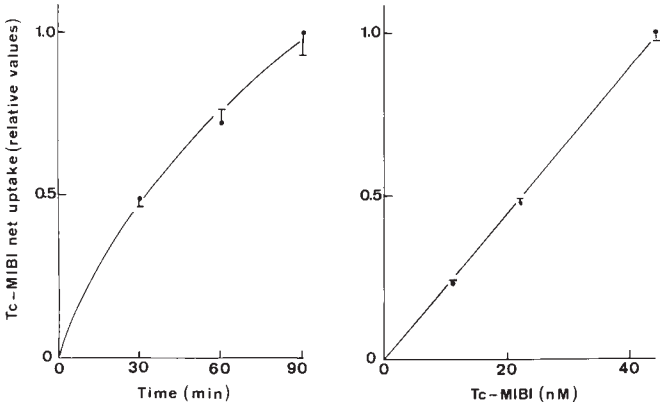

Fig. 1. Time-course (left) and concentration dependency (right) for the uptake of Tc-MIBI by pancreatic islets incubated for 30–90 min in the presence of 22 n*M* Tc-MIBI (left) or for 90 min at increasing concentrations (11–44 n*M*) of Tc-MIBI (right). Mean values (±SEM) refer to 8–16 individual determinations, and are expressed relative to the mean highest value recorded in each series of experiments.

The time-course and concentration dependency for Tc-MIBI uptake are illustrated in Fig. 1. Whether in the absence or presence of D-glucose (16.7 m*M*), the net uptake of Tc-MIBI after a 30-min incubation already represented about half the value recorded after a 90-min incubation. Over a 90-min incubation, the uptake of Tc-MIBI was virtually proportional to its concentration (11–44 n*M*), such being again the case both in the absence or presence of D-glucose.

Whether in the absence or presence of D-glucose (16.7 m*M*), the net uptake of Tc-MIBI was abolished at a low temperature or in the presence of mitochondrial poisons. Thus, at 4°C, the Tc-MIBi uptake averaged 4.4 ± 1.4% ($n = 8$) of the corresponding control value found at 37°C. Likewise, in the presence of 2.0 m*M* KCN, the measurements represented no more than 7.1 ± 1.1% ($n = 10$) of their corresponding mean control values. In the presence of 16.7 m*M* D-glucose, the association of antimycin A and rotenone (10.0 μ*M* each) also decreased Tc-MIBI uptake to 7.8 ± 1.6% ($n = 4$) of the paired control value.

In media deprived of $CaCl_2$ and containing 0.5 m*M* EGTA, the net uptake of Tc-MIBI by islets exposed to 16.7 m*M* D-glucose was not significantly different ($p > 0.5$) from that found, within the same experiment, at normal extracellular Ca^{2+} concentration (1.0 m*M*), with mean values of 3.08 ± 0.22 (no Ca2+; $n = 4$) and 3.23 ± 0.13 (1.0 m*M* Ca^{2+}; $n = 5$) fmol/islet.

When the concentration of extracellular K^+ was raised from 5.0 to 30.0 m*M*, by isoosmolar substitution of NaCl by KCl, the uptake of Tc-MIBI was decreased to 68.2 ± 5.2% ($n = 8$; $p < 0.005$) of the mean corresponding control value (5.0 m*M* K^+).

At normal K^+ concentration (5.0 m*M*), the uptake of Tc-MIBI was, as a rule, slightly increased by 16.7 m*M* D-glucose relative to basal value. For instance, in a series of 6 experiments conducted under the usual conditions (a 90-min incubation in the presence of 22 n*M* Tc-MIBI), the measurements made in the presence of the hexose averaged 113.9 ± 2.5% ($n = 26$) of the mean basal value (100.0 ± 6.1%; $n = 26$) recorded within the same experiment ($p < 0.05$). Likewise, in another series of 6 experiments conducted either over shorter incubation times (30–60 min) in the presence of 22 n*M* Tc-MIBI or over a 90-min incubation in the presence of 11 or 44 n*M* Tc-MIBI, the results found in the presence of D-glucose (16.7 m*M*) averaged 111.9 ± 2.5% ($n = 24$) of the mean corresponding basal value (100.0 ± 2.5%; $n = 26$), yielding a p value below 0.005. When pooling together the results of these two series of experiments, the glucose-stimulated uptake of Tc-MIBI was 13.0 ± 3.7% higher ($p < 0.001$; df = 98) than basal value.

Unexpectedly, the enhancing action of D-glucose on Tc-MIBI uptake was, in relative terms, more pronounced at 30.0 than 5.0 m*M* K^+. Indeed, at the high K^+ concentration, the measurements made in islets incubated for 90 min in the presence of 2.8 and 16.7 m*M* D-glucose averaged, respectively, 112.2 ± 6.2% ($n = 15$; $p > 0.1$) and 146.0 ± 3.4% ($n = 19$; $p < 0.001$) of the mean basal value found within the same experiments (100.0 ± 4.2%; $n = 19$).

The uptake of Tc-MIBI stimulated by 16.7 m*M* D-glucose was slightly, but not significantly ($p < 0.07$), decreased to 90.7 ± 1.9% ($n = 5$) of its paired control value by 10.0 m*M* D-mannoheptulose. Although failing to affect significantly Tc-MIBI uptake at normal K^+ concentration (data not shown), 2-ketoisocaproate (10.0 m*M*) augmented such an uptake ($p < 0.005$) to 130.4 ± 8.0% ($n = 15$) of the mean corresponding basal value (100.0 ± 4.8%; $n = 15$) in the presence of 30 m*M* K^+. The association of L-leucine and L-glutamine (10.0 m*M* each) increased the net uptake of Tc-MIBI by 28.6 ± 5.2% and 28.1 ± 15.7% (d.f. = 8 in both cases) at 5 and 30 m*M* K^+, respectively, only the former increment achieving statistical significance ($p < 0.001$). When tested at a 240.0-m*M* concentration, D-fructose also slightly increased Tc-MIBI uptake by 11.4 ± 4.9% ($p < 0.05$) and 7.2 ± 7.7% ($p > 0.3$; df = 8 in both cases) at 5 and 30 m*M* K^+, respectively.

When groups of 100 islets each were preincubated for 90 min at 37°C in the presence of 16.7 m*M* D-glucose and 22 n*M* Tc-MIBI and then placed in a perifusion chamber, the Tc-MIBI fractional outflow rate progressively decreased from 4.34 ± 0.35 to 0.54 ± 0.03 $10^{-2} \cdot min^{-1}$ over 44 min of perifusion in the absence of any exogenous nutrient. No obvious change in Tc-MIBI outflow could be detected when the concentration of D-glucose was raised from 0 to 16.7 m*M* from min 46 to 70 inclusive (Fig. 2). In these experiments, the output of insulin averaged 0.47 ± 0.03 μU/min/islet before the introduction of D-glucose (min 42–45) and 0.96 ± 0.03 μU/min during exposure to the hexose (min 46–70), yielding a paired increase of 0.49 ± 0.05 μU/min/islet ($n = 4$ in all cases; $p < 0.005$).

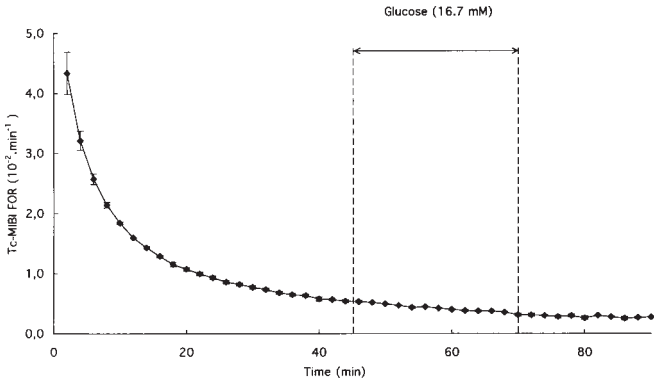

Fig. 2. Tc-MIBI fractional outflow rate (FOR) from pancreatic islets preincubated for 90 min at 37°C in the presence of 22 n*M* Tc-MIBI and 16.7 m*M* D-glucose and then perifused for 90 min at 37°C, the concentration of D-glucose being raised from 0 to 16.7 m*M* (vertical dotted lines) between the 46th and 70th min. Mean values (±SEM) refer to four individual experiments.

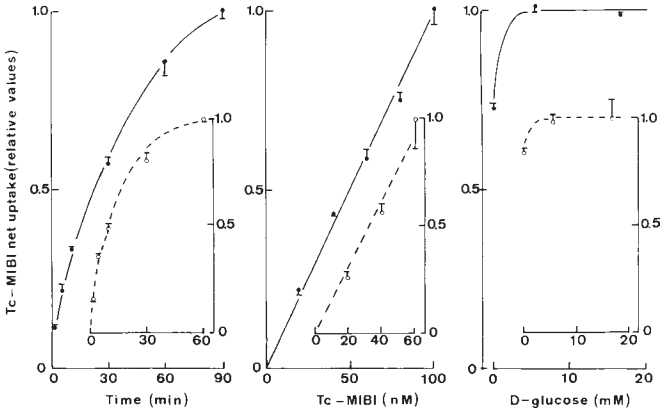

Fig. 3. Time-course (left), concentration dependency (middle), and D-glucose responsiveness (right) of Tc-MIBI uptake by either parotid cells (closed circles and solid lines) or MCF-7 cells (open circles and dashed lines). Except if otherwise indicated, the cells were incubated for 60 min in the presence of 44 n*M* Tc-MIBI and 16.7 m*M* D-glucose. Mean values (±SEM) refer to three to nine individual determinations. Presentation is the same as in Fig. 1.

Parotid Cells and MCF-7 Cells

The results of experiments conducted in parotid and MCF-7 cells are summarized in Fig. 3. After a 60-min incubation in the presence of 16.7 m*M* D-glucose, the net uptake of Tc-MIBI (44 n*M*) averaged, in parotid and MCF-7 cells, respectively, 5.92 ± 0.38 (n = 20) and 2.75 ± 0.41 (n = 27) fmol/10^3 cells. In both cell types, the time-course for Tc-MIBI uptake indicated a progressive increase toward an equilibrium value (Fig. 3, left panel). Over a 60-min incubation, it was virtually proportional to the concentration of Tc-MIBI (20–100 n*M*; Fig. 3, middle panel). Over the same incubation time, a concentration of 5.6 mM D-glucose was sufficient to increase Tc-MIBI uptake above basal value to a maximal level, no further increase in uptake being observed when the concentration of the hexose was raised to 16.7 m*M* (Fig. 3, right panel).

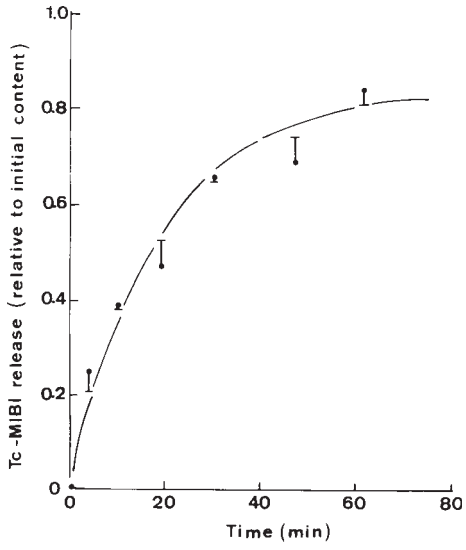

Fig. 4. Time-course for the release of Tc-MIBI during incubation of MCF-7 cells prelabeled over a 60-min preincubation in the presence of 44 n*M* Tc-MIBI and 5.6 m*M* D-glucose. Mean values (±SEM) refer to three individual determinations.

The efflux of Tc-MIBI from prelabeled MCF-7 cells yielded a fractional outflow rate close to 5.3×10^{-2}/min for a rapidly exchangeable pool representing about 84% of the total amount of Tc-MIBI taken up by the cells during preincubation (Fig. 4).

Discussion

The present results indicate that Tc-MIBI is efficiently taken up by pancreatic islets, parotid cells, and MCF-7 cells. Under the same experimental conditions, the absolute value for the net uptake of Tc-MIBI by pancreatic islets was of the same order of magnitude as that found in parotid or MCF-7 cells, taking into account the fact that each islet contains about 10^3 cells. The time-course and concentration dependency for Tc-MIBI uptake also displayed comparable patterns in these three cell types.

The high uptake of Tc-MIBI by parotid cells is consistent with clinical studies, indicating accumulation of the lipophilic cation in parotid glands.

In all cell types, the net uptake of Tc-MIBI was increased by D-glucose. The concentration dependency for the latter effect was not identical, however, in pancreatic islets and in parotid or MCF-7 cells. In the latter two cell types, a concentration of 5.6 m*M* D-glucose was sufficient to increase Tc-MIBI uptake to a close-to-maximal value. In pancreatic islets, the hexose-induced increment in Tc-MIBI uptake above basal value was about four times higher ($p < 0.001$) at 16.7 m*M* than 2.8 m*M* D-glucose, in which case it failed to achieve statistical significance. In islets exposed to 30 m*M* K^+, the deamination product of L-leucine, 2-ketoisocaproate, and the association of L-leucine and L-glutamine (10.0 m*M* in all cases) also increased Tc-MIBI uptake, while D-fruc-

tose (240.0 m*M*) exerted a more modest effect. Taken as a whole, these findings indicate that the net uptake of Tc-MIBI, as measured after extensive washing of the cells or islets, depends on the availability of endogenous ATP. The enhancing action of exogenous nutrients indeed paralleled their rate of utilization in the different cell types *(32–34)* and, in the case of islets, also grossly paralleled the rate of insulin release evoked by each nutrient or association of nutrients *(32,35)*.

The fact that the stimulation by nutrients of Tc-MIBI net uptake was, as a rule, more marked at 30 m*M* K^+ than at 5 m*M* K^+ is consistent with the view that they normally affect such an uptake in a dual manner, i.e., decreasing uptake as a result of plasma membrane depolarization, but increasing mitochondrial sequestration. At a high extracellular K^+ concentration, which depolarizes the plasma membrane and decreases Tc-MIBI uptake, only the latter component of the response to nutrients remains operative.

Further support for the ATP dependency of Tc-MIBI uptake by pancreatic islets is provided by the experiments conducted either at low temperature or in the presence of mitochondrial poisons. It should be realized that in these experiments, the environmental factors under consideration also impair the production of ATP linked to the catabolism of endogenous nutrients *(36,37)*.

It is currently thought that Tc-MIBI penetrates into cells by passive diffusion across the phospholipid domain of the plasma membrane and then accumulates into mitochondria, its net uptake being modulated by its extracellular concentration and the potential of both the plasma and mitochondrial membranes.

The present findings are consistent with such a view, since the uptake of Tc-MIBI was:

1. Increased as a function of its extracellular concentration.
2. Decreased at a high extracellular K^+ concentration, a procedure resulting in depolarization of the plasma membrane *(38)*.
3. Enhanced in the presence of exogenous nutrients known to cause hyperpolarization of the mitochondrial inner membrane in isolated pancreatic B-cells *(31)*.

Moreover, it failed to be affected in the absence of extracellular Ca^{2+}, indicating that the stimulation of Tc-MIBI uptake by nutrient secretagogs was not merely the consequence of increased insulin output.

No change in Tc-MIBI fractional outflow rate from prelabeled islets could be detected in prelabeled perifused islets exposed to a rise in extracellular D-glucose concentration from 0 to 16.7 m*M*, despite efficient stimulation of insulin release under these experimental conditions. This could reflect opposite effects of plasma membrane depolarization and mitochondrial hyperpolarization on the movements of Tc-MIBI. It might also suggest that the polarization of membranes at the mitochondrial level controls the uptake rather than release of Tc-MIBI by these organelles.

Despite the latter negative finding and despite the rather modest relative changes in Tc-MIBI net uptake caused by D-glucose or other nutrient secretagogs, this lipophilic cation could represent a useful tool to assess changes in nutrient catabolism in islet cells. For reasons mentioned above, it may be advantageous to conduct such experiments at 30 m*M* K^+. Moreover, considering the methodological limitations in the measurement of O_2 uptake by isolated islets, the present procedure may be most helpful in detecting interference of nonnutrient insulinotropic agents with the metabolism of endogenous nutrients in islets deprived of any exogenous fuel.

Materials and Methods

Tc-MIBI was prepared over a 10-min incubation at 100°C of 1.0 mg tetrakis (2-methoxy-isobutyl-isonitrile) dissolved in 3 mL of sterile solution containing 0.2 Ci sodium [^{99m}Tc]pertechnetate and placed in a sealed vial with low atmospheric pressure. After cooling, the efficiency of labeling (≥90%) was assessed by thin-layer chromatography. A diluted stock solution of Tc-MIBI (0.5 μ*M*, 100 μCi/mL) was then prepared in a vial that had first contained a solution of bovine serum albumin (10 mg/mL).

Pancreatic islets and parotid cells were prepared from fed female Wistar rats (B & K Universal Ltd., Hull, UK) by methods described elsewhere *(39,40)*. MCF-7 cells were cultured in MEM medium containing 10% (v/v) fetal calf serum, gentamicin, and fungizone (Gibco BRL; Life Technologies, Merelbeke, Belgium).

For measuring Tc-MIBI net uptake, groups of 50 islets each were incubated for 30–90 min at 37°C in 0.1 mL of a bicarbonate-buffered medium *(39)* containing bovine serum albumin (5 mg/mL) and Tc-MIBI. After incubation, the medium was removed, and the islets washed 4 times at 4°C with 1.0 mL of bicarbonate-buffered medium *(41)*, the islets being eventually examined for their radioactive content. A comparable procedure was used to study the uptake of Tc-MIBI by parotid or MCF-7 cells, using groups of 247 ± 12 and 400 ± 83 10^3 cells each, respectively (n = 5 in both cases).

In the islets, the release of Tc-MIBI during the third and fourth wash represented, respectively, 5.54 ± 0.72 and 3.76 ± 0.43% of the paired final radioactive islet content, the latter percentage averaging 68.3 ± 3.8% ($p < 0.04$, as compared to unity) of the former one (n = 6 in all cases).

The efflux of Tc-MIBI from prelabeled islets was monitored by perifusion, the experimental design being comparable to that used for measuring ^{45}Ca efflux from perifused islets *(42)*. The efflux of Tc-MIBI from prelabeled MCF-7 cells was assessed by measuring the radioactive content of the extracellular medium at selected times during the final incubation. The chemical identity of effluent radioactive material was not assessed in this study.

All results, including those already mentioned, are presented as mean values (±SEM) together with the number of

individual determinations. The statistical significance of differences between mean values was assessed by use of Student's t-test.

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References

1. Li, Q.-S., Frank, T. L., Franceschi, D., Wagner H. N., Jr., and Becker, L. C. (1988). *J. Nuclear Med.* **29,** 1539–1548.
2. Kayden, D. S., Mattera, J. A., Zaret, B. L., and Wackers, F. J. Th. (1988). *J. Nuclear Med.* **29,** 1865–1867.
3. Wackers, F. J. Th., Berman, D. S., Maddahi, J., Watson, D. D., Beller, G. A., Strauss, H. W., et al. (1989). *J. Nuclear Med.* **30,** 301–311.
4. Dilsizian, V., Rocco, T. P., Strauss, H. W., and Boucher, C. A. (1989). *J. Am. Coll. Cardiol.* **14,** 1673–1677.
5. O'Doherty, M. J., Kettle, A. G., Wells, P., Colins, R. E. C., and Coakley, A. J. (1992). *J. Nuclear Med.* **33,** 313–318.
6. McBiles, M., Lambert, A. T., Cote, M. G., and Kim, S. Y. (1995). *Sem. Nuclear Med.* **XXV,** 221–234.
7. Blocklet, D., Martin, P., Schoutens, A., Verhas, M., Hooghe, L., and Kinnaert, P. (1997) *Eur. J. Nuclear Med.* **24,** 46–51.
8. Khalkhali, I., Mena, I., and Diggles, L. (1994). *Eur. J. Nuclear Med.* **21,** 357–362.
9. Waxman, A. D. (1997). *Semin. Nuclear Med.* **XXVII,** 40–54.
10. Duran Cordobes, M., Starzec, A., Delmon-Moingeon, L., Blanchot, C., Kouyoumdjian, J.-C., Prèvost, G., et al. (1996). *J. Nuclear Med.* **37,** 286–289.
11. Aktolun, C., Bayhan, H., and Kir, M. (1992). *Clin. Nuclear Med.* **17,** 171–176.
12. Aktolun, C., Bayhan, H., Pabuccu, Y., Bilgic, H., Acar, H., and Koylu, R. (1994). *Eur. J. Nuclear Med.* **21,** 973–979.
13. Kao, C.-H., Wang, S.-J., Lin, W.-Y., Hsu, C.-Y., Liao, S.-Q., and Yeh, S.-H. (1993). *Eur. J. Nuclear Med.* **20,** 249–254.
14. Chiti, A., Maffioli, L. S., Infante, M., Grasselli, G., Incarbone, M., Gasparini, M. D., et al. (1996). *J. Nuclear Med.* **37,** 938–942.
15. O'Tuama, L. A., Packard, A. B., and Treves, S. T. (1990). *J. Nuclear Med.* **31,** 2040–2041.
16. O'Tuama, L. A., Treves, S. T., Larar, J. N., Packard, A. B., Kwan, A. J., Barnes, P. D., et al. (1993). *J. Nuclear Med.* **34,** 1045–1051.
17. Baillet, G., Albuquerque, L., Chen, Q., Poisson, M., and Delattre, J.-Y. (1994) *Eur. J. Nuclear Med.* **21,** 1061–1066.
18. Caner, B., Kitapçl, M., Unlü, M., Erbengi, G., Çalikoglu, T., Gögüs, T., et al. (1992). *J. Nuclear Med.* **33,** 319–324.
19. Reiman, R. E. and Fetters, J. K. (1996). *J. Nuclear Med.* **37,** 471–473.
20. El-Shirbiny, A. M., Yeung, H., Imbriaco, M., Michaeli, J., Macapinlac, H., and Larson, S. M. (1997). *J. Nuclear Med.* **38,** 1208–1210.
21. Nguyen, K., Waxman, A., Gupta, P., Delriego, J., Durie, B., Look, R., et al. (1996). *J. Nuclear Med.* **37,** 269P (abstract).
22. Maublant, J. C., Gachon, P., and Moins, N. (1988). *J. Nuclear Med.* **29,** 48–54.
23. Piwnica-Worms, D., Kronauge, J. F., and Chiu, M. L. (1990). *Circulation* **82,** 1826–1838.
24. Chiu, M. L., Kronauge, J. F., and Piwnica-Worms, D. (1990). *J. Nuclear Med.* **31,** 1646–1654.
25. Maublant, J. C., Zhang, Z., Rapp, M., Ollier, M., Michelot, J., and Veyre, A. (1993). *J. Nuclear Med.* **34,** 1949–1952.
26. Carvalho, P. A., Chiu, M. L., Kronauge, J. F., Kawamura, M., Jones, A. G., Holman, B. L., et al. (1992). *J. Nuclear Med.* **33,** 1516–1521.
27. Delmon-Moingeon, L. I., Piwnica-Worms, D., Van den Abbeele, A. D., Holman, B. L., Davison, A., and Jones, A. G. (1990). *Cancer Res.* **50,** 2198–2202.
28. Backus, M., Piwnica-Worms, D., Hockett, D., Kronauge, J., Lieberman, M., Ingram, P., et al. (1993). *Am. J. Physiol.* **265,** C178–C187.
29. Chernoff, D. M., Strichartz, G. R., and Piwnica-Worms, D. (1993). *Biochim. Biophys. Acta* **1147,** 262–266.
30. Henquin, J. C., Gembal, M., Detimary, P., Gao, Z. Y., Warnotte, C., and Gilon, P. (1994). *Diab. Métab.* **20,** 132–137.
31. Duchen, M. R., Smith, P. A., and Ashcroft, F. M. (1993). *Biochem. J.* **294,** 35–42.
32. Hutton, J. C. and Malaisse, W. J. (1980). *Diabetologia* **18,** 395–405.
33. Malaisse, W. J., Sener, A., Malaisse-Lagae, F., Welsh, M., Matthews, D. E., Bier, D. M., et al. (1982). *J. Biol. Chem.* **257,** 8731–8737.
34. Sener, A., Rasschaert, J., Zähner, D., and Malaisse, W. J. (1988). *Int. J. Biochem.* **20,** 595–598.
35. Sener, A., Somers, G., Devis, G., and Malaisse, W. J. (1981). *Diabetologia* **21,** 135–142.
36. Malaisse-Lagae, F., Sener, A., Lebrun, P., Herchuelz, A., Leclercq-Meyer, V., and Malaisse, W. J. (1982). *C. R. Acad. Sci. (Paris)* **294 [III],** 605–607.
37. Malaisse, W. J., Hutton, J. C., Kawazu, S., Herchuelz, A., Valverde, I., and Sener, A. (1979). *Diabetologia* **16,** 331–341.
38. Herchuelz, A., Thonnart, N., Carpinelli, A., Sener, A., and Malaisse, W. J. (1980). *J. Pharmacol. Exp. Ther.* **215,** 213–220.
39. Malaisse-Lagae, F. and Malaisse, W. J. (1984). In: *Methods in Diabetes Research.* Larner, J. and Pohl, S. L. (eds.). Wiley, New York, pp. 147–152.
40. Mangos, J. A., Mesherry, N. R., Butcher, F., Irwin, K., and Barber, T. (1975). *Am. J. Physiol.* **229,** 553–559.
41. Malaisse-Lagae, F. and Malaisse, W. J. (1971). *Endocrinology* **88,** 72–80.
42. Herchuelz, A. and Malaisse, W. J. (1978). *J. Physiol. (London)* **283,** 409–424.