**Endocrine,** *vol. 11, no. 2, 189–193, October 1999* 0969–711X/99/11:189–193/$11.25 

# Short- and Long-Term Effects of β-Cellulin and Transforming Growth Factor-α on β-Cell Function in Cultured Fetal Rat Pancreatic Islets

Åke Sjöholm* and Henrik Kindmark

*Department of Molecular Medicine (L6:01B), Endocrine and Diabetes Unit, The Rolf Luft Center for Diabetes Research, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden*

**The polypeptide β-cellulin, identified in conditioned media from insulinoma cell cultures and produced by pancreatic islet cells, was recently identified as a possible autocrine growth factor for the pancreatic islet β-cell. In this study, we investigated the short- and long-term actions of β-cellulin, and the structurally related transforming growth factor-α (TGF-α), on β-cell function in fetal rat pancreatic islets in vitro. We found that neither β-cellulin nor TGF-α (10 n*M* each), in contrast to glucose (20 m*M*), acutely influenced β-cell levels of cytosolic-free $Ca^{2+}$. Additionally, whereas glucose markedly increased short-term (60-min) insulin release, neither β-cellulin nor TGF-α (10 n*M* each) influenced the rate of hormone secretion at basal (3 m*M*) or stimulatory (20 m*M*) concentrations of glucose. Likewise, long-term (24-h) exposure of islets to a high glucose concentration significantly augmented the secretion of insulin. This effect was slightly potentiated by TGF-α (10 n*M*), but not β-cellulin (10 n*M*), at high (but not low) glucose concentrations. Conversely, the islet insulin content was not significantly affected by β-cellulin or TGF-α at any glucose concentration tested. We conclude that, although β-cellulin is produced by islet cells, the peptide does not seem to be of importance for the regulation of insulin production by isolated pancreatic ß-cells.**

**Key Words:** Pancreatic islet; insulin secretion; β-cellulin; transforming growth factor.

## Introduction

Insulin, secreted exclusively by the pancreatic islet β-cell, is the key hormone in maintenance of normoglycemia; consequently, defects in insulin secretion ultimately may result in diabetes mellitus *(1,2)*. Glucose is a cardinal stimulator of pancreatic β-cell insulin secretion, but the hexose is also an important stimulus for β-cell mitogenesis *(3–8)*. Among other regulators of β-cell replication and function, growth hormone is a potent stimulator of these functions both in vivo and in vitro *(2–5,7)*.

Another peptide growth factor, named β-cellulin, was recently identified as an autocrine or juxtacrine factor produced by insulinoma and mouse islet cells *(9)*. β-Cellulin is a 32-kDa glycoprotein belonging to the epidermal growth factor (EGF) superfamily of growth factors, and seems to be conspiciously abundant in the pancreas *(9–11)*. The peptide, which appears to be proteolytically processed from a larger transmembrane precursor, has been structurally well characterized, and its carboxy-terminal domain was found to share 50% sequence homology with that of rat transforming growth factor-α (TGF-α) *(10)*. Like EGF and TGF-α, β-cellulin is a potent mitogenic stimulus for vascular smooth muscle cells *(9)*. In this study, we evaluated the possibility that β-cellulin and TGF-α may influence short- or long-term β-cell function and insulin production by isolated rat pancreatic islets.

## Results

Figure 1 reveals that acutely raising the ambient glucose concentration from 3 m*M* to 20 m*M* for 60 min evoked a significant stimulation of short-term insulin release. Coaddition of β-cellulin or TGF-α (10 n*M* each) did not influence the rate of insulin released in basal (3 m*M*) or maximally stimulatory (20 m*M*) concentrations of glucose.

Because changes in cytosolic $Ca^{2+}$ regulate insulin secretion *(12–15)*, dynamic changes in $[Ca^{2+}]_i$ were monitored in β-cells loaded with the fluorescent probe Fura-2. It is evident from Fig. 2 that neither β-cellulin (10 n*M*) nor TGF-α (10 n*M*) at basal (3 m*M*) glucose affected $[Ca^{2+}]_i$, whereas raising the glucose concentration to 20 m*M* elicited a brisk rise in $[Ca^{2+}]_i$, thus mirroring the actions of the peptides and the sugar on short-term insulin release. This confirms that the islet cell preparations were functionally intact.

When measuring insulin secretion to the medium in the long term, it was found that islets maintained in 20 m*M*

Received June 4, 1999; Revised August 2, 1999; Accepted August 2, 1999.
Author to whom all correspondence and reprint requests should be addressed: Dr. Ake Sjöholm, Department of Molecular Medicine, Rolf Luft Center For Diabetes Research, Karolinska Institute, Karolinska Hospital (L6:01B), SE-171 76, Stockholm, Sweden. E-mail: ake@enk.ks.se

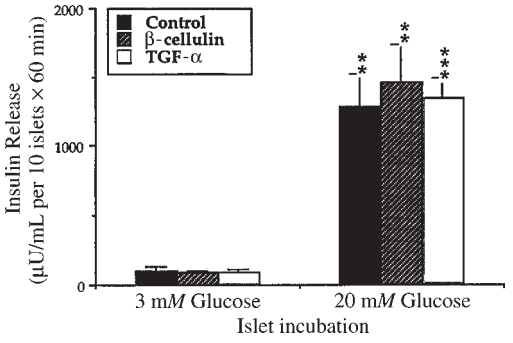


Fig. 1. Short-term effects of β-cellulin, TGF-α, and glucose on β-cell insulin secretion. Fetal rat pancreatic islets were cultured for 7 d in RPMI-1640 medium with 1% fetal calf serum (FCS) in 11.1 m*M* glucose. Insulin release, measured by radioimmunoassay (RIA), was studied in duplicate batches of 10 islets exposed to a 60-min incubation in 3 or 20 m*M* glucose, in the presence or absence of β-cellulin (10 n*M*) or TGF-α (10 n*M*). Bars represent means ± SEM for five observations. ** and ***: $p < 0.01$ and $p < 0.001$, respectively, for a chance difference vs islets incubated in 3 m*M* glucose using analysis of variance (ANOVA).

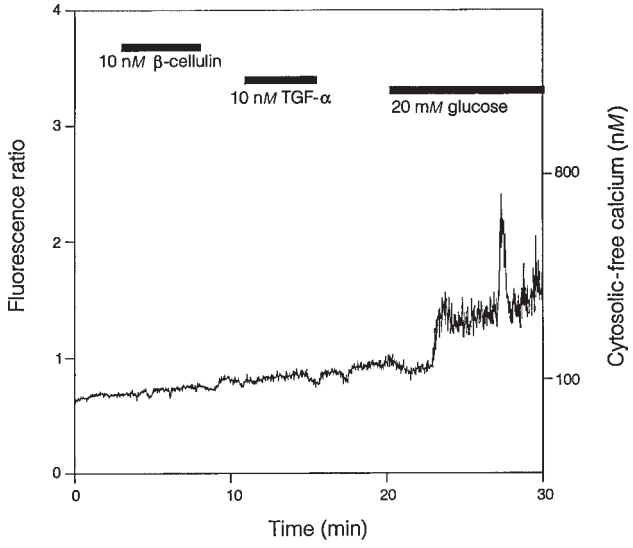


Fig. 2. Glucose, but not β-cellulin or TGF-α, increases β-cell cytoplasmic-free $Ca^{2+}$. Islet cell monolayers on glass cover slips were loaded for 30 min with 1 μ*M* fura-2 AM in culture media at 37°C. Clusters of two to four β-cells were identified in the microscope and selected for $Ca^{2+}$ measurements. Fluorescence measurements were performed at 37°C in an inverted fluorescence microscope, using excitation and emission wavelengths of 340/380 and 510 nm, respectively. Cells were superfused at a flow rate of 0.5 mL/min. All traces shown are typical for experiments repeated with at least three different cell preparations, and $[Ca^{2+}]_i$ is expressed as the ratio of the fluorescence measured at 340 and 380 nm. The ambient glucose concentration (3 or 20 m*M*) is shown at the base the figure. β-cellulin or TGF-α (10 n*M*) is present as indicated by the horizontal bars.

glucose secreted significantly more insulin than those cultured in 3 m*M* glucose (Fig. 3). At the low glucose concentration, neither β-cellulin nor TGF-α (10 n*M* each) affected insulin release. However, at 20 m*M* glucose, TGF-α (10 n*M*) slightly but significantly potentiated the secretory output of insulin in response to the sugar. The islet insulin content was not significantly altered by any of the peptides after 24 hr of culture (not shown).

## Discussion

Circulating polypeptide growth factors constitute important regulators by which mitogenesis and specific functions of various cells can be controlled through binding of the peptides to distinct cell-surface receptors *(16)*. Among the most extensively studied growth factors are the members of the EGF superfamily *(17,18)*. EGF is abundant in plasma and is an important growth factor for many cell types (reviewed in refs. *17* and *18*). An initial study *(19)* suggested that the pancreatic β-cell responds mitogenically to EGF. However, this could not be confirmed by two independent reports *(5,20)*. Nevertheless, EGF receptors, or at least EGF-binding sites, have been localized in the pancreas *(21)*, albeit not unambiguously to the β-cell.

The role of TGF-α, which shares some 40% sequence homology with EGF and functions through the EGF receptor, has been studied in transgenic mice *(22,23)*. In these studies, TGF-α overexpression in the pancreas promoted proliferation of both acinar cells and fibroblasts, leading to a massive interstitial fibrosis and florid acinoductular metaplasia, whereas no consistent abnormalities in islets were noted. In concordance with this, and our present and previous findings *(24)*, in another transgenic mouse model, TGF-α overexpression in the pancreas also failed to increase islet cell mass *(25,26)*. On the other hand, this certifies the

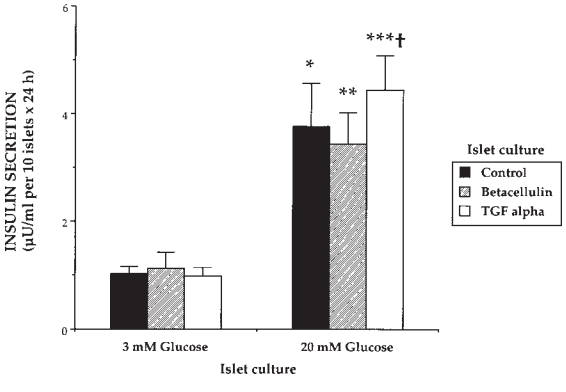


Fig. 3. Long-term effects of β-cellulin, TGF-α, and glucose on β-cell insulin secretion. Fetal rat pancreatic islets were cultured for 7 d in RPMI-1640 medium with 1% FCS in 11.1 m*M* glucose. Insulin secretion to the medium over 24 hr, measured by RIA, was studied in batches of 10 islets cultured in 3 or 20 m*M* glucose, in the presence or absence of β-cellulin (10 n*M*) or TGF-α (10 n*M*). Bars represent means ± SEM for five observations. *, **, and *** denote $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, for a chance difference vs islets cultured in 3 m*M* glucose; and †, denotes $p < 0.05$ vs islets cultured in 20 m*M* glucose without peptide, using ANOVA.

purity of the islet preparations, which, on electron microscopical examination *(27)*, were found to contain >90% β-cells and no fibroblast-like elements or acinar cells.

Here we have examined the effects of a novel growth factor in the EGF superfamily, β-cellulin, on islet β-cell function and compared it to another member of this group, TGF-α. β-Cellulin was originally isolated from conditioned media from insulinoma cell cultures and is able to promote mitogenesis of fibroblast-like cell types *(9)*. Additionally, it is produced by mouse pancreatic islet cells, suggesting that the peptide may exert a physiological role as an autocrine or juxtacrine factor regulating hormone secretion by the insulin-producing pancreatic β-cell *(9)*. It was the aim of this study to determine whether β-cellulin or TGF-α exert any short- or long-term actions on islet β-cell function in vitro. Our results, however, indicate that this seems not to be the case, at least not in cultured rat β-cells. Thus, although these cells responded excellently to glucose stimulation in terms of increased $[Ca^{2+}]_i$ and augmented insulin output, no discernable effects of the peptide growth factors β-cellulin or TGF-α could be detected even at such high concentrations as 10 n*M*, which promote fibroblast proliferation maximally. The only positive finding in this study was that TGF-α slightly amplified the long-term secretory response to high glucose. The mechanisms by which this small stimulatory effect occurs remain elusive at this stage, but could involve recruitment of protodifferentiated precursor cells of ductular origin in the fetal islets into mature β-cells, as has been reported in transgenic mouse models *(25)*.

Like the others members of the EGF family, β-cellulin is a ligand for the receptor tyrosine kinases encoded by the *erb*B gene family, more specifically for the EGF receptor and *erb*B-4 *(26)*. Additionally, through heterodimerization and cross-phosphorylation, β-cellulin may also activate *erb*B-3. Previous studies have shown that β-cellulin may exert a differentiating effect on certain cell lines. For instance, the amylase-secreting rat pancreatic AR42J cell line possesses exocrine and neuroendocrine properties *(28)*. When these cells were incubated with β-cellulin, a small portion of the cell population was found to stain positively for insulin and express insulin mRNA *(28)*. Additionally, glucokinase and the facilitative glucose transporter protein GLUT-2 were induced in these cells by β-cellulin *(28)*. Moreover, functional differentiation was acquired, including regulated insulin secretion stimulated by potassium, hypoglycemic sulfonylureas, carbachol, and glucagon-like peptide 1, thus resembling the situation in the native β-cell *(28)*.

In another model system, alpha TC1 clone 6–derived transfectant cells expressing the islet-specific transcription factor PDX-1 were cultured in the presence of β-cellulin *(29)*. This treatment made these normally insulin-negative cells express mRNAs for both insulin and glucokinase *(29)*. As in the AR42J cells, this effect of β-cellulin could not be mimicked by EGF or TGF-α, suggesting that β-cellulin may signal through a distinct, specific cell-surface receptor, a possibility also indirectly supported by our present results.

In a recent article *(30)*, it was also shown that human β-cellulin can function as a growth factor for the relatively differentiated rat insulinoma cell line INS-1 under serum-starved conditions, e.g., half-maximal stimulation of proliferation obtained with 25 p*M* β-cellulin. However, β-cellulin failed to affect insulin production by these cells, thus resembling the results of our present study.

In conclusion, the present study shows that islet β-cell function — in terms of $Ca^{2+}$ handling and insulin secretion — is not significantly altered by β-cellulin in the short or long term in vitro. Future experiments will reveal whether islet β-cellulin expression is modulated by physiological stimuli or inhibitors of β-cell mitogenesis and insulin production.

## Materials and Methods

### *Reagents and Chemicals*

Human recombinant β-cellulin was the kind gift of Dr. Yuen W. Shing and Prof. Judah Folkman, Harvard Medical School, Boston, MA, and synthetic rat TGF-α was obtained from Sigma, St. Louis, MO. Collagenase type CLS (EC 3.4.24.3) was purchased from Boehringer-Mannheim, Mannheim, Germany. Culture medium RPMI-1640, FCS, L-glutamine, benzylpenicillin, and streptomycin were from Flow, Irvine, UK. Antiporcine insulin serum was raised in guinea pigs in our laboratory *(31)*. Crystalline rat insulin was from Novo, Copenhagen, Denmark, and porcine $^{125}$I-insulin was made in our laboratory. All other chemicals of analytical grade were obtained from E. Merck, Darmstadt, Germany.

### *Fetal Islet Preparation and Culture*

Pregnant Wistar rats purchased from B & K Universal (Sollentuna, Sweden) were killed by cervical dislocation on d 21 of gestation and the fetuses rapidly removed. Fetal rat islets were prepared from pancreatic glands as previously described *(27,32–35)*. Briefly, the pancreata were finely chopped and digested for a short time with collagenase. The carefully washed digest was plated in culture dishes allowing cell attachment (Nunc, Roskilde, Denmark) and cultured for 5 d at 37°C in a humidified atmosphere of 5% $CO_2$ in ambient air in RPMI-1640 medium containing 11.1 m*M* of glucose, 10% FCS, 2 m*M* L-glutamine, 100 U/mL of benzylpenicillin, and 0.1 mg/mL of streptomycin. At the end of the culture period, groups of islets were transferred to fresh media containing 1% FCS and cultured free-floating overnight, a procedure that minimizes fibroblast proliferation. Spherical islets, free of connective tissue, were then selected under a stereomicroscope and used for the different analyses described next.

### *$[Ca^{2+}]_i$ Measurements*

Islets were cultured as just described. Monolayers of cells were prepared as described *(36)* by shaking in a $Ca^{2+}$-free medium containing EGTA and cultured

overnight on plastic cover slips. They were then loaded for 30 min with 1 μ*M* fura-2 AM in culture media at 37°C. After rinsing, the cover slips were placed as the bottom of an open perifusion chamber (150 μL-vol) connected to a two-channel peristaltic pump, allowing constant superfusion of cells. The chamber was mounted on a thermostatically controlled stage of an inverted fluorescence microscope (Zeiss Axiovert 35M, Berlin, Germany) controlled by a microscope system processor (MSP 21, Zeiss). Fluorescence measurements were performed at 37°C, using excitation and emission wavelengths of 340/380 and 510 nm, respectively. Cells were superfused at a flow rate of 500 μL/min with a buffer (pH 7.4) containing 125 m*M* NaCl, 5.9 m*M* KCl, 1.3 m*M* $CaCl_2$, 1.2 m*M* $MgCl_2$, 3 m*M* D-glucose, 25 m*M* HEPES, and 1 mg/mL of bovine serum albumin (BSA). Additions of peptides and other substances were made in the same buffer. Clusters of two to four β-cells were localized with the microscope and selected for $Ca^{2+}$ measurements. Occasional fibroblast-like elements were identified, but were avoided. All traces shown are typical for experiments repeated with at least three different cell preparations, and $[Ca^{2+}]_i$ is expressed as the ratio of the fluorescence measured at 340 and 380 nm.

### *Insulin Secretion and Islet Insulin Content*

For short-term insulin release experiments, duplicate batches of 10 islets were selected and preincubated at 37°C for 45 min in a bicarbonate buffer *(37)*, supplemented with 2 mg/mL of BSA, 3 m*M* glucose, and 10 m*M* HEPES (pH 7.4). The preincubation media were discarded and incubations continued for another 60 min in fresh buffer now containing the desired test substances. Media were frozen for subsequent analysis of their insulin concentration *(31)*.

For long-term insulin secretion studies, groups of 10 islets each were cultured for 24 hr in 48-well plates containing 500 μL of RPMI-1640 medium with 1% FCS, 3 or 20 m*M* glucose, and β-cellulin or TGF-α (10 n*M* each). Aliquots of culture media were frozen for subsequent insulin analysis by RIA *(31)*. The islet insulin content (extracted from sonicates overnight at 4°C in 70% ethanol plus 0.18 *M* HCl) was measured radioimmunologically *(31)*.

### *Statistical Analysis*

Results presented are derived from five independent experiments performed on different days. Means ± SEM were calculated and groups of data compared using ANOVA.

## Acknowledgments

We thank Elvi Sandberg for skillful technical assistance. Human recombinant β-cellulin was graciously donated by Prof. Judah Folkman and Dr. Yuen W. Shing, Harvard Medical School, Boston, MA, to whom we are also indebted for valuable advice and discussions. Å.S. is the recipient of the 1994 Eli Lilly/EASD Research Fellowship in Diabetes and Metabolism. Financial support was received from the Swedish Medical Research Council, Torsten and Ragnar Söderberg Foundations, Barndiabetesfonden, Funds of the Karolinska Institute, Tore Nillson's Fund, Novo-Nordisk Sweden Pharma AB, the Swedish Society of Medicine, Åke Wiberg's Foundation, Magn. Bergvall's Fund, Harald and Harald and Civeta Jeanssonn Foundations, The Swedish Diabetes Association, Fredrik and Inger Thuring's Foundation and Syskonen Svensson's Fund.

## References

1. Efendić, S., Luft, R., and Wajngot, A. (1984). *Endocrine Rev.* **5,** 395–410.
2. Sjöholm, Å. J. (1996). *Intern. Med.* **239,** 211–220.
3. Sjöholm, Å. (1993). *Am. J. Physiol.* **264,** C501–C518.
4. Sjöholm, Å. and Hellerström, C. (1991). *Am. J. Physiol.* **260,** C1046–C1051.
5. Sjöholm, Å., Welsh, N., Sandler, S., and Hellerström, C. (1990). *Am. J. Physiol.* **259,** C828–C833.
6. Sjöholm, Å., Funakoshi, A., Efendić, S., Östenson, C.-G., and Hellerström, C. (1991). *Endocrinology* **128,** 3277–3282.
7. Sjöholm, Å. (1992). *FEBS Lett.* **311,** 85–90.
8. Sjöholm, Å. (1997). *Diabetes* **46,** 1141–1147.
9. Shing, Y., Christofori, G., Hanahan, D., Ono, Y., Sasada, R., Igarashi, R., and Folkman, J. (1993). *Science* **259,** 1604–1607.
10. Watanabe, T., Shintani, A., Nakata, M., Shing, Y., Folkman, J., Igarashi, K., and Sasada, R. (1994). *J. Biol. Chem.* **269,** 9966–9973.
11. Seno, M., Tada, H., Kosaka, M., Sasada, R., Igarashi, K., Shing, Y., Folkman, J., Ueda, M., and Yamada, H. (1996). *Growth Factors* **13,** 181–191.
12. Newgard, C. B. and McGarry, J. D. (1995). *Ann. Rev. Biochem.* **64,** 689–719.
13. Sjöholm, Å., Arkhammar, P., Welsh, N., Bokvist, K., Rorsman, P., Hallberg, A., Nilsson, T., Welsh, M., and Berggren, P.-O. (1993). *J. Clin. Invest.* **92,** 1910–1917.
14. Arkhammar, P., Juntti-Berggren, L., Larsson, O., Welsh, M., Nånberg, E., Sjöholm, Å., Köhler, M. and Berggren, P.-O. (1994). *J. Biol. Chem.* **269,** 2743–2749.
15. Larsson, O., Barker, C. J., Sjöholm, Å., Carlqvist, H., Michell, R. H., Bertorello, A., Nilsson, T., Honkanen, R., Mayr, G. W., Zwiller, J., and Berggren, P.-O. (1997). *Science* **278,** 471–474.
16. Rozengurt, E. (1986). *Science* **234,** 161-166.
17. Carpenter, G. and Cohen, S. (1990) *J. Biol. Chem.* **265,** 7709–7712.
18. Burgess, A. W. (1989). *Br. Med. Bull.* **45,** 401–420.
19. Chatterjee, A. K., Sieradzki, J., and Schatz, H. (1986). *Horm. Metab. Res.* **18,** 873–874.
20. Brelje, T. C. and Sorensen, R. L. (1991). *Endocrinology* **128,** 45–57.
21. Chabot, J.-G., Walker, P., and Pelletier, G. (1987). *Pancreas* **2,** 653–657.
22. Jhappan, C., Stahle, C., Harkins, R. N., Fausto, N., Smith, G. H., and Merlino, G. T. (1990). *Cell* **61,** 1137–1146.
23. Sandgren, E. P., Luetteke, N. C., Palmiter, R. D., Brinster, R. L. and Lee, D. C. (1990) *Cell* **61,** 1121–1135.
24. Sjöholm, Å. (1996) *Biosci. Rep.* **16,** 415-423.
25. Wang, T. C., Bonner-Weir, S., Oates, P. S., Chulak, M. B., Simon, B., Merlino, G. T., Schmidt, E. V., and Brand, S. J. (1993). *J. Clin. Invest.* **92,** 1349–1356.
26. Riese, D. J., II, Bermingham, Y., van Raaij, T. M., Buckley, S., Plowman, G. D., and Stern, D. F. (1996). *Oncogene* **12,** 345–353.

27. Hellerström, C., Lewis, N. J., Borg, H., Johnson, R., and Freinkel, N. (1979). *Diabetes* **28,** 769–776.
28. Mashima, H., Ohnishi, H., Wakabayashi, K., Mine, T., Miyagawa, J.-I., Hanafusa, T., Seno, M., Yamada, H., and Kojima, I. (1996). *J. Clin. Invest.* **97,** 1647–1654.
29. Watada, H., Kajimoto, Y., Miyagawa, J., Hanafusa, T., Hamaguchi, K., Matsuoka, T., Yamamoto, K., Matsuzawa, Y., Kawamori, R., and Yamasaki, Y. (1996). *Diabetes* **45,** 1826-1831.
30. Huotari, M.-A., Palgi, J., and Otonkoski, T. (1998) *Endocrinology* **139,** 1494–1499.
31. Grill, V., Pigon, J., Hartling, S. G., Binder, C., and Efendić, S. (1990). *Metabolism* **39,** 251–258.
32. Sjöholm, Å. (1991). *FEBS Lett.* **294,** 257–260.
33. Sjöholm, Å. (1995). *FEBS Lett.* **367,** 283–286.
34. Sjöholm, Å. (1995). *Biochem. Biophys. Res. Commun.* **214,** 224–229.
35. Sjöholm, Å. (1991). *FEBS Lett.* **289,** 249–252.
36. Lernmark, Å. (1974). *Diabetologia* **10,** 431–438.
37. Krebs, H. A. and Henseleit, K. (1932). *Hoppe-Seyler's Z. Physiol. Chem.* **210,** 33–66.