Insulin and Analogue Effects on Protein Degradation in Different Cell Types

DISSOCIATION BETWEEN BINDING AND ACTIVITY*

Janet Fawcett‡‡, Frederick G. Hamel¶¶, Robert G. Bennett¶¶, Zoltan Vajo‡, and William C. Duckworth**

From the ‡Endocrinology Section, Carl T. Hayden Veterans Administration (VA) Medical Center, Phoenix, Arizona 85012, the ¶¶Research Service, VA Medical Center, Omaha, Nebraska 68105, the Division of Diabetes, Endocrinology and Metabolism, University of Nebraska Medical Center, Omaha, Nebraska 68198, and the **Molecular and Cellular Biology Program, Arizona State University, Tempe, Arizona 85287

In adult animals, the major effect of insulin on protein turnover is inhibition of protein degradation. Cellular protein degradation is under the control of multiple systems, including lysosomes, proteasomes, calpains, and giant protease. Insulin has been shown to alter proteasome activity in vitro and in vivo. We examined the inhibition of protein degradation by insulin and insulin analogues (LysB28ProB29-insulin (LysPro), AspB19-insulin (B10), and GluB4GlnB16PheB17-insulin (EQF)) in H4, HepG2, and L6 cells. These effects were compared with receptor binding. Protein degradation was examined by release of trichloroacetic acid-soluble radioactivity from cells previously labeled with [3H]leucine. Short- and intermediate-lived proteins were examined. H4 cells bound insulin with an EC50 of 4.6 × 10⁻¹⁰ M. LysPro was similar. The affinity of B10 was increased 2-fold; that of EQF decreased 15-fold. Protein degradation inhibition in H4 cells was highly sensitive to insulin (EC50 = 4.2 × 10⁻¹⁰ and 1.6 × 10⁻⁹ M, short- and intermediate-lived protein degradation, respectively) and analogues. Despite similar binding, LysPro was 11- to 18-fold more potent than insulin at inhibiting protein degradation. Conversely, although EQF showed lower binding to H4 cells than insulin, its action was similar. The relative binding potencies of analogues in HepG2 cells were similar to those in H4 cells. Examination of protein degradation showed insulin, LysPro, and B10 were equivalent while EQF was less potent. L6 cells showed no difference in the binding of the analogues compared with insulin, but their effect on protein degradation was similar to that seen in HepG2 cells except B10 inhibited intermediate-lived protein degradation better than insulin. These studies illustrate the complexities of cellular protein degradation and the effects of insulin. The effect of insulin and analogues on protein degradation vary significantly in different cell types and with different experimental conditions. The differences seen in the action of the analogues cannot be attributed to binding differences. Post-receptor mechanisms, including intracellular processing and degradation, must be considered.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Endocrinology (CS/111E), Veterans Administration Medical Center, 650 E. Indian School Rd., Phoenix, AZ 85012. Tel.: 602-277-5551 (ext. 6690); Fax: 602-200-6004; E-mail: janet.fawcett@med.va.gov.

The major effect of insulin on whole body protein turnover is inhibition of protein degradation (1). Insulin also stimulates the synthesis of selected proteins and overall protein synthesis under certain conditions such as growth and development (2, 3). In the adult animal, however, total protein synthesis is not increased by insulin, and the protein anabolic effect of the hormone is actually an anti-catabolic property, i.e. inhibition of degradation (1).

The mechanisms of cellular protein degradation and the control of these processes are poorly understood. Currently, the major degradative systems are considered to be the lysosome, the proteasome, and various cytoplasmic and cellular proteases such as the calpain family and a recently described giant protease (4–7), which may functionally overlap proteasome activities. In general, lysosomes appear responsible for degradative processing of most endocytosed proteins and for autophagy under extreme catabolic states (4). The proteasome is a multifunctional organelle with multiple forms, e.g. 20 S and 26 S, which degrades abnormal and targeted proteins (ubiquitin, ATP-dependent pathway) and has various specific functions (antigen processing, etc.) (5). Calpains participate in Ca²⁺-mediated degradative activity (6). Various other proteases have specialized or uncertain roles but have a relatively minor contribution to cellular protein balance.

Overall, cellular protein degradation can also be divided according to the half-lives of the proteins. This is particularly relevant, because many of the techniques for assessing protein turnover involve prelabeling with radioactive amino acids. The duration of labeling, chase, and subsequent release can provide data on turnover of different classes of proteins (e.g. short-lived versus long-lived).

Protein degradation is altered by many factors, including hormones, amino acid levels, metabolic substrates (e.g. glucose), ions, and others (8–14). In addition, tissues such as liver and muscle may interact in regulating degradation in the alternate tissue; for example, amino acids from muscle breakdown may alter hepatic protein degradation. With this degree of complexity and interaction, it is not surprising that the specific effects of insulin on protein degradation and the mechanisms of those effects are not well understood. We (15–17) and others (18) have shown that insulin alters proteasomal activity in broken cell preparations and in intact cells. In recent work, we have shown effects of insulin on ubiquitin-mediated protein degradation (19). The present studies examine the inhibition of protein degradation by insulin and various insulin analogues in three different cell types under different conditions. We have compared these effects to the binding of the different peptides.
**Materials**—The rat hepatoma cell line, H4-II-E, the human hepatoma cell line, HepG2, and the rat skeletal muscle cell line, L6, were purchased from the ATCC (Rockville, MD). Culture media were from Sigma (St. Louis, MO), fetal calf serum (FCS) and gentamicin were from Life Technologies Inc. (Gaithersburg, MD). Biosynthetic human insulin, LysB28,ProB29-insulin (LysPro), AspB10-insulin (B10), and GluB4,GlnB16,PheB17-insulin (EQF) and 125I-insulin (labeled in the A14 tyrosine position) were gifts of Dr. R. Chance (Lilly Research Laboratories). [3H]Leucine was from Amersham Pharmacia Biotech (Piscataway, NJ). All other chemicals were of at least reagent grade.

**Cell Culture**—L6 myoblasts were plated in 24-well culture dishes at a density of 7000 cells/cm². Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FCS and 10 μg/ml gentamicin. Cells were incubated for 37 °C in an atmosphere of 5% CO₂/95% air. The medium was changed every 2–3 days. L6 myoblasts spontaneously differentiate upon confluency and fuse to form multinucleated myotubes (20). Cells are used when differentiated (10 days after plating). H4 cells and HepG2 cells were grown in 24-well plates (starting density = 3.4 × 10⁴ cells/cm²). The growth medium consisted of Eagle’s minimum essential medium (MEM) with 10% FCS and 10 μg/ml gentamicin. Cells were incubated at 37 °C in an atmosphere of 5% CO₂/95% air. The medium was changed every 2–3 days, and the cells were used when confluent (~5 days).

**Binding Assay**—Binding of 125I-insulin to cells was carried out at 4 °C for 3 h. Radioactivity (10–20,000 cpm) was added with either insulin or analogues in serum-free medium containing 0.1% BSA and 2 mM TES, pH 7.5. After incubation unbound material was removed and the cells were washed twice in PBS, pH 7.4. Solubilized cells were counted in a gamma-counter.

**Intermediate-lived Protein Degradation**—Protein degradation was measured as described by Gunn et al. (21) for liver cells with slight modifications. For L6 cells, on day 9 after plating, the growth medium was removed from the cells and replaced with leucine-free DMEM containing 10% FCS and 10 μg/ml gentamicin and 1 μCi/ml [3H]leucine. Cells were incubated for 18 h to allow labeling of cellular proteins with [3H]leucine. The labeling medium consisted of Eagle’s minimum essential medium (MEM) containing 2 μCi unlabeled leucine, 20 mM TES, pH 7.5, and 0.1% BSA and incubated for 3 h at 37 °C (chase time). For some experiments DMEM, which normally contains 20 mM glucose, was replaced with DMEM containing 5 mM glucose (low glucose medium). The incubation was stopped by placing the cells on ice and adding an equal volume of 6 M acetic acid containing 2% Triton X-100 to solubilize the cells. Aliquots of the cell/medium mix were analyzed for protein degradation by precipitation in 10% (final concentration) trichloroacetic acid. Protein degradation was taken as the percent trichloroacetic acid-soluble radioactivity. Assays in hepatoma cells were carried out as described for L6 cells except the cell labeling medium was leucine-free MEM with 10% FCS, 10 μg/ml gentamicin, and 1 μCi/ml leucine. The incubation/chase medium consisted of MEM with 20 mM TES, pH 7.5, 0.1% BSA, and 2 mM leucine. All incubations were identical to the muscle cells.

**Short-lived Protein Degradation**—The effect of insulin on short-lived protein degradation was investigated essentially as described for inter-

---

**TABLE I**

Displacement of [125I] iodoinsulin binding by insulin and analogues

| Binding Ligand | H4 hepatocytes | HepG2 hepatocytes | L6 myotubes | Mean of Three Experiments (nM) |
|----------------|----------------|-------------------|-------------|-------------------------------|
| Insulin        | 4.6            | 4.9               | 24.9        | 82.1                          |
| LysPro B10     | 5.3            | 2.4               | 30.3        | 44.1                          |
| B10            | 2.0            | 0.64              | 16.7        | 60.1                          |
| EQF            | 82.1           | 44.1              | 60.1        |                               |

*p < 0.01 versus insulin.

*p < 0.05 versus insulin.

**EXPERIMENTAL PROCEDURES**

**FIG. 1.** Binding of 125I-insulin to H4 cells. Cells were incubated for 3 h at 4 °C with 10,000 cpm 125I-insulin with and without unlabeled insulin or analogues. Binding was determined as described under “Experimental Procedures.” The data show the mean of three individual experiments carried out in triplicate.

**FIG. 2.** Degradation of short-lived proteins in H4 cells. The dose-response to insulin and the analogues LysPro (A), B10 (B), and EQF (C) are shown. Cells were labeled overnight with [3H]leucine. Cells were then washed, and medium containing excess unlabeled leucine was added with and without insulin or analogues. Cells were incubated for 3 h at 37 °C. Protein degradation was determined as described under “Experimental Procedures.” Cells degraded 15.2 ± 0.6%/3 h of protein in the absence of insulin or analogues. Data show mean ± S.E. of 3–12 experiments.

---

1 The abbreviations used are: FCS, fetal calf serum; LysPro, LysB28,ProB29-insulin; B10, AspB10-insulin; EQF, GluB4,GlnB16,PheB17-insulin; DMEM, Dulbecco’s modified Eagle’s medium; MEM, Eagle’s minimal essential medium; BSA, bovine serum albumin; TES, 2-(3-hydroxyethyl)aminomethanesulfonic acid.
mediate-lived protein degradation except there was no chase period before the addition of insulin or analogues. Following labeling for 18 h with radioactive leucine, cells were rinsed twice with incubation/chase medium, insulin or analogues were added, and the cells incubated at 37 °C for 3 h.

Statistical Analysis—Comparisons between the different conditions were by analysis of variance with Dunnett’s multiple comparison post test. \( p \leq 0.05 \) was taken as significant.

RESULTS

H4-II-E Hepatoma cells—Because insulin effects on cellular function are initiated by receptor binding, the binding of \( ^{125}\text{I}\)iodoinsulin and the effects of native insulin and various analogues were examined. Fig. 1 shows the competitive displacement of \( ^{125}\text{I}\)iodoinsulin by native insulin and three analogues, LysPro, B10, and EQF. Insulin and LysPro are similar. B10 has a 2-fold increased affinity and EQF a 15-fold reduced affinity as compared with insulin. The EC50 values are shown in Table I.

Fig. 2A shows that H4 cells are very sensitive to the inhibition of protein degradation by insulin (Table II), as has also been seen for other actions of insulin (22). LysPro and B10 are even more effective (\( p < 0.001 \) versus insulin). Despite the significant differences in binding, EQF had an effect similar to insulin on protein degradation (Table II). The studies shown in Fig. 2 were done with overnight labeling and a 3-h incubation with insulin or analogues in complete medium as described under “Experimental Procedures.” Several aspects of the results are notable, including the very high sensitivity to insulin and the differences in activity of the analogues relative to their binding potencies as compared with insulin.

The studies were repeated using a modification of the experimental protocol. In the approach shown in Fig. 2 the degradation of both short- and intermediate-lived proteins is measured. To remove the contribution of the rapidly turning over proteins and to focus on proteins with longer half-lives, a 3-h chase period was added, and the incubation with insulin or analogues was extended to 4 h (Fig. 3). Under these conditions the curves

| Cell type | Assay type | Insulin EC50 | LysPro EC50 \( \times 10^\text{nm} \) | B10 EC50 \( \times 10^\text{nm} \) | EQF EC50 \( \times 10^\text{nm} \) |
|-----------|------------|--------------|----------------|----------------|----------------|
| H4        | Short-lived| 0.042        | 0.0023 \( b \) | 0.0041 \( b \) | 0.069          |
|           | Intermediate-lived| 0.16 | 0.014 \( b \) | 0.023 \( c \) | 0.13          |
| HepG2     | Short-lived| 1.4          | 1.3           | 0.55           | 24.6 \( d \) |
|           | Intermediate-lived| 1.3 | 0.92         | 1.9           | 43.0          |
| L6        | Short-lived| 4.0          | 1.4           | 1.7           | 24.0          |
|           | Intermediate-lived| 6.4 | 4.6          | 1.0           | 14.2          |

\( a \) Short-lived: overnight labeling, 3h incubation; intermediate-lived: overnight labeling, 3h chase, 4h incubation.

\( b \) \( p < 0.001 \) versus insulin.

\( c \) \( p < 0.05 \) versus insulin.

\( d \) \( p < 0.01 \) versus insulin.
are shifted to the right, indicating lesser sensitivity for insulin and LysPro (Fig. 3A). Despite this, LysPro remains more effective than insulin (Table II). B10 (Fig. 3B) is significantly more effective than insulin under these conditions (Table II). EQF (Fig. 3C) remains equivalent to insulin (Table II). Thus, discrepancies between binding and activity for LysPro and EQF, but not for B10, remain. For this reason, studies were done in a different cell line.

**HepG2 Cultured Hepatocytes**—Fig. 4 shows competitive displacement of [125I]iodoinsulin by insulin and analogues from HepG2 cells. The pattern is similar to H4 cells with insulin and LysPro being equivalent and with B10 having an increased and EQF a marked decrease in apparent affinity (Table I).

Fig. 5 compares the effectiveness of the analogues with insulin on the inhibition of short-lived protein degradation in HepG2 cells. Insulin, LysPro (Fig. 5A), and B10 (Fig. 5B) are very similar (Table II). EQF (Fig. 5C) is much less effective (p < 0.01).

To examine intermediate-lived proteins, a 3-h chase and a 4-h incubation were done (Fig. 6). In these studies, the dose-response curve for EQF was different from insulin (Fig. 6C), and the EC50 was significantly higher (Table II). Insulin, LysPro, and B10 were equivalent.

**L6 Cells**—Fig. 7 shows binding of [125I]-insulin to L6 myotubes and displacement by insulin and analogues. The EC50 values are shown in Table I. Although the affinities followed the same relative order as with other cells, the absolute differences were less and no statistical significance was seen among the materials. Protein degradation was examined for short-lived (Fig. 8) and intermediate-lived (Fig. 9) proteins. Figs. 8A and 9A show insulin and LysPro to be equal, Fig. 9B shows an increased potency of B10, and EQF is less potent (Figs. 8C and 9C) (Table II).

**DISCUSSION**

Although the effect of insulin to inhibit protein degradation is well established in both *in vivo* and *in vitro* studies, the mechanisms by which the hormone has this effect and the proteolytic pathways involved are unclear. Protein degradation is a complex set of processes involving multiple pathways and multiple classes of proteins with different turnover rates. Insulin has variable effects on the different processes. The pres-
ent series of studies demonstrates this complexity. We compared the effects of insulin on protein degradation under different conditions with several insulin analogues in various cell types. The rapidly absorbed analogue LysPro (23) was examined along with two other analogues that have altered binding and processing characteristics. B10 has an increased binding affinity (24), but reduced susceptibility to cellular processing and degradation (25). The B10 position is an early cleavage site for the degradation of insulin (26) and the Asp for His substitution alters cellular processing in several ways, including slower receptor dissociation and intracellular processing (25). EQF has several substitutions that decrease both binding and susceptibility to degradation (27). The B16 site, which is altered in EQF insulin, is one of the earliest cleavage sites in insulin (26). This study confirms the increased binding affinity of B10 (24), the reduced receptor binding of EQF (27), and the unaltered binding of LysPro. Similar results were seen with H4 hepatocytes, HepG2 hepatocytes, and L6 myotubes.

These studies illustrate the complexities in cellular protein degradation and the effects of insulin. The results varied with study conditions and cell type. H4 cells were very sensitive to insulin. Under conditions that measured the turnover of both short- and intermediate-lived proteins, an extremely sensitive insulin effect was seen \( \text{EC}_{50} = 4.2 \times 10^{-11} \text{M} \). When a preincubation was added to eliminate rapidly turning over proteins, the \( \text{EC}_{50} \) increased 4-fold \( (1.6 \times 10^{-10} \text{M}) \) but remained very sensitive (22). The binding affinity is typical \( (4.6 \times 10^{-9} \text{M}) \) (28).
This shows a dissociation between binding and activity. The biological effects of insulin and analogues on protein degradation relative to binding are shown in Table III. No consistent relationship between binding and biological activity on protein degradation by the analogues was seen. This is in contrast to the high correlation between insulin receptor binding and glucose transport (29) and lipogenesis (30–34). This is true for the analogues used here (29, 31, 33–35) and other insulin analogues (29–35). We also found that glucose incorporation into glycogen in L6 cells and H4 cells correlate with insulin analogues (29–35). We also found that glucose incorporation into glycogen in L6 cells and H4 cells correlate with receptor binding.2

Of equal note is that the results varied in different cell types and with different experimental conditions. In general, H4 cells responded better to the analogues than to insulin, and HepG2 cells responded less well to the analogues. Relative to binding, LysPro was 13–21 times more effective than insulin in H4 cells but only half as effective in HepG2 cells. EQF was up to 22 times more effective in H4 cells but less than half as effective in HepG2 cells. The simple conclusion is that differences in action of insulin analogues on protein degradation cannot be attributed solely to their receptor binding.

These results illustrate the importance of factors beyond receptor binding in the actions of insulin. LysPro insulin binds to the insulin receptor with an affinity identical to insulin (Ref. 23 and our results). The effects of this analogue on glucose metabolism are identical to insulin (34, 36). Other effects, including cell growth and mitogenesis, are also similar to insulin (27, 34). B10 has an increased affinity for the insulin receptor and correspondingly greater effects on glucose metabolism and on mitogenesis (29, 34). The latter effect has been attributed to increased IGF-I receptor binding (29).

In the present study LysPro was more potent than insulin in decreasing protein degradation in H4 cells but not HepG2 or L6 cells (Table II). B10 had a greater effect than insulin in H4 cells but not in HepG2 cells despite the increased binding of B10 in HepG2 cells. EQF had a markedly decreased binding affinity in both hepatic cell lines but had effects similar to insulin on protein degradation in H4 cells. In HepG2 cells EQF was less potent than insulin in inhibiting protein degradation. Overall, there was a very poor correlation between binding and inhibition, which extended across all cell types for all analogues.

Previous studies have shown that biological activity (glucose transport and metabolism, cell growth, and mitogenesis) and insulin receptor binding activity correspond closely for insulin and most analogues (24, 27, 29–35). In the present study of insulin effects on protein degradation, these correlations break down. The activities of the analogues relative to binding vary among different cell types and with different assay conditions (Table III). In H4 cells all of the analogues tended to have increased activity relative to binding, when compared with insulin. EQF had a considerably greater effect than insulin on intermediate-lived proteins relative to binding. In contrast, in HepG2 cells, another cultured hepatocyte line, the analogues tended to have less activity relative to binding, as compared with insulin. Again there were differences between effects on intermediate- and short-lived proteins. EQF was much less effective on short-lived protein degradation and B10 less effective on intermediate lived proteins. LysPro was very similar to insulin. Lastly, in L6 cells, EQF was less effective than insulin on short-lived protein degradation but equally effective when intermediate protein degradation was measured. LysPro and B10 were similar to insulin when activity relative to binding was considered. This lack of correlation demonstrates that post-receptor binding events must be involved.

The conclusion that the control of protein degradation by insulin requires more than just receptor binding is supported by previous literature. Dranžin’s group (37) and our laboratory (38) reported that cellular insulin processing and degradation were required for the effect of insulin on protein degradation. We have shown direct effects of insulin on isolated proteasomal activity (15, 16, 19), raising the possibility that intracellular insulin or insulin degradation products (39) may play a role in mediating some of the effects of insulin on protein degradation. In studies of cellular processing of the analogues used in this study, the correlation between cellular processing and effect on protein degradation is much greater than that between binding and activity.3 The present study has shown that the effects of insulin and various insulin analogues on protein degradation vary significantly in different cell types and with different experimental conditions. These differences cannot be explained by alterations in receptor binding. Post-receptor mechanisms, including intracellular processing and degradation, deserve evaluation.

REFERENCES
1. Rooyackers, O. E., and Nair, K. S. (1997) Annu. Rev. Nutr. 17, 457–485
2. Kimball, S. R., Horetsky, R. L., and Jefferson, L. S. (1998) Am. J. Physiol. 274, C221–C228
3. Thompson, M. G., and Palmer, R. M. (1998) Cell. Signal. 10, 1–11
4. Bohley, P., and Seglen, P. O. (1992) Exp. Cell Res. 194, 425–430
5. Coux, O., Tanaka, K., and Goldberg, A. L. (1996) Annu. Rev. Biochem. 65, 801–847
6. Sorimachi, H., Ishii, S., and Suzuki, K. (1997) Biochem. J. 328, 713–722
7. Yao, T., and Cohen, R. E. (1999) Curr. Biol. 9, R543–R547
8. Breyman, R. J., and Bond, J. S. (1986) Annu. Rev. Physiol. 51, C141–C152
9. Mortimore, G. E. (1982) Nutr. Rev. 40, 1–12
10. Mortimore, G. E., Poso, A. R., and Lardeux, B. R. (1989) Diabetes Metab. Rev. 5, 49–70
11. Garlick, P. J., McNurlan, M. A., Barks, T., Lang, C. H., and Gelato, M. C. (1998) J. Nutr. 128, 3565–3569
12. Fulka, R. M., Li, J. B., and Goldberg, A. L. (1975) J. Biol. Chem. 250, 290–298
13. Kettelhut, I. C., Wing, S. S., and Goldberg, A. L. (1988) Diabetes/Metabolism Rev. 4, 751–772
14. Mitch, W. E., and Goldberg, A. L. (1996) N. Engl. J. Med. 335, 1897–1905
15. Bennett, R. G., Hamel, F. G., and Duckworth, W. C. (1997) Diabetes 46, 197–203
16. Duckworth, W. C., Bennett, R. G., and Hamel, F. G. (1994) J. Biol. Chem. 269, 24375–24380
17. Hamel, F. G., Bennett, R. G., Harmon, K. S., and Duckworth, W. C. (1997) Biochem. Biophys. Res. Commun. 234, 671–674
18. Li, B. G., Fang, C. H., and Hasselgren, P. (2000) Int. J. Biochem. Cell Biol. 32, 677–685
19. Bennett, R. G., Hamel, F. G., and Duckworth, W. C. (2000) Endocrinology 141, 2508–2517

2 J. Fawcett, F. G. Hamel, R. G. Bennett, Z. Vajo, and W. C. Duckworth, unpublished observation.

3 J. Fawcett, F. G. Hamel, R. G. Bennett, Z. Vajo, and W. C. Duckworth, manuscript in preparation.
20. Mandel, J.-L., and Pearson, M. L. (1974) *Nature* 251, 618–620
21. Gunn, J. M., Clark, M. G., Knowles, S. E., Hopgood, M. F., and Ballard, F. J. (1977) *Nature* 266, 58–60
22. Kelley, D. S., Becker, J. E., and Potter, V. (1978) *Cancer Res.* 38, 4591–4600
23. Hallemann, F., and Hoenkstra, J. B. L. (1997) *N. Eng. J. Med.* 337, 176–183
24. Sliker, L. J., Brooke, G. S., DiMarchi, R. D., Flora, D. B., Green, L. K., Hoffmann, J. A., Long, H. B., Fan, L., Shields, J. E., Sundell, K. L., Surface, P. L., and Chance, R. E. (1997) *Diabetologia* 40, S54–S61
25. Hanel, F. G., Siford, G. L., Fawcett, J., Chance, R. E., Frank, B. H., and Duckworth, W. C. (1998) *Endocrine Rev.* 19, 688–624
26. Sliker, L. J., Brooke, G. S., Chance, R. E., Fan, L., Hoffmann, J. A., Howey, D. C., Long, H. B., Mayer, J., Shields, J. E., Sundell, K. L., and DiMarchi, R. D. (1994) in *Current Directions in Insulin-like Growth Factor Research* (LeRoith, D., and Raizada, M. K., eds) Vol. 343, pp. 25–32, Plenum Press, New York
27. Ballard, F. J., Wong, S. S., Knowles, S. E., Partridge, N. C., Martin, T. J., Wood, C. M., and Gunn, J. M. (1980) *J. Cell. Physiol.* 105, 335–346
28. Hansen, B. F., Danielsen, G. M., Drejer, K., Sørensen, A. R., Wiberg, P. C., Klein, H. H., and Lundemose, A. G. (1996) *Biochem. J.* 315, 271–279
29. Chu, Y. C., Zong, L., Burke, G. T., and Katsoyannis, P. G. (1992) *J. Protein Chem.* 11, 571–577
30. Burke, G. T., Hu, S. Q., Ohta, N., Schwartz, G. P., Zong, L., and Katsoyannis, P. G. (1990) *Biochem. Biophys. Res. Commun.* 173, 982–987
31. Svoboda, I., Brandenburg, D., Barth, T., Gattner, H. G., Jiracek, J., Veleg, J., Blaha, I., Uhnk, K., Kasirka, V., Pespeck, J., and Hrbas, P. (1994) *Biol. Chem. Hoppe-Seyler* 375, 373–378
32. Vslund, A., Brange, J., Drejer, K., Jensen, I., Markussen, J., Ribel, U., Sørensen, A. R., and Schlichtkrull, J. (1991) *Diabet. Med.* 8, 839–847
33. Kurtzhals, P., Schaffer, L., Sørensen, A., Kristensen, C., Jonassen, I., Schmid, C., and Trub, T. (2000) *Diabetes* 49, 999–1005
34. Howey, D. C., Bowsher, R. R., Brunelle, R. L., and Woodworth, J. R. (1994) *Diabetes* 43, 396–402
35. Draznin, B., and Trowbridge, M. (1982) *J. Biol. Chem.* 257, 11988–11993
36. Peavy, D. E., Edmondson, J. W., and Duckworth, W. C. (1984) *Endocrinology* 114, 753–760
37. Hart, F. G., Fawcett, J., and Duckworth, W. C. (2000) *Diabetes* 49, A26 (abstr.)