GLUT-4 Phosphorylation and Its Intrinsic Activity

MECHANISM OF Ca²⁺-INDUCED INHIBITION OF INSULIN-STIMULATED GLUCOSE TRANSPORT

Najma Begum, Wayne Leitner, Jane E-B. Reusch, Karl E. Sussman, and Boris Draznin

From the Department of Medicine and Research Service of the Veterans Affairs Medical Center and the University of Colorado Health Sciences Center, Denver, Colorado 80220

In this study, we examined the influence of high levels of cytosolic calcium on phosphorylation status and function of GLUT-4 in isolated rat adipocytes. Intracellular calcium was elevated by exposing adipocytes to either extracellular ATP (1.6 mM) or thapsigargin (100 nM). Both agents increased cytosolic calcium 2-3-fold. While basal glucose uptake was unaffected, both ATP and thapsigargin reduced insulin-stimulated glucose transport by 40-70% (p < 0.05). Neither ATP nor thapsigargin affected GLUT-4 content or its translocation from the low density micromeres to the plasma membrane (PM). In contrast, GLUT-4 immunoprecipitated from the PM of adipocytes exposed to either ATP or thapsigargin was phosphorylated to a greater extent than the GLUT-4 isolated from control cells. ATP and thapsigargin also abolished insulin-stimulated phosphorylation of GLUT-4. At the same time, GLUT-4 intrinsic activity was significantly reduced in adipocytes with high levels of cytosolic calcium (p < 0.05). Preincubation of adipocytes with cAMP antagonist, RpcAMP (10⁻⁴ M), and calcium channel blocker, nitrendipine (30 μM), improved the ability of insulin to dephosphorylate GLUT-4 and restored insulin-stimulated GLUT-4 intrinsic activity. We conclude that elevated levels of cytosolic calcium interfere with insulin's ability to dephosphorylate GLUT-4, thus reducing its intrinsic activity.

Recent observations from this laboratory indicated that high levels of cytosolic calcium, [Ca²⁺], in insulin target cells rendered these cells less sensitive to insulin (1-3), thus contributing to insulin resistance. The mechanism of high [Ca²⁺]-induced insulin resistance appears to involve post-receptor steps of insulin action (4). The insulin-initiated phosphorylation cascade begins with activation of the tyrosine kinase of the insulin receptor. Subsequent steps are believed to result in a site-specific phosphorylation and activation of a phosphoserine phosphatase-1 by insulin (5-7). Phosphoserine phosphatase-1 dephosphorylates a number of the intracellular substrates of insulin action including glycogen synthase and GLUT-4 (8-10). Sustained levels of [Ca²⁺], in the insulin target cells inhibit phosphoserine phosphatase-1 activity, at least in part, by the phosphorylation and activation of the inhibitor 1 (11). This effect of high [Ca²⁺], appears to be mediated largely via a cAMP-dependent pathway (11). Inhibition of phosphoserine phosphatase-1 activity results in an impaired dephosphorylation of several insulin-sensitive enzymes and proteins, such as glycogen synthase and GLUT-4 (4, 10). Previous studies from this and other laboratories (12-15) as well as the accompanying article (16) indicate that phosphorylation of GLUT-4 decreases its intrinsic activity.

In this study, we attempted to dissect further the mechanism of high [Ca²⁺]-induced insulin resistance. We used the following two different approaches to elevate [Ca²⁺]: in normal rat adipocytes: extracellular ATP and thapsigargin, a potent inhibitor of Ca²⁺-ATPase (17). We evaluated the effects of these agents on glucose transport, phosphorylation, and distribution of GLUT-4, as well as on GLUT-4 intrinsic activity.

EXPERIMENTAL PROCEDURES

Materials

Porcine insulin was a gift from Eli Lilly Co. [¹H]2-Deoxyglucose, [³²P]orthophosphoric acid, and [¹⁴C]glucose were purchased from Du Pont-New England Nuclear. Collagenase was obtained from Worthington Biochemical Corporation (Freehold, NJ). Reagents for SDS-polyacrylamide gel electrophoresis and immunoblotting were purchased from Bio-Rad; adenosine triphosphate, di-thiothreitol, phenylmethylsufonyl fluoride, leupeptin, benzamidine, bovine serum albumin and 30 mg/di glucose buffer containing 3% bovine serum albumin and 30 mg/di glucose and incubated with either ATP (0.5-1.6 mM) or thapsigargin (25-200 nM) for 30 min at 37 °C.

Methods

Isolated adipocytes from male Sprague-Dawley rats (body weight 150-160 g) were prepared by collagenase digestion (18). Aliquots (1-3 ml) of adipocytes were resuspended in 7-10 ml of Krebs-HEPES buffer containing 3% bovine serum albumin and 30 mg/di glucose and incubated with either ATP (0.5-1.6 mM) or thapsigargin (25-200 nM) for 30 min at 37 °C.

In some experiments, a cAMP antagonist, RpcAMP (10⁻⁴ M), or a calcium channel blocker, nitrendipine (30 μM), were added 10 min prior to the addition of ATP or thapsigargin. At the end of the incubation period, adipocytes were processed for the measurements of [Ca²⁺], CAMP, basal and insulin-stimulated 2-deoxyglucose transport, quantitation of GLUT-4 content, and phosphorylation status in the plasma membrane (PM) and low density microsomal (LDM) fractions as well as GLUT-4 intrinsic activity in the plasma membranes.

Measurement of [Ca²⁺].—Adipocytes were loaded with 3 μM Fura-2AM at 37 °C for 45 min as described earlier (1-3). [Ca²⁺] was
monitored at the excitation wavelength of 340 nm (Fura-2-Ca\(^{2+}\) complex) and 380 nm (free Fura-2AM) and emission at 510 nm, using a Turner spectrofluorometer (1-3).

**Measurement of cAMP Levels—** Intracellular cAMP was extracted from control and experimental adipocytes with ice-cold 5% trichloroacetic acid. Trichloroacetic acid was removed by successive extractions with ether. The levels were then measured by non-acetylation from control and experimental adipocytes with ice-cold 5% trichloroacetic acid.

**Estimation of Glycogen Phosphorylase Activity—** Control, ATP-, or thapsigargin-treated adipocytes were divided into 2 aliquots and incubated with and without insulin (25 ng/ml), followed by the subcellular fractionation as described in the accompanying article (16). PM fractions were reconstituted in transport buffer and used for the assay of intrinsic activity using \[^{14}C\]glucose as described by Weber et al. (22).

**Protein Assay—** The amount of protein was determined by the method of Bradford (23) or by bicinchoninic acid (21).

**Statistics—** The results are presented as mean ± S.E. of four to six individual experiments performed in duplicate. Paired or unpaired Student’s t test was used to compare the mean values among the groups as indicated.

**RESULTS—** In previous studies, we employed either sequential depolarization of cells with 40 mM K\(^+\) or treatment with parathyroid hormone (PTH) (20 ng/ml) to maintain elevations in [Ca\(^{2+}\)], above 200 nM (3, 11, 12). To prove the specificity of [Ca\(^{2+}\)], effects and to investigate the mechanisms of its action, it was important to induce and maintain [Ca\(^{2+}\)], at elevated levels by other mechanisms. In this study, we used extracellular ATP or thapsigargin to induce sustained elevations in [Ca\(^{2+}\)]. ATP elevated [Ca\(^{2+}\)], by increasing mobilization from intracellular pools as well as by increasing [Ca\(^{2+}\)], influx through the action of plasma membrane (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase that acts as a Ca\(^{2+}\) pump (24, 25). Thapsigargin increases [Ca\(^{2+}\)], by inhibiting the intracellular Ca\(^{2+}\)-ATPase (17, 26).

Incubation of isolated rat adipocytes with either ATP (1.6 mM) or thapsigargin (100 nM) increased [Ca\(^{2+}\)], 2–3-fold (Table I). These increases were not affected by either a calcium channel blocker, nitrendipine (30 μM), or a cAMP antagonist, RpcAMP (10 μM). Exposure of adipocytes to the extracellular ATP also resulted in a 3-fold increase in the intracellular cAMP levels (Table I). In contrast, thapsigargin did not affect cellular cAMP levels.

The presence of 1.6 mM ATP did not alter the basal glucose uptake. In contrast, ATP reduced insulin-stimulated glucose transport by 70% (Table II). The ID\(_{50}\) for ATP was 0.4 mM. Despite the inherent instability of ATP and its likely susceptibility to tissue ATPases, this inhibition was effective after

### Table I

| Experiment | [Ca\(^{2+}\)] nm | cAMP fmoc/tube |
|------------|------------------|----------------|
| 1          |                  |                |
| None (control) | 86.48 ± 12.28 | 826.5 ± 35.77 |
| ATP (1.6 mM) | 261.15 ± 92.47 | 224.8 ± 100.39 |
| ATP + Nitrrendipine | 236.64 ± 44.56 | 2068.0 ± 113.39 |
| ATP + RpcAMP | 212.82 ± 30.15 |                  |
| 2          |                  |                |
| None (control) | 121.68 ± 11.35 | 826.5 ± 35.77 |
| Thapsigargin (100 nM) | 256.25 ± 22.59 | 871.8 ± 29.36 |
| Thapsigargin + Nitrrendipine | 244.79 ± 26.38 | 616.2 ± 121.75 |
| Thapsigargin + RpcAMP | 316.43 ± 33.83 |                  |
TABLE II

Effect of ATP and thapsigargin on insulin-stimulated glucose transport

Adipocytes were exposed to ATP (1.6 mM) or thapsigargin (a = 25 nM; b = 100 nM) in the presence or in the absence of either nitrendipine or RpcAMP. Insulin (25 ng/ml) was added and the cells were incubated for 30 min followed by glucose transport assay. Details are given under "Experimental Procedures." Results are the mean ± S.E. of six experiments performed in duplicate. *p < 0.05 versus control. **p < 0.05 versus ATP. §p < 0.05 versus thapsigargin (25 nM). §§p < 0.05 versus thapsigargin (100 nM).

| Experiment | Insulin-stimulated glucose transport (% above basal) |
|------------|---------------------------------|
| 1 None (control) | 700 ± 220 |
| ATP | 213 ± 33* |
| ATP + nitrendipine | 224 ± 34* |
| ATP + RpcAMP | 491 ± 75** |
| 2 None (control) | 1196 ± 94 |
| Thapsigargin (a) | 849 ± 123* |
| Thapsigargin (b) | 740 ± 65* |
| Thapsigargin + nitrendipine | 767 ± 109* |
| Thapsigargin (a) + RpcAMP | 1992 ± 163§ |
| Thapsigargin (b) + RpcAMP | 941 ± 79§ |

FIG. 1. Effect of elevated [Ca2+]i on GLUT-4 protein content and its distribution in subcellular fractions (a representative autoradiogram). Control, ATP-, or thapsigargin-treated adipocytes were divided into two parts and incubated with or without insulin (25 ng/ml) followed by subcellular fractionation to obtain LDM and PM. Proteins (10 μg) were subjected to SDS-polyacrylamide gel electrophoresis and Western blotting with GLUT-4 antibody (R820) followed by detection with chemiluminescent substrate. Lanes 1–4, control cells incubated with (lanes 2 and 4) or without (lanes 1 and 3) insulin (lanes 1 and 2, LDM; lanes 3 and 4, PM). Lanes 5–8, ATP-treated cells incubated with (lanes 6 and 8) or without (lanes 5 and 7) insulin (lanes 5 and 6, LDM; lanes 7 and 8, PM). Lanes 9–12 thapsigargin-treated cells incubated with (lanes 10 and 12) or without (lanes 9 and 11) insulin (lanes 9 and 10, LDM; lanes 11 and 12, PM). Similar results were obtained in three different experiments.

Exposure of cells for only a few minutes and persisted after removal of ATP from the medium (data not shown). Nitrendipine was ineffective in preventing the ATP-induced inhibition of the insulin-stimulated glucose uptake, whereas RpcAMP restored insulin’s effect by 60%. Neither nitrendipine nor RpcAMP affected the basal glucose uptake (not shown).

Treatment of adipocytes with thapsigargin also resulted in a 38% decrease in insulin-stimulated glucose uptake (Table II). The half-maximal effect of thapsigargin was observed at 10 nM. Although thapsigargin did not increase cellular cAMP content (Table I), the presence of RpcAMP completely prevented the thapsigargin-induced inhibition of glucose transport (Table II).

We then examined the effects of ATP and thapsigargin on GLUT-4 cellular distribution, phosphorylation, and its intrinsic activity. Neither ATP nor thapsigargin affected GLUT-4 content or its translocation from the LDM to the PM fraction (Fig. 1). In contrast, studies with 32P-labeled subcellular fractions demonstrated that GLUT-4 immunoprecipitated from the PM of adipocytes exposed to either ATP (Fig. 2A) or thapsigargin (Fig. 2B) was phosphorylated to a greater extent than the GLUT-4 isolated from control cells. This increase in GLUT-4 phosphorylation was seen both in basal and insulin-stimulated states. In control preparations, insulin treatment resulted in significant dephosphorylation of PM GLUT-4. This effect of insulin was abolished in adipocytes treated with ATP or thapsigargin. Results of four independent experiments are summarized in Table III. ATP and thapsigargin increased GLUT-4 specific activity (expressed as the amount of 32P/unit of protein determined by Western blotting) by 30–40% in the basal and insulin-stimulated states. RpcAMP partially prevented ATP and thapsigargin’s effect on GLUT-4 phosphorylation (Table III). Phosphorylation of the LDM GLUT-4 was also increased by either ATP or thapsigargin.

Since our concurrent studies indicated that enhanced phosphorylation of GLUT-4 diminished its intrinsic activity (16), we examined GLUT-4 intrinsic activity in adipocytes treated with either ATP or thapsigargin. Adipocytes were incubated with either ATP or thapsigargin for 30 min followed by a treatment with and without insulin (25 ng/ml). At the end of the incubation time, the PM vesicles were isolated from the control and experimental cells for the measurements of D-[3H]glucose uptake. The ability of GLUT-4 to transport [3H]glucose into the PM vesicles was taken as a measure of its intrinsic activity. ATP and thapsigargin did not alter GLUT-4 intrinsic activity in the absence of insulin, but both agents blocked the insulin-stimulated increases in the intrinsic activity of GLUT-4 (Figs. 3 and 4). Preincubation of adipocytes with RpcAMP (10−4 M) or with RpcAMP and nitrendipine (30 μM) restored insulin-stimulated intrinsic activity of GLUT-4 to normal (Fig. 4).

Discussion

The major finding of this study is that two dissimilar agents that raise [Ca2+], by independent mechanisms inhibited insulin-stimulated glucose transport in normal rat adipocytes, increased phosphorylation of GLUT-4, and inhibited GLUT-4 intrinsic activity. There was no effect of increasing [Ca2+]i on GLUT-4 intracellular localization or translocation to the plasma membrane in response to insulin. These observations lend further support to our previous reports that high levels of [Ca2+], render insulin target cells resistant to insulin (3, 11, 12).

These metabolic effects appear to be specific and causally related to high levels of [Ca2+]. In the present studies, two agents which increased [Ca2+], by different mechanisms induced similar metabolic abnormalities. In previous experi-
Table III
Specific activity of GLUT-4 in control, ATP-, and thapsigargin-treated adipocytes

Autoradiograms of $^{32}$P-labeled GLUT-4 and Western blots of GLUT-4 protein were scanned for optical density and areas beneath the peaks corresponding to GLUT-4 were determined. Specific activities in arbitrary units were calculated by dividing the values of $^{32}$P peaks by those of protein peaks. To compare results from different experiments, specific activity of control LDM was assigned to the peaks corresponding to GLUT-4 were determined. Specific activities in arbitrary units were calculated by dividing the values of $^{32}$P peaks by cell ATP or thapsigargin, nor thapsigargin stimulates Ca$^{2+}$ influx via voltage-dependent channels, nitrendipine predictably failed to exert any effect upon ATP or thapsigargin's actions. In contrast, the cAMP antagonist (RpcAMP) was partially effective in blocking the detrimental effects of sustained levels of Ca$^{2+}$; induced by ATP and thapsigargin. The fact that RpcAMP was effective in preventing thapsigargin's action, while levels of cAMP remained normal, suggests that calcium's action upon GLUT-4 phosphorylation and activity is likely to be mediated via a CAMP-dependent pathway. The precise mechanism of the convergent effects of Ca$^{2+}$ and cAMP is incompletely understood. A similar convergence was described recently by Sheng et al. (27) and Ginty et al. (28). These authors demonstrated that Ca$^{2+}$ phosphorylates transcription factor CREB (CAMP response element-binding protein) at the same site as does CAMP, but presumably via different kinases. Alternatively, high levels of Ca$^{2+}$, which activate a number of Ca$^{2+}$-dependent kinases such as calcium-calmodulin-dependent kinase (23), calcium-phospholipid-dependent kinase (29), etc. Partial restoration of insulin action by the cAMP antagonist may suggest an involvement of the Ca$^{2+}$-dependent kinases that are not inhibited by RpcAMP. Further work is needed to identify the mechanism of cross-talk between Ca$^{2+}$ and cAMP in their effects on phosphoserine phosphatase-1 phosphorylation and activity.

Sustained levels of Ca$^{2+}$, not only increased GLUT-4 phosphorylation in the basal state but also prevented the insulin-induced dephosphorylation of GLUT-4 occurring under normal circumstances (10, 15). Insulin promotes rapid translocation of GLUT-4 from the LDM pool to the PM (10, 12, 14, 30-32) and dephosphorylates GLUT-4 in both compartments (10, 13, 14). It is possible that insulin induces translocation of GLUT-4 and thereby simply dilutes the pool of the phosphorylated PM transporter. This possibility appears to be unlikely, however, since insulin dephosphorylates both PM and LDM GLUT-4 (10). It appears that phosphorylation of GLUT-4 with either calcium or cAMP does not affect the ability of insulin to translocate glucose transporters to the plasma membrane (1). However, phosphorylated GLUT-4 has a lower intrinsic activity than the GLUT-4 dephosphorylated normally by insulin. The influence of GLUT-4 phosphorylation on its reinternalization (13, 14) remains to be determined.

The mechanism whereby high levels of Ca$^{2+}$, inhibit dephosphorylation of GLUT-4 remains incompletely understood. We have previously shown that high levels of Ca$^{2+}$ inhibit the overall phosphoserine phosphatase activity in adipocytes and skeletal muscle (11). This inhibition can occur in one of the two possible ways. In one sequence, high levels of Ca$^{2+}$ stimulate phosphorylation and activation of the

| Treatment | No insulin | + insulin | No insulin | + insulin |
|-----------|------------|-----------|------------|-----------|
| LDM       |            |           |            |           |
| None      | 1.00       | 0.65 ± 0.12* | 1.1 ± 0.50  | 0.60 ± 0.23* |
| ATP       | 1.43 ± 0.16** | 1.25 ± 0.20** | 1.60 ± 0.32** | 1.45 ± 0.40** |
| ATP + RpcAMP | 1.20 ± 0.10*** | 0.89 ± 0.20*** | 1.30 ± 0.40*** | 1.0 ± 0.35*** |
| Thapsigargin | 1.5 ± 0.25§ | 1.40 ± 0.50§ | 1.29 ± 0.50§ | 1.35 ± 0.20§ |
| Thapsigargin + RpcAMP | 1.10 ± 0.20§§ | 0.85 ± 0.20§§ | 1.0 ± 0.20§§ | 0.70 ± 0.23§§ |

PM

| Treatment | No insulin | + insulin |
|-----------|------------|-----------|
| None      | 1.00       | 0.65 ± 0.12* |
| ATP       | 1.43 ± 0.16** | 1.25 ± 0.20** |
| ATP + RpcAMP | 1.20 ± 0.10*** | 0.89 ± 0.20*** |
| Thapsigargin | 1.5 ± 0.25§ | 1.40 ± 0.50§ |
| Thapsigargin + RpcAMP | 1.10 ± 0.20§§ | 0.85 ± 0.20§§ |

Experiments were performed with calcium ionophores (3, 11) and PTH (15) to induce similar changes in Ca$^{2+}$, and observed comparable alterations in glucose transport and GLUT-4 phosphorylation. The calcium channel blocker (nitrendipine) prevented the increases in Ca$^{2+}$, induced by K$^+$ and PTH and restored phosphorylation of GLUT-4 and insulin-stimulated glucose uptake (15). Because neither extracellular ATP nor thapsigargin stimulates Ca$^{2+}$ influx via
phosphorylation of phosphoserine phosphatase-1 and thus reduces its enzymatic activity (33, 34). The effect of calcium on inhibitor 1 appears to be mediated via cAMP-dependent pathways (11). Alternatively, high levels of [Ca\textsuperscript{2+}] may promote direct phosphorylation of phosphoserine phosphatase-1 at the cAMP-sensitive sites (sites 1, 2, and 3). Phosphorylation of site 2 of the regulatory subunit of phosphoserine phosphatase-1 leads to dissociation of the catalytic subunit from the regulatory subunit of this enzyme (35). The enzyme loses its activity when the catalytic subunit released into the cytosol is bound by the activated inhibitor 1 (33, 34). This mechanism of phosphoserine phosphatase-1 inactivation by cAMP has been demonstrated in skeletal muscle in the elegant experiments of Hubbard and Cohen (33, 34) and Dent et al. (35). Whether or not a similar mechanism is operational in adipocytes is not known. Of note, the role of [Ca\textsuperscript{2+}] in the phosphorylation of the phosphoserine phosphatase-1 regulatory subunit has not yet been demonstrated.

The present study confirms our previous observations that phosphorylation of GLUT-4 does not interfere with its translocation from the intracellular pool to the plasma membrane (1). In contrast, phosphorylated GLUT-4 appears to exhibit reduced intrinsic activity. If the intrinsic activity of GLUT-4 is increased by insulin-stimulated dephosphorylation (via phosphorylation and activation of phosphoserine phosphatase-1), then an inhibitory effect of high [Ca\textsuperscript{2+}] on phosphoserine phosphatase-1 may be responsible for the diminished GLUT-4 intrinsic activity in adipocytes treated with ATP or thapsigargin.

In summary, high levels of [Ca\textsuperscript{2+}] in insulin target cells stimulate phosphorylation of GLUT-4 either directly or indirectly by inhibiting its dephosphorylation. Increased phosphorylation of GLUT-4 interferes with its intrinsic activity. This inhibition of GLUT-4 intrinsic activity results in diminution of insulin-stimulated glucose transport in these cells. Thus, we conclude that high levels of [Ca\textsuperscript{2+}] induce insulin resistance at the post-receptor steps of insulin action by interfering with normal dephosphorylation of insulin-sensitive substrates.

Acknowledgments—We thank Gloria Smith for excellent secretarial assistance. We are indebted to Drs. Cushman and Simpson, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, for input, encouragement, and help with setting up the methodology for studying glucose transporter intrinsic activity.

REFERENCES
1. Draznin, B., Lewis, D., Houlder, N., Adamo, M., Garvey, T. W., LeRoith, D., and Sussman, K. (1989) Endocrinology 125, 2341-2349
2. Draznin, B., Sussman, K., Mao, M., Lewis, D., and Sherman, N. (1987) J. Biol. Chem. 262, 14385-14388
3. Draznin, B., Sussman, K. E., Eckel, R. H., Kao, M., Yost, T., and Sherman, N. (1988) J. Clin. Invest. 82, 1549-1552
4. Begum, N., Sussman, K. E., and Draznin, B. (1991) Cell Calcium 12, 423-430
5. Dent, P., Lavoine, A., Nakielny, S., Caudwell, F. B., Watt, P., and Cohen, P. (1990) Nature 348, 302-306
6. Hubbard, M. J., and Cohen, P. (1989) Eur. J. Biochem. 180, 457-465
7. Möller, D., and Flur, J. S. (1981) New Engl. J. Med. 325, 938-948
8. Cohen, P., and Cohen, P. T. W. (1989) J Biol. Chem. 264, 21435-21438
9. Alemamy, S., Pelch, S., Breazer, C., and Cohen, P. (1980) Eur. J. Biochem. 116, 101-110
10. Begum, N., and Draznin, B. (1992) J. Clin. Invest. 90, 1254-1262
11. Begum, N., Sussman, K. E., and Draznin, B. (1990) J. Biol. Chem. 267, 5969-5983
12. James, D. E., Hiken, J., and Lawrence, J. C. Jr. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8368-8372
13. Lawrence, J. C. Jr., Hiken, J. F., and James, D. E. (1990) J. Biol. Chem. 265, 19769-19775
14. Coovers, S., Jaspers, S., and Pasceri, M. (1991) J. Biol. Chem. 266, 9271-9275
15. Reusch, J. E. B., Begum, N., Sussman, K. E., and Draznin, B. (1991) Endocrinology 129, 3269-3273
16. Reusch, J. E. B., Sussman, K. E., and Draznin, B. (1993) J. Biol. Chem. 268, 3346-3351
17. Lyttone, J., Westlin, M., and Hanley, M. R. (1991) J. Biol. Chem. 266, 17097-17097
18. Rodbell, M. (1964) J. Biol. Chem. 239, 375-380
19. Volker, T. V., Viratello, O. M., Delaige, M. A., and Laborisse, J. (1985) Anal. Biochem. 144, 247-253
20. Smith, F. K., Krohn, R. I., Hermanns, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Gokee N. M., Olson, B. J., and Klenk, D. C. (1989) Anal. Biochem. 150, 2324-2332
21. Weber, T. M., Joost, H. G., Simpson, I. A., and Cushman, S. W. (1988) Insulin Receptors (Kahn, C. R., and Harrison, L. C., eds) p. 171, Alan R. Liss, Inc., New York
22. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254
23. Law, S. Y. (1988) J. Biol. Chem. 263, 7850-7856
24. Macir, M. J., Garcia-Rodriguez, C., and Grinstein, S. (1991) J. Biol. Chem. 266, 20809-20862
25. Sheng, M., McFadden, G., and Greenberg, M. E. (1991) Neuron 4, 157-183
26. Ginty, D. D., Glowacka, D., Rader, D. S., Hidaka, H., and Wagner, J. A. (1991) J. Biol. Chem. 266, 14754-14758
27. Cohen, P. (1980) Proc. Soc. Exp. Biol. 151, 115-144
28. Simpson, I. A., and Cushman, S. W. (1988) Annu. Rev. Biochem. 55, 1059-1089
29. Cushman, M. J., and Wardzala, L. J. (1980) J. Biol. Chem. 255, 4158-4162
30. Suzuki, K., and Kono, T. (1988) Proc. Natl. Acad. Sci. U. S. A. 77, 2542-2545
31. Hubbard, M. J., and Cohen, P. (1989) Eur. J. Biochem. 186, 701-709
32. Hubbard, M. J., and Cohen, P. (1989) Eur. J. Biochem. 186, 711-716
33. Dent, P., Campbell, D. G., Caudwell, F. B., and Cohen, P. (1990) FEBS Lett. 259, 281-285