Role of Serine/Threonine Protein Phosphatases in Insulin Regulation of Na\(^{+}/K\(^{+}\)-ATPase Activity in Cultured Rat Skeletal Muscle Cells*  

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Louis Ragolia‡§§, Basil Cherpalis‡§§, Malathi Srinivasan‡§§, and Najma Begum‡§§  
From ‡§§The Diabetes Research Laboratory, Winthrop University Hospital, Mineola, New York 11501 and the §§School of Medicine, State University of New York, Stony Brook, New York 11704  

In this study, we examined the potential role of serine/threonine protein phosphatase-1 (PP-1) and PP-2A in the mechanism of Na\(^{+}/K\(^{+}\)-ATPase activation by insulin in the rat skeletal muscle cell line L6. Incubation of L6 cells with insulin caused a time- and dose-dependent stimulation of ouabain-sensitive plasma membrane Na\(^{+}/K\(^{+}\)-ATPase activity. Pretreatment with okadaic acid (OA; 0.1–1 \(\mu\)M) or calyculin A (1 \(\mu\)M) blocked insulin's effect on Na\(^{+}/K\(^{+}\)-ATPase activation. Low concentrations of OA that specifically inhibit PP-2A were ineffective. Immunoprecipitation of the enzyme from \(^{32}\)P-labeled cells with an antibody directed against the \(\alpha\)-1 subunit of the enzyme revealed a 60% decrease in 110-kDa protein phosphorylation in insulin-treated cells. The presence of calyculin A blocked insulin-mediated dephosphorylation of Na\(^{+}/K\(^{+}\)-ATPase, whereas low concentrations of OA were ineffective. To further confirm the role of PP-1, we used L6 cell lines that overexpress the glycogen/SG-associated regulatory subunit of PP-1, PP-1\(\alpha\). Overexpression of PP-1\(\alpha\) resulted in a 3-fold increase in insulin-stimulated PP-1 catalytic activity. This was accompanied by a 30% increase in basal Na\(^{+}/K\(^{+}\)-ATPase activity and a >2-fold increase in insulin's effect on pump activity. Inhibition of phosphatidylinositol-3 kinase with wortmannin blocked insulin-stimulated PP-1 activation as well as the dephosphorylation and activation of Na\(^{+}/K\(^{+}\)-ATPase. We conclude that insulin regulates the activity of Na\(^{+}/K\(^{+}\)-ATPase by promoting dephosphorylation of the \(\alpha\) subunit via an insulin-stimulated PP-1 and that phosphatidylinositol-3 kinase-generated signals may mediate insulin activation of PP-1 and Na\(^{+}/K\(^{+}\)-ATPase.

Na\(^{+}/K\(^{+}\)-ATPase is a ubiquitous enzyme essential for the maintenance of electrolyte balance, preservation of membrane potential, and control of cellular volume in all tissues (1). This enzyme is composed of a 112-kDa catalytic \(\alpha\) subunit, of which there are three isoforms, and a 35-kDa \(\beta\) subunit (two isoforms) responsible for targeting the \(\alpha\) subunit to the plasma membrane and maintaining its structural integrity (1–2). Recent studies on purified enzyme preparations as well as intact cells indicate that Na\(^{+}/K\(^{+}\)-ATPase activity may be regulated acutely by phosphorylation/dephosphorylation reactions (2–5), whereas the long-term regulation exerted by certain hormones is mediated by changes in gene expression (2, 6). Thus, phosphorylation of the catalytic subunit (\(\alpha\) subunit) of Na\(^{+}/K\(^{+}\)-ATPase by protein kinase A and protein kinase C (PKC) in vitro inhibits enzymatic activity (7). The exact link between the physiological effects and the direct phosphorylation of Na\(^{+}/K\(^{+}\)-ATPase is unclear, however.

Insulin acutely stimulates the activities of ouabain-sensitive \(\alpha\)-1 and \(\alpha\)-2 isoforms of Na\(^{+}/K\(^{+}\)-ATPase in rat skeletal muscle and adipocytes (2, 8–10). In adipocytes, the acute effects of insulin on the Na\(^{+}/K\(^{+}\) pump are due to an increase in the V\(_{\text{max}}\) of the \(\alpha\)-2 isoform and a decrease in the K\(_{\text{mNa}^{+}}\) of the \(\alpha\)-1 isoform (8). In skeletal muscle, insulin promotes translocation of the \(\alpha\)-2 and \(\beta\)-1 subunits to the plasma membranes (9–10). The exact molecular mechanism by which insulin regulates Na\(^{+}/K\(^{+}\) pump activity in these cell types is unclear. In view of the recent reports that Na\(^{+}/K\(^{+}\)-ATPase activity can be regulated by phosphorylation/dephosphorylation mechanisms, we have examined potential roles of serine/threonine protein phosphatase 1 (PP-1) and PP-2A in the mechanism of insulin activation of Na\(^{+}/K\(^{+}\)-ATPase in cultured L6 rat skeletal muscle cells by using two complementary approaches. In the first approach, L6 cells were pretreated with okadaic acid or calyculin A, two cell-permeable inhibitors of PP-1 and PP-2A, followed by insulin treatment and assay of Na\(^{+}/K\(^{+}\)-ATPase activity in plasma membranes. In the second approach, cellular levels of the glycogen/SG-associated regulatory subunit of PP-1 (PP-1\(\alpha\)) were altered by gene transfer techniques to stimulate PP-1 catalytic activity, and the effect of PP-1\(\alpha\) overexpression on insulin-stimulated Na\(^{+}/K\(^{+}\)-ATPase activity was examined. The premise for these studies is our recent finding that overexpression of PP-1\(\alpha\) increases cellular responsiveness to insulin via the activation of PP-1 catalytic function (11). We hypothesized that if PP-1 is indeed involved in insulin activation of Na\(^{+}/K\(^{+}\)-ATPase, then increasing the catalytic activity of PP-1 via its regulatory subunit should increase the activation of Na\(^{+}/K\(^{+}\)-ATPase by enhancing dephosphorylation of the \(\alpha\) subunit.

The results presented in this study indicate that dephosphorylation of the \(\alpha\) subunit of Na\(^{+}/K\(^{+}\)-ATPase by insulin-stimulated PP-1 may be one of the mechanisms by which insulin regulates the activation of the Na\(^{+}/K\(^{+}\) pump in L6 cells.  

MATERIALS AND METHODS

Materials

Cell culture reagents, fetal bovine serum, LipofectAMINE™, calyculin A (cal-A), G418, phosphorylase \(b\), phosphorylase kinase, and antibiotics were purchased from Life Technologies, Inc. [\(\gamma\)\(^{32}\)P]ATP (specific

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‡ To whom correspondence should be addressed: The Diabetes Research Laboratory, Winthrop University Hospital, 259 First St., Mineola, NY 11501. Tel.: 516-663-3915; Fax: 516-663-4780; E-mail: diabetes96@nol.com.

1 The abbreviations used are: PKC, protein kinase C; PP, protein phosphatase; OA, okadaic acid; cal-A, calyculin A; PBS, phosphate-buffered saline; PI3, phosphatidylinositol-3; PKC, protein kinase B; TPA, 12-O-tetradecanoylphorbol-13-acetate.
activity \geq 3000 \text{ Ci/mmol}, [\text{32P}]\text{orthophosphoric acid, and } ^{125}\text{I}-\text{protein A were purchased from Du Pont NEN. Electrophoresis and protein assay reagents were from Bio-Rad. Okadaic acid was from Moana Bioproducts (Honolulu, HI). Oubain, pyruvate kinase, lactate dehydrogenase, ATP, NADH, phospho(en)pyruvate, and all other reagents were from Sigma. Monoclonal antibodies against \alpha-1 (McK1) and \alpha-2 (McB2) subunits of Na\(^+/K\(^+\)-ATPase were kindly given by Dr. Kathleen Swadener (Massachusetts General Hospital, Charlestown, MA). Porcine insulin was a kind gift from Eli Lilly Co. (Indianapolis, IN). The Lac Switch™ inducible mammalian expression system vector pOPi3 was purchased from Stratagene (La Jolla, CA).}

**Experimental Procedures**

**Cell Culture**—Spontaneously fusing L6 rat skeletal muscle cells (a kind gift from Dr. Amira Klip, The Hospital for Sick Children, Toronto, Canada) were seeded in 100-mm dishes at a density of 1 \times 10^6 cells/ml and maintained in \alpha-minimum Eagle’s medium containing 2% fetal bovine serum and 1% antibiotic/antimycotic mixture in an atmosphere of 5% CO\(_2\) at 37 °C as described previously (12). Completely differentiated myotubes were used for all experiments after a 16–18-h starvation in serum-free Dulbecco’s modified Eagle’s medium.

**Generation of Stable Cell Lines Overexpressing PP-1G**—Stable L6 cell lines overexpressing PP-1G were generated by transfection of \(L_6\) myoblasts with PP-1G cDNA using an isopropyl-1-thio-\(\beta\)-galactopyranoside while maintaining expression vector as detailed in our recent publication (11). Experiments were performed on myotubes after a 40-h induction with isopropyl-1-thio-\(\beta\)-galactopyranoside. Normal control represents transfection with an empty expression vector. As detailed in our recent publication (11), transfection per se did not affect the extent of differentiation of \(L_6\) cells as monitored by analysis of myogenin protein and cell morphology.

**Insulin Treatment and Isolation of Crude Plasma Membranes**—Serum-starved myotubes were fed with serum-free medium containing 5 mM glucose and incubated at 37 °C for 1 h before treatment with insulin or other agents. Identical dishes were pulsed with insulin (0.1–1000 nM) for 0–30 min. In some experiments, to evaluate the effects of phosphatase inhibitors on Na\(^+/K\(^+\)-ATPase enzymic activity, the cells were preincubated with okadaic acid (0.1–1000 nM), caly-A (1 \mu M), or tautomycin (3 mM) for 30 min, followed by the addition of insulin (100 nM) for 15 min. At the end of the incubation period, the medium was removed, and the cells were rinsed three times with ice-cold PBS and finally scraped off the dishes in 1–2 ml of ice-cold isolation buffer containing 15 mM Tris base; 5 mM EGTA; 300 mM monomannitol, pH 7.0, with 10 \mu M each of aprotinin, leupeptin, antipain, and soybean trypsin inhibitor; 1 mM benzamidine; and 1 mM phenylmethylsulfonyl fluoride. The cells were sonicated for 10 s and centrifuged at 8000 \( \times \) g for 20 min at 4 °C. The supernatants were centrifuged at 100,000 \( \times \) g for 20 min to pellet the membrane fraction (13). The crude plasma membranes were reconstituted in 200 \mu M of ATP assay buffer containing 100 mM iimidazole, 5 mM MgCl\(_2\), 100 mM NaCl, 150 mM NaCl, 1 mM EGTA, 2 mM sodium azide, and a mixture of the protease inhibitors indicated above. Aliquots of these membrane preparations were used for the assay of proteins and Na\(^+/K\(^+\)-ATPase activity. Unless otherwise stated, all enzymatic assays were performed immediately after the isolation of membrane fractions.

**Assay of Na\(^+/K\(^+\)-ATPase Activity**—Enzyme activity was measured by monitoring the hydrolysis of ATP to ADP using the coupled assay of Barnett (14). Oxidation of NADH to NAD\(^+\) was followed on a Beckman DU 640 spectrophotometer at a wavelength of 340 nm. Specific Na\(^+/K\(^+\)-ATPase activity was determined by immuno-precipitating the subunit of Na\(^+/K\(^+\)-ATPase by Dephosphorylation. The stimulation of Na\(^+/K\(^+\)-ATPase by Insulin—In the initial series of experiments, we studied the kinetics and insulin dose-response of Na\(^+/K\(^+\)-ATPase activity using the enzymatic assay described under “Materials and Methods.” In L6 cell membrane preparations, ouabain-sensitive Na\(^+/K\(^+\)-ATPase activity represents \(-40\%\) of total ATPase activity. Acute exposure of L6 myotubes to 100 nM insulin for 15 min resulted in an approximate 2-fold stimulation of Na\(^+/K\(^+\)-ATPase activity over the basal values (insulin versus basal values, 104.6 ± 8.6 \text{ versus} 53.1 ± 4.83 \mu M of NAD\(^+)\text{ formed/mg protein/h, respectively.} Insulin treatment did not affect ouabain-resistant enzyme activity. Kinetic studies indicate that the half-maximal insulin effect was observed within 5 min of insulin addition, with a peak activation at 15 min with 100 nM insulin (Fig. 1). The effect of insulin on pump activation was transient, returning to basal values at 30 min.

The stimulation of Na\(^+/K\(^+\)-ATPase activity by insulin in concentration dependent with an EC\(_{50}\) of <1 nM insulin, and a maximum effect was seen at a concentration of 100 nM insulin (Fig. 2).
Insulin treatment decreased the amount of 32Pi inhibition of insulin's effect on Na+/K+-ATPase. Preincubation of L6 cells with okadaic acid (1 μM), caly-A (1 μM), or tautomycin (3 nM) for 30 min followed by insulin treatment for 15 min caused a greater than 85% inhibition of insulin's effect on Na+/K+-ATPase activation (Fig. 3). The addition of phosphatase inhibitors 5 min after insulin treatment and a further incubation for 15 min completely reversed insulin's effect on pump activity (Fig. 3; p < 0.05) and decreased the enzyme activity below control levels. OA and caly-A alone caused ~20% decrease in basal Na+/K+-ATPase activity.

Dose-response analysis of OA on Na+/K+-ATPase activation revealed that pretreatment of cells with low concentrations of OA (0.1–10 nM), which are known to specifically inhibit PP-2A (12, 18, 19), did not affect insulin's stimulation of pump activation in L6 cells (Fig. 4). As the concentration of OA was increased to concentrations that inhibit both PP-1 and PP-2A, there was a reduction in insulin's effect on Na+/K+-ATPase. At a concentration of 1 μM that is known to inhibit both PP-1 and PP-2A, OA blocked insulin-stimulated Na+/K+-ATPase activity by 80% (Fig. 4). This data further confirms the results shown in Fig. 3 with tautomycin, another cell-permeable phosphatase inhibitor that completely inhibits insulin-stimulated Na+/K+-ATPase activity. Asterisks denote p < 0.05 versus control; **, p < 0.05 versus insulin. Addition of phosphatase inhibitors at 5 min after insulin stimulation reverses insulin's effect on Na+/K+-ATPase activity. L6 cells were treated with insulin for 5 min, followed by the addition of phosphatase inhibitors for 15 min.

**Fig. 2.** Dose-response of insulin on Na+/K+-ATPase activity. Cells were exposed to varying concentrations of insulin for 15 min, followed by an enzyme assay. Results are the mean of two different experiments, each performed on duplicate dishes.

**Fig. 3.** Phosphatase inhibitors block insulin stimulation of Na+/K+-ATPase activity. L6 cells were pretreated with OA (1 μM), caly-A (1 μM), or tautomycin (3 nM) for 30 min, followed by the addition of insulin (100 nM) for 15 min. Results are the mean ± S.E. of 10 independent experiments performed in duplicate. Asterisks: *, p < 0.05 versus control; **, p < 0.05 versus insulin. Addition of phosphatase inhibitors at 5 min after insulin stimulation reverses insulin's effect on Na+/K+-ATPase activity. L6 cells were treated with insulin for 5 min, followed by the addition of phosphatase inhibitors for 15 min.

**Fig. 4.** Effect of various concentrations of OA on insulin-stimulated Na+/K+-ATPase activity. L6 cells were pretreated with various doses of OA (0–1000 nM) for 30 min before insulin treatment, followed by the assay of Na+/K+-ATPase activity in plasma membranes. Results are the mean ± S.E. of three separate experiments performed in duplicate and are expressed as the percentage of insulin-stimulated Na+/K+-ATPase activity. Asterisks (**) denote p < 0.05 versus insulin.

centrations of OA were ineffective in blocking insulin-induced dephosphorylation (compare lane 4 versus lane 2). The insulin-induced decrease in the phosphorylation status of the α subunit seen in Fig. 5α was not due to variations in the amount of α subunit protein immunoprecipitated from insulin-treated samples (Fig. 5B). Quantitation of the extent of phosphorylation of the α subunit by densitometric analyses of the autoradiograms from different experiments after normalization for α subunit proteins revealed a 60% decrease in phosphorylation status of the 110-kDa α subunit in insulin-treated cells when compared with that of controls (Fig. 5C). Pretreatment with caly-A prevented insulin-mediated reductions in α subunit phosphorylation and caused an increase in the phosphorylation of the catalytic subunit above the basal levels. In contrast, low concentrations of OA did not effectively prevent insulin-stimulated dephosphorylation.

**Overexpression of PP-1α Subunit Increases Basal and Insulin-stimulated Na+/K+-ATPase Activity**—The results of the metabolic labeling studies of the α subunit of Na+/K+-ATPase...
The results presented in this study indicate that the acute stimulatory effects of insulin on Na$^+/K^-$-ATPase activity are mediated by the dephosphorylation of its catalytic $\alpha$ subunit. The activation mechanism observed in the present study is in addition to the previously reported effects of insulin on the $\alpha$-1 isoform of Na$^+/K^-$-ATPase. For this, we used purified preparations of Na$^+/K^-$-ATPase. Lane order is identical to that of Fig. 6A. C, quantitation of the specific activity of the Na$^+/K^-$-ATPase $\alpha$-1 subunit. Autoradiograms of $^{32}$P-labeled $\alpha$-1 subunit and Western blots of $\alpha$-1 protein were scanned for optical density. Specific activities from preparations were assigned a value of 1, and the rest of the data were expressed relative to control specific activity. Results are the mean ± S.E. of four independent experiments. $\ast$, $p < 0.05$ versus control.

The effects of insulin on the dephosphorylation and activation of the $\alpha$ subunit of Na$^+/K^-$-ATPase seem to be mediated via a complex dephosphorylation/activation mechanism. This observation together with our recent studies that treatment of L6 cells with insulin results in a rapid activation of a particulate form of protein phosphatase-1 (PP-1), which is similar to glycerogen/SR-associated PP-1 (PP-1G) present in skeletal muscle; Ref. 20) led us to examine the role of PP-1 in insulin-mediated dephosphorylation and activation of Na$^+/K^-$-ATPase. For this, we used the recently generated L6 cell lines (clones S29 and S34 that overexpress the glycogen/SR-associated regulatory subunit of PP-1, PP-1G). As detailed in our recent publication (11), overexpression of PP-1G did not alter cell growth or morphology but resulted in a small increase in basal PP-1 activity and a >2-fold increase in insulin-stimulated PP-1 catalytic activity when compared with wild-type L6 cells and neo controls carrying an empty expression vector. This increase in PP-1 activity was accompanied by a 30% increase in the basal Na$^+/K^-$-ATPase activity (when compared with wild-type L6 cells and neo controls) and a 2-fold increase in insulin-stimulated ouabain-sensitive Na$^+/K^-$-ATPase activity (Fig. 6). Ouabain-resistant Na$^+/K^-$-ATPase activity was not affected by overexpression of PP-1G (data not shown).

**Inhibition of PI3 Kinase by Wortmannin Abrogates Insulin-stimulated PP-1 Activation and Na$^+/K^-$-ATPase Activity**—To gain insight into the intracellular upstream signaling components that mediate insulin’s effect on PP-1 and Na$^+/K^-$-ATPase activation, we pretreated L6 cells with wortmannin, a selective and potent inhibitor of PI3 kinase. Pretreatment with wortmannin blocked insulin’s effects on PP-1 activation (Fig. 7A) and prevented Na$^+/K^-$-ATPase stimulation by insulin (Fig. 7B) as well as dephosphorylation of its $\alpha$ subunit (Fig. 7B, inset). Wortmannin alone did not affect the basal activities of PP-1 or Na$^+/K^-$-ATPase. In contrast, rapamycin, a p70S6Kinase inhibitor at a concentration of 20 ng/ml did not block insulin’s effect on PP-1 activation and Na$^+/K^-$-ATPase activity (data not shown).

**DISCUSSION**

The results presented in this study indicate that the acute stimulatory effects of insulin on Na$^+/K^-$-ATPase activity are mediated by the dephosphorylation of its catalytic $\alpha$ subunit. The activation mechanism observed in the present study is in addition to the previously reported effects of insulin on the $K_{Na}^{0.5}$ and $V_{max}$ of the $\alpha$-1 and $\alpha$-2 isoforms, respectively, in adipocytes and the subcellular redistribution of the enzyme in skeletal muscle cells (7–10).

The effects of insulin on the dephosphorylation and activation of the $\alpha$ subunit of Na$^+/K^-$-ATPase seem to be mediated via the activation of PP-1. This is supported by the kinetics of insulin-activated Na$^+/K^-$-ATPase and PP-1, insulin dose-response data, the inhibition of insulin’s effect on pump activity by high concentrations of OA and caly-A, and, most importantly, the absence of inhibition by low concentrations of OA, which specifically inhibit PP-2A (18, 19). Thus, these results provide evidence that Na$^+/K^-$-ATPase catalytic activity is regulated by insulin in vivo via a complex dephosphorylation/phosphorylation mechanism involving PP-1 activation and presumably inactivation of protein kinase A.

Recent work by a number of laboratories using a variety of cell types has shown that phosphorylation of the Na$^+/K^-$-ATPase $\alpha$ subunit by agonists or hormones that elevate protein kinase A, PKC, phospholipase A2, and intracellular calcium will all inhibit its catalytic activity (21–23). However, the inhibition observed by these agonists seems to be cell type specific. Thus, PKC activation results in an elevation of pump activity in rat primary skeletal muscle cells (2). Nonetheless, studies with purified preparations of Na$^+/K^-$-ATPase $\alpha$ subunit have shown that incubation with protein kinase A or PKC results in
that insulin specifically activates PP-1 and simultaneously inhibits PP-2A activity in L6 cells (12), and low concentrations of OA fail to block insulin-stimulated pump activation. Agents that cause PP-1 activation also cause stimulation of Na⁺/K⁺-ATPase activity. We have recently shown that acute stimulation of L6 cells with TPA, a PKC activator, results in PP-1 activation and that the effects of insulin and TPA on PP-1 activation were not additive, suggesting a common mechanism via PKC activation (25). In addition, down-regulation of PKC by chronic treatment with TPA, as well as inhibition of PKC with synthetic inhibitors, blocks insulin-stimulated PP-1 activation (25). Although we did not measure Na⁺/K⁺-ATPase activity in TPA-treated L6 cells, recent studies with primary cultures of rat skeletal muscle have demonstrated an activation of ouabain-sensitive Na⁺/K⁺-ATPase by TPA and insulin (26), and these effects could be reduced by the inhibition or down-regulation of PKC. Thus, it seems that PP-1 not only plays a major role in glycogen synthesis but also has a role in the regulation of ion channels. These studies provide a new mechanism for the in vivo hormonal regulation of intracellular Na⁺ and K⁺ homeostasis, cell volume, and resting membrane potential in skeletal muscle.

We have previously reported that insulin resistance induced by elevations in intracellular calcium, as well as streptozotocin diabetes, is accompanied by inhibition of PP-1 activities in adipocytes and skeletal muscle (27–29). Interestingly, both of these conditions were accompanied by reductions in Na⁺/K⁺-ATPase activity in adipocytes, skeletal muscle, and aortic tissue (13, 29–32). This lends further support to the observation that inhibition of PP-1 results in impaired activation of Na⁺/K⁺-ATPase, and this defect may contribute to the early muscle fatigue associated with diabetic complications such as neuropathy.

In intact skeletal muscle, Na⁺/K⁺-ATPase units have been found to be present in either latent or intracellular storage pools (2). Insulin is known to increase the number of Na⁺/K⁺-ATPase units on the plasma membrane in skeletal muscle but not in adipose tissue (2, 9–10). It is presently not known whether PP-1 plays a role in insulin regulation of subcellular distribution of Na⁺/K⁺-ATPase by promoting reinsertion into the plasma membrane and/or increasing its pumping efficiency. Additional studies are warranted to understand the role of PP-1 in insulin-mediated translocation of Na⁺/K⁺-ATPase from the intracellular compartment to the plasma membrane.

To examine the upstream insulin signaling components that mediate insulin's effect on PP-1 as well as Na⁺/K⁺-ATPase activation, we pretreated L6 cells with nanomolar concentrations of wortmannin, a selective inhibitor of PI3 kinase (33). This inhibitor not only blocked the activation of PP-1 by insulin but also prevented insulin's effects on Na⁺/K⁺-ATPase pump phosphorylation and therefore blocked activation. Thus it seems that insulin signaling via a PI3 kinase pathway stimulates PP-1, presumably by activating PKC and/or protein kinase B (c-Akt) (the downstream target of PI3 kinase), which in turn may increase phosphorylation of PP-1α, leading to activation of PP-1. Earlier studies from this laboratory have shown that insulin- as well as TPA-mediated activation of PP-1 was accompanied by phosphorylation of PP-1α and also that insulin-mediated PP-1α phosphorylation/activation could be prevented by inhibitors of PKC (25). Also, recent studies have demonstrated a role for PKB (c-Akt) in insulin-mediated inhibition of glycogen synthase kinase-3. It is not known presently whether or not PKB (c-Akt) is the upstream activator of PP-1.

In summary, the results of the present studies indicate that acute regulation of Na⁺/K⁺-ATPase by insulin in cultured skeletal muscle cells is mediated by dephosphorylation of the α
subunit via an activated PP-1 G. Activation of PP-1 G and Na\(^+\)/K\(^-\)-ATPase by insulin is dependent in part upon PI3 kinase-generated signals.

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