Akt1 kinase and dynamics of insulin resistance in denervated muscles in vivo

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Turinsky, Jiri, and Alice Damrau-Abney. Akt1 kinase and dynamics of insulin resistance in denervated muscles in vivo. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1425–R1430, 1998.—Basal and insulin-stimulated activity of Akt1 kinase and uptake of 2-deoxy-d-glucose (2-DG) were measured in soleus (slow-twitch) and plantaris (fast-twitch) muscles of rats at 1 and 3 days after sectioning the sciatic nerve in one hindlimb of the animals. At 1 day after surgery, the insulin-stimulated activity of Akt1 kinase in denervated soleus and plantaris muscles remained unchanged, but the insulin-stimulated 2-DG uptake by these muscles was reduced by 71 and 61%, respectively, compared with the corresponding muscles of the contralateral sham (control) hindlimb. At 3 days, the insulin-stimulated activity of Akt1 kinase in the denervated soleus and plantaris muscles was 86 and 71% lower, respectively, than in their sham counterparts. At this time point, the denervated soleus muscles showed no increase in 2-DG uptake in response to insulin. In contrast, the denervated plantaris muscle exhibited the same absolute level of insulin-stimulated 2-DG uptake as the sham plantaris muscle; however, the insulin-induced increment in 2-DG uptake was reduced by 60%, whereas basal 2-DG uptake was increased by 251% compared with the sham plantaris muscle. None of the denervated muscles showed a decrease in the abundance of Akt1 kinase. The results demonstrate that the causes of insulin resistance in denervated muscles are dependent on time after surgery. Initially, they involve only mechanisms downstream of Akt1 kinase (day 1), whereas at day 3 they also involve mechanisms upstream of, and including, Akt1 kinase.

protein kinase Bα; 2-deoxyglucose uptake; soleus muscle; plantaris muscle; slow-twitch and fast-twitch muscles

STIMULATION OF GLUCOSE uptake by insulin is initiated by insulin binding to its disulfide-linked heterotrameric receptor consisting of two extracellular α-subunits and two transmembrane β-subunits. Insulin binding to the α-subunit rapidly results in receptor autophosphorylation and activation of an intrinsic tyrosine kinase associated with the β-subunit (7). The receptor tyrosine kinase phosphorylates insulin receptor substrates (IRS), which enables the regulatory subunit (p85) of phosphatidylinositol 3-kinase to bind to IRS. The binding of p85 to IRS then results in the activation of the catalytic subunit (p110) of phosphatidylinositol 3-kinase (7). The product of the phosphatidylinositol 3-kinase reaction, phosphatidylinositol 3,4,5-trisphosphate, may serve as a link to the activation of 3-phosphoinositide-dependent protein kinase-1 (9), which in turn phosphorylates Akt1 kinase, also referred to as protein kinase B or RAC kinase, at Thr-308 (2). The role of Akt1 kinase in mediating the insulin-induced stimulation of glucose uptake is suggested by the observation that transfection of a constitutively active Akt1 kinase into 3T3-L1 adipocytes has an insulin-like effect on the translocation of GLUT-4 transporter to the plasma membrane and glucose uptake (13). The regulation of Akt1 kinase activity is incompletely understood. The full activation of Akt1 kinase requires phosphorylation of both Thr-308 and Ser-473 (1). Because phosphoinositide-dependent protein kinase-1 phosphorylates Akt1 kinase only on Thr-308 (2), an additional, so far unknown, serine kinase is believed to participate in the regulation of Akt1 kinase activity.

A single hindlimb denervation in the rat is a useful and highly reproducible model of insulin resistance. In this model, muscles of the denervated hindlimb develop insulin resistance, whereas muscles of the contralateral sham hindlimb respond to insulin in a normal fashion and serve as an internal control (16). Interruption of nerve supply to skeletal muscle results in the development of insulin resistance characterized by a decreased ability or inability of insulin to stimulate the transport of sugars (4, 6, 16), glycogen synthesis (4), or amino acid transport (16) in the affected muscles. The signs of insulin resistance in the denervated rat muscles in vivo can be observed as early as 3 h after sectioning the nerve (the earliest time tested) (16). We have previously demonstrated that both slow-twitch and fast-twitch muscles exhibit a progressive lowering of insulin-induced glucose uptake in vivo during the first 24 h after sectioning the sciatic nerve but that the two kinds of muscles differ in the manifestations of insulin resistance at 3–17 days after denervation (16). During the latter period, the slow-twitch muscles become completely unresponsive to stimulation with insulin, whereas the fast-twitch muscles show a normal glucose uptake when stimulated by insulin. However, the insulin-induced increment in glucose uptake is reduced because it is superimposed on already elevated basal glucose uptake.

The present study was undertaken to investigate how closely the insulin-stimulated activity of Akt1 kinase parallels the insulin-stimulated glucose uptake in hindlimb muscles of rats after a single hindlimb denervation. This study extends observations on insulin receptor tyrosine kinase and phosphatidylinositol 3-kinase activities in denervated soleus and plantaris muscles reported recently (11).

EXPERIMENTAL PROCEDURES

Materials. Anti-human Akt1/PKBα plekstrin homology domain sheep polyclonal antibody, horseradish peroxidase...
and 2 µCi [U-14C]sucrose with or without 0.1 U bovine insulin on
the total tissue 3H radioactivity (in dpm) and the amount of
radioactivity present in the tissue extracellular ([14C]sucrose) were from New
England Nuclear (Boston, MA). [U-14C]sucrose was obtained from ICN Pharmaceutical
Costa Mesa, CA). Microcystin was from Calbiochem (San Diego, CA). Leupeptin, pepstatin,
as well as other chemicals were from Sigma (St. Louis, MO).

Animals. The experiments were performed on adult male Sprague-Dawley rats weighing 225–240 g. All experimental
procedures were approved by the Institutional Animal Care and Use Committee and the institutional veterinarian
and strictly adhered to the National Institutes of Health Guide for
the Care and Use of Laboratory Animals [Department of
Health and Human Services Publication No. (NIH) 85–23,
Revised 1985]. The right hindlimb of each rat was denervated
under ether anesthesia, as previously described (11, 16–19).
Briefly, the thigh muscles were bluntly separated from the
lateral side, and a 5-mm segment of the sciatic nerve was
excised. On the contralateral (sham) hindlimb, the sciatic
nerve was visualized in the same way, but not touched. The
skin wounds were closed with surgical clips and coated with
disinfectant (Betadine). No bleeding was associated with the
surgery, and no infection was observed during the subsequent
recovery period. The denervation had no effect on daily food
intake during the experimental period. The rats were studied
at 1 and 3 days after surgery. At these test times, muscles
from the denervated hindlimb display minimal, if any, changes
in muscle mass or extracellular fluid volume (16). All animals
were fasted overnight (18 h) before the experiment and were
anesthetized with 50 mg/kg body wt pentobarbital sodium
given intraperitoneally at the time of the experiment.

Cellular uptake of 2-deoxy-D-glucose. Glucose uptake by
individual hindlimb muscles in vivo was assessed by cellular
accumulation of labeled 2-deoxy-D-glucose as described previ-
ously (11, 16, 17). Pentobarbital sodium-anesthetized animals
were injected with 10 µCi 2-deoxy-D-1,2-3H(N)glucose and 2 µCi [U-14C]sucrose with or without 0.1 U bovine insulin in
0.4 ml of 0.3% defatted bovine serum albumin intravenously via
the dorsal vein of the penis. The animals were killed immediately by rapid
exsanguination 25 min after the injection of labeled sub-
stances. Blood was collected, and soleus and plantaris muscles
were excised from both hindlimbs. The muscles and serum
were digested separately in tissue solubilizer (Solvable, New
England Nuclear), and the 3H and 14C radioactivities were
determined by liquid scintillation counting. Cellular uptake
of 2-deoxy-D-glucose was calculated as the difference between
the total tissue 3H radioactivity (in dpm) and the amount of
3H radioactivity present in the tissue extracellular ([14C]su-
crose) space.

Activity and abundance of Akt1 kinase. Pentobarbital
sodium-anesthetized animals were injected with 0.4 ml of
0.1% bovine serum albumin with or without 0.1 U bovine insulin intravenously
via the dorsal vein of the penis. A preliminary study indicated that
the insulin-stimulated activity of Akt1 kinase peaked in
both soleus and plantaris muscles at 5 min and remained
above control level for at least 15 min (data not shown). This
time course is in agreement with studies by Cross et al. (10).
Therefore, soleus and plantaris muscles were quickly excised
at 5 min after the intravenous injection. In each rat, the
muscles were excised only from one hindlimb, either the
denervated hindlimb or the contralateral sham hindlimb, to
ensure that the muscles were removed exactly at the 5-min
interval. Each excised muscle was immediately frozen in
liquid nitrogen and subsequently kept at −85°C until further
use. Each muscle was powdered under liquid nitrogen within
24 h. The powder was transferred into a 6-ml polypropylene
tube containing 0.5 ml of ice-cold buffer A/100 mg muscle.
The composition of buffer A was 50 mM Tris, pH 7.5, 1 mM EDTA,
1 mM EGTA, 0.5 mM NaVO₃, 0.1% 2-mercaptoethanol, 1% Triton X-100, 50 mM NaF, 5 mM sodium pyrophosphate, 10
mM sodium β-glycerol phosphate, 0.1 mM phenylmethylsulfo-
nyl fluoride, 1 µg/ml aprotinin, 1 µg/ml pepstatin, 1 µg/ml
leupeptin, and 1 µM microcystin. The suspension was homog-
enized using three 10-s bursts of a Polytron homogenizer set
at 75% of maximum power. The test tube with the homog-
enate was kept in an ice bath during the homogenization.
After centrifugation at 13,000 g and 4°C for 15 min, the
supernatant of muscle homogenate was separated, frozen in
liquid nitrogen, and kept at −85°C until further use.

The activity of Akt1 kinase was measured in immunopre-
cipitates from supernatants of muscle homogenates. Unless
indicated otherwise, all steps were performed in chilled ice,
and centrifugations were done at 4°C. Protein G-Sepharose
(30 µl of packed volume) in 250 µl of buffer A was agitated
with 4 µg anti-human Akt1/PKBα pleckstrin homology do-
main sheep polyclonal antibody overnight at 4°C. The anti-ody-protein G-Sepharose complex was washed three times
with buffer A and then allowed to react with 500 µg muscle
homogenate supernatant protein (Bradford protein assay
using bovine serum albumin as a standard) under constant agitation at 4°C for 90 min. The enzyme-
antibody-protein G-Sepharose complex was subsequently incubated with 40 µl
of assay dilution buffer containing 10 µM cAMP-dependent
protein kinase inhibitor peptide, 100 µM Akt/PKB specific
substrate peptide, 125 µM ATP (with 10 µCi [γ-32P]ATP per
reaction mixture), and 19 mM MgCl₂ for 10 min at 30°C
with continuous shaking. The 40-µl supernatant was then trans-
ferred into another tube, mixed with 20 µl of 40% TCA, and
incubated at room temperature for 5 min. After mixing, 40 µl
of TCA precipitate was transferred into another tube, mixed with 200 µl of
phosphocellulose paper and allowed to bind to it for 30 s
before immersing the square in 0.75% phosphoric acid. The
collected phosphocellulose squares were washed three times
with 0.75% phosphoric acid and once with acetone for 5 min
per wash under continuous mixing. The radioactivity of each
square was determined by scintillation counting. Radioactiv-
ity of samples that did not contain Akt1 kinase (enzyme
blank) was subtracted from measured radioactivities.

To determine the abundance of Akt1 kinase, aliquots of
supernatants of muscle homogenates were prepared for SDS-
PAGE by diluting them in electrophoresis-reducing sample
buffer and heating them at 98°C for 4 min. Aliquots corre-
sponding to 10 µg protein were subjected to SDS-PAGE using
4% stacking gels and 8% resolving gels and transferred
electrophoretically to pure nitrocellulose membranes (Bio-
Rad, Hercules, CA). The membranes were washed twice with
water and blocked under constant agitation in freshly pre-
pared Tris-buffered saline, pH 7.4, containing 0.05% Tween
20 and 3% nonfat dry milk (TBS-T-MILK) at 21°C for 30 min.
The membranes were then agitated overnight at 4°C in
TBS-T-MILK containing 0.75 µg/ml anti-human Akt1/PKBα
pleckstrin homology domain sheep IgG. After being washed
twice with water, the membranes were agitated at 21°C for 90
min in PBS, pH 7.4, with 3% nonfat dry milk containing
a rabbit-anti-sheep HRP-conjugated IgG at a dilution of 1:1,500.

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twice with water, the membranes were agitated at 21°C for 90
min in PBS, pH 7.4, with 3% nonfat dry milk containing
a rabbit-anti-sheep HRP-conjugated IgG at a dilution of 1:1,500.
The nitrocellulose membranes were subsequently washed in water (twice) then in PBS-0.05% Tween 20 for 3–5 min, and finally in four or five changes of water. Blots were detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL). Bands corresponding to Akt1 kinase were quantified by video densitometry (Bioimage 60S; Millipore, Bedford, MA). Data evaluation. The results are expressed as means ± SE. Statistical significance was assessed using ANOVA followed by the Student-Newman-Keuls multiple-comparison test.

RESULTS

Soleus muscle 1 day after denervation. In agreement with previous studies (11, 16), the denervated soleus muscle exhibited a 49% (P < 0.005) lower basal 2-deoxy-D-glucose uptake and a 71% (P < 0.002) lower insulin-stimulated 2-deoxy-D-glucose uptake in vivo compared with the contralateral sham soleus muscle (Fig. 1A). Our previous studies have shown that these changes occur in the absence of any alteration in insulin binding, basal and insulin-stimulated tyrosine kinase activity of the insulin receptor, basal and insulin-stimulated activity of phosphatidylinositol 3-kinase, and the abundance of GLUT-1 and GLUT-4 in the denervated soleus muscle (11). The present study extends these observations by assessing the activity of Akt1 kinase (Fig. 1B). Basal activity of Akt1 kinase in muscles was very low and was not statistically different from background phosphorylation (enzyme blank). Administration of insulin resulted in pronounced increases in the activities of Akt1 kinase in both sham and denervated soleus muscles, and there was no difference in the magnitude of the insulin-stimulated Akt1 kinase activity between the sham and denervated soleus muscles. Despite this similarity, the denervated soleus muscle exhibited a 29% (P < 0.04) greater abundance of Akt1 kinase than the contralateral sham soleus muscle (Fig. 1C).

Plantaris muscle 1 day after denervation. Basal 2-deoxy-D-glucose uptake by the sham plantaris muscle did not differ from that in sham soleus muscle, but the insulin-induced uptake by the sham plantaris muscle was 50% (P < 0.02) lower than that in the sham soleus muscle (Fig. 1A). The denervated plantaris muscle exhibited no change in basal 2-deoxy-D-glucose uptake, but its insulin-stimulated uptake was reduced by 61% (P < 0.001) compared with the contralateral sham plantaris muscle (Fig. 1A). Our previous studies have demonstrated that the insulin resistance of the denervated plantaris muscle at this time point is associated with unchanged insulin binding, unchanged basal and insulin-stimulated activities of tyrosine kinase of the insulin receptor and of phosphatidylinositol 3-kinase, and unchanged abundance of GLUT-1 and GLUT-4 (11). Figure 1, B and C, also shows that the insulin resistance of the denervated plantaris muscle occurs in the absence of any alteration in the insulin-induced activity of Akt1 kinase or its abundance in the denervated plantaris muscle.

Aside from the absence of effects of denervation, it is noteworthy that insulin-stimulated activities of Akt1 kinase in sham and denervated plantaris muscles were 39 and 44% (P < 0.05) lower, respectively, than those of sham and denervated soleus muscles (Fig. 1B). Also, the abundance of Akt1 kinase in sham and denervated plantaris muscles was 43 and 58% (P < 0.006) lower, respectively, than those of sham and denervated soleus muscles (Fig. 1C).

Soleus muscle 3 days after denervation. At this time point, the denervated soleus muscle exhibited a 60% (P < 0.03) lower basal 2-deoxy-D-glucose uptake than the contralateral sham soleus muscle and was completely unresponsive to stimulation with insulin (Fig. 2A). Our previous findings have shown that insulin
binding was not significantly altered at this interval, but the denervated soleus muscle exhibited a 35% decrease in insulin-induced tyrosine kinase activity of the insulin receptor, a 63% decrease in insulin-stimulated activity of phosphatidylinositol 3-kinase, and a pronounced decrease in the tissue abundance of GLUT-4 transporter compared with the contralateral sham soleus muscle (11). As depicted in Fig. 2, B and C, the denervated soleus muscle also shows an 86% (P < 0.001) decrease in insulin-stimulated Akt1 kinase activity compared with the contralateral sham counterpart, whereas the abundance of Akt1 kinase in the denervated soleus muscle appears to be, in fact, increased 43% (P = 0.06) compared with the sham soleus muscle.

Plantaris muscle 3 days after denervation. In agreement with studies on rats 1 day after a single hindlimb denervation, sham plantaris muscle from 3-day postsurgery rats (Fig. 2A) showed a 38% lower basal 2-deoxy-D-glucose uptake and a 44% lower insulin-stimulated uptake compared with the adjacent sham soleus muscle (P < 0.05).

The denervated plantaris muscle exhibited a 251% (P < 0.001) elevation in basal 2-deoxy-D-glucose uptake and an unchanged absolute level of insulin-induced 2-deoxy-D-glucose uptake compared with the contralateral sham plantaris muscle. It should be noted, however, that the insulin-induced increment in uptake in the denervated plantaris muscle, although statistically significant (P < 0.03), was reduced by 60% (P < 0.007) compared with the contralateral sham plantaris muscle. Although insulin binding is not altered in the plantaris muscle 3 days after denervation (11), we have previously observed a 44% lower insulin-stimulated tyrosine kinase activity of the insulin receptor, a 41% lower insulin-stimulated activity of phosphatidylinositol 3-kinase, diminished abundance of GLUT-4, and increased abundance of GLUT-1 in the denervated plantaris muscle compared with its sham counterpart (11). As shown in Fig. 2B, the insulin-stimulated activity of Akt1 kinase in the denervated plantaris muscle is 71% (P < 0.002) lower than that in the contralateral sham plantaris muscle. The abundance of Akt1 kinase in the denervated plantaris muscle appeared to be 63% higher than in its sham counterpart, but the difference was not statistically significant (Fig. 2C).

It should be noted that sham muscles of rats 3 days after a single hindlimb denervation (Fig. 2, B and C) exhibited the same difference in insulin-stimulated activity and abundance of Akt1 kinase as slow-twitch and fast-twitch muscles of rats studied 1 day after surgery (Fig. 1, B and C). As shown in Fig. 2, B and C, the insulin-stimulated activity of Akt1 kinase and the abundance of this enzyme in the sham plantaris muscle were 50% (P < 0.001) and 60% (P < 0.04) lower, respectively, than those in the sham soleus muscle.

DISCUSSION

The present study has been focused on Akt1 kinase, which is believed to act distal to phosphatidylinositol 3-kinase (2) and which has been reported to induce the translocation of GLUT-4 to the plasma membrane with the resulting increase in glucose uptake in 3T3-L1 adipocytes (13). The results of the present study represent the first information on the abundance and activity of Akt1 kinase in the denervated, insulin-resistant skeletal muscles. This new information and our previous observations on denervated muscles (11, 16) are summarized in Table 1. At 1 day after denervation, both soleus and plantaris muscles exhibit a pronounced decrease in the ability of insulin to stimulate 2-deoxy-D-glucose uptake.
Table 1. Effects of denervation on parameters of insulin action involved in glucose uptake by soleus and plantaris muscles 1 and 3 days after denervation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 Day Postdenervation</th>
<th>3 Days Postdenervation</th>
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<tbody>
<tr>
<td></td>
<td>Soleus muscle</td>
<td>Plantaris muscle</td>
</tr>
<tr>
<td>Insulin binding</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Basal receptor activity</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Insulin-stimulated receptor activity</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>p85 protein amount</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Basal PI 3-kinase activity</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Insulin-stimulated PI 3-kinase activity</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Akt1 kinase protein amount</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Insulin-stimulated Akt1 kinase activity</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>GLUT-1 protein amount</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>GLUT-4 protein amount</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Basal 2-deoxyglucose uptake</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Insulin-induced increment in 2-deoxyglucose uptake</td>
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PI, phosphatidylinositol; NC, no change. Number of arrows is proportional to magnitude of change induced by denervation.

This insulin resistance occurs without any alteration in insulin binding and insulin-stimulated activity of the insulin receptor tyrosine kinase (11). This agrees with observations of others (5). There is also no alteration in the insulin-stimulated activity of phosphatidylinositol 3-kinase (11). The present study demonstrates that there is also no change in the ability of insulin to stimulate the activity of Akt1 kinase. These data indicate that 1 day after denervation there is a clear dissociation between the markedly diminished 2-deoxy-D-glucose uptake in response to insulin and the upstream events involved in the stimulation of glucose uptake by insulin. It is unclear how many signaling steps exist between the activation of Akt1 kinase and the appearance of GLUT-4 at the plasma membrane. Because the abundance of GLUT-1 and GLUT-4 in 1-day postdenervation muscles is not different from control muscles (11), the site of the defect(s) underlying the insulin resistance at this interval is in signaling steps downstream from Akt1 kinase.

At 3 days after denervation, the parameters relevant to insulin signaling in muscles differ from day 1, and there is also a difference in manifestations of insulin resistance between soleus and plantaris muscles (Table 1). At 3 days after denervation, soleus muscle is unresponsive to insulin and does not show any increase in 2-deoxy-D-glucose uptake after the administration of the hormone. In contrast, plantaris muscle exhibits a pronounced increase in basal 2-deoxy-D-glucose uptake and a reduced increment in uptake in response to insulin. The sum of these two components results in uptake that does not differ from the absolute level of insulin-stimulated 2-deoxy-D-glucose uptake in control muscles. Despite the different manifestations of insulin resistance in denervated soleus and plantaris muscles at the 3-day interval, both soleus and plantaris muscles exhibit comparable decreases in insulin-stimulated activities of insulin receptor tyrosine kinase (−35 and −44%, respectively) and phosphatidylinositol 3-kinase (−65 and −41%, respectively) (11). The present study demonstrates that these muscles also show comparable decreases in insulin-stimulated activity of Akt1 kinase (−87 and −71%, respectively). Even though the degree of inhibition of insulin-stimulated activity of Akt1 kinase in denervated soleus and plantaris muscles could virtually account for the degree of reduction in the insulin-induced increment in 2-deoxy-D-glucose uptake in these respective muscles, there is another level of regulation contributing to the observed changes.

Studies by this laboratory have shown a pronounced decrease in the content of GLUT-4 in soleus and plantaris muscles 3 days after denervation (11). In addition, an increase in GLUT-1 abundance in plantaris muscle has been observed at this interval (11). These findings agree with observations by Henriksen et al. (12), Block et al. (3), and Coderre et al. (8). The increase in GLUT-1 abundance is consistent with the augmented basal 2-deoxy-D-glucose uptake in plantaris muscle at 3 days after denervation.

The present study also demonstrates that insulin-stimulated 2-deoxy-D-glucose uptake by slow-twitch and fast-twitch muscles is directly proportional to the abundance and insulin-stimulated activity of Akt1 kinase in the given muscle. Thus soleus muscle, a slow-twitch muscle, which exhibits a high abundance and insulin-stimulated activity of Akt1 kinase, also shows a high insulin-stimulated 2-deoxy-D-glucose uptake. In contrast, plantaris muscle, a fast-twitch muscle, which has, on the average, 52% lower abundance of Akt1 kinase and 45% lower insulin-stimulated activity of Akt1 kinase compared with soleus, also exhibits 47% lower insulin-stimulated 2-deoxy-D-glucose uptake than soleus muscle. This close, direct correlation between the magnitude of insulin-stimulated activity of Akt1 kinase and the level of glucose uptake in muscles with different fiber populations provides indirect support for the role of Akt1 kinase in insulin-stimulated glucose uptake by skeletal muscles in vivo.

Because decreased availability of energy diminishes cellular responsiveness to insulin (20), we have previously measured ATP and related substances in calf muscles of rats 3 days after a single hindlimb denervation (16). The denervated calf muscles, frozen in situ, exhibited slightly higher ATP and creatine phosphate levels and unchanged ADP and AMP levels compared with the contralateral sham muscles, demonstrating that denervated muscles are not energy deficient (16). It is also noteworthy that insulin fails to stimulate 2-deoxy-D-glucose uptake and glycogen synthesis in soleus muscles of hindlimbs immobilized for 1 day (14). This suggests that denervation-induced insulin resistance in muscle may be because of muscle inactivity rather than denervation per se.
Perspectives

The single hindlimb denervation model has several important advantages for studying the mechanisms of insulin resistance. The development of insulin resistance is rapid and highly reproducible. The presence, in the same animal, of muscles that exhibit insulin resistance (denervated hindlimb) and muscles that respond to insulin in a normal fashion (contralateral sham, control hindlimb) provides an internal control and decreases variability of results. Perfusion of both hindlimbs with the same blood in vivo eliminates differences in plasma concentrations of metabolic substrates, hormones, and cytokines as potential causal or contributing factors in the development of insulin resistance. This creates “cleaner” conditions for investigating cellular mechanisms underlying the denervation-induced insulin resistance in muscle. To date, there is no scientific explanation for insulin resistance in muscles at 1 day after denervation. Consequently, further studies on denervated muscles have a potential to provide qualitatively new information on the mechanism of insulin action.

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