Intrinsic Differences of Insulin Receptor Kinase Activity in Red and White Muscle*

David E. James‡§†, Antonio Zorzano‡, Marianne Böni-Schnetzler§, Raphael A. Nemenoff||, Alvin Powers‡|, Paul F. Pileh‡‡, and Neil B. Ruderman‡

From the ‡Division of Diabetes and Metabolism and Departments of Medicine (University Hospital) and §Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118 and the ¶Diabetes Unit, Howard Hughes Medical Institute, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114

The sensitivity and responsiveness of glucose uptake and glycogen synthesis to insulin are 3–4-fold greater in red than in white skeletal muscle (James, D. E., Jenkins, A. B., and Kraegen, E. W. (1985) Am. J. Physiol. 248, E567–E574). In the present study, the insulin receptor tyrosine kinase activity has been examined in red and white muscle of rats. Partially purified insulin receptors were obtained from muscle following solubilization in detergent, ultracentrifugation, and lectin affinity chromatography. Total insulin receptor number per gram of tissue was slightly higher in red (30%) than in white muscle. In contrast, basal and insulin-stimulated autophosphorylation, normalized for receptor number, were 2.3-fold higher in red muscle. A similar difference was observed in the ability of partially purified receptors to phosphorylate the exogenous substrate polyglutamate/tyrosine. The integrity of the insulin receptor preparation in the two fiber types was identical as determined by affinity cross-linking of [125I-Tyr]insulin to the receptor. Mixing partially purified receptors from red and white muscle resulted in an additive response for exogenous substrate phosphorylation, suggesting that the difference in tyrosine kinase activity was not due to the presence of an inhibitor or activator.

The results suggest that there are differences in the insulin receptors of red and white muscles that lead to discordance in their basal and insulin-stimulated intrinsic tyrosine kinase activity. The correlation between these differences and insulin action in red and white muscle supports the concept that the insulin receptor tyrosine kinase activity is involved in the initiation of insulin action.

It is well established that skeletal muscle is the quantitatively most important site of insulin-stimulated glucose disposal (1, 2). However, there is considerable heterogeneity of insulin action among skeletal muscles of different fiber composition (2–5). Red (primarily oxidative) muscle exhibits 3–4-fold higher sensitivity and maximal responsiveness to insulin, with respect to 2-deoxyglucose uptake (2, 4), glycogen synthesis (2, 4), and amino acid uptake (5), than does white (primarily glycolytic) muscle. This heterogeneity has been demonstrated in vivo, in both conscious (2) and anesthetized rats (6) and in vitro in isolated muscle incubations (3) and hindquarter perfusions (4). These differences cannot be entirely attributed to extrinsic factors, such as blood flow (6), and may well reflect differences in intracellular insulin action. Elucidation of the factor(s) that contribute to this heterogeneity may yield important information concerning the regulation and/or modulation of insulin action in skeletal muscle and the whole body.

Previous studies using isolated muscle preparations have reported higher insulin binding in the soleus, a predominantly red muscle, than in the extensor digitorum longus, a predominantly white muscle (3). However, it is not clear whether the difference in binding is adequate to account for the greater effect of insulin in red muscle. In particular, in view of the existence of spare receptors, it is unlikely that the increase in receptor number in red muscle is sufficient to account for increased maximal insulin responsiveness. Much is now known about the structure of the insulin receptor purified from mammalian tissue (reviewed in 7 and 8). It is lodged in the plasma membrane of the cell as a tetramer, consisting of two distinct subunits. The α subunit has a molecular weight of 130,000–135,000 and contains the insulin binding domain. The β subunit, with a molecular weight of 90,000–95,000, has been shown to possess insulin-dependent, tyrosine-specific protein kinase activity. It is an attractive hypothesis that this kinase may be involved in coupling insulin binding to subsequent metabolic events, since protein kinase-mediated phosphorylation reactions are known to modify and regulate the activity of many cellular enzymes (9, 10). Furthermore, augmentation of receptor autophosphorylation by insulin is sufficiently rapid to account for subsequent metabolic changes such as increased glucose transport (11).

Few studies have examined the structure/function of the insulin receptor kinase activity in skeletal muscle. Stimulation of kinase activity has been observed in soluble, partially purified insulin receptor preparations from muscle (12, 13). In addition, LeMarchand-Brustel et al. (13) reported decreased insulin receptor kinase activity in muscle of obese insulin-resistant mice that was not accounted for by a change in insulin receptor number. A dissociation between insulin binding and receptor kinase activity has also been found in mixed muscle from rats with streptozotocin-induced diabetes (14). These studies support the hypothesis that the tyrosine

* This work was supported in part by Grants AM19514, AM30425, and AM36424 from the United States Public Health Service. 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.

Dedicated to George F. Cahill on his 60th birthday.

‡Recipient of a Fogarty International Research Fellowship from the National Institutes of Health. To whom correspondence may be addressed: Dept. of Biochemistry, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

§Recipient of a Research Career Development Award from the United States Public Health Service. To whom correspondence may also be addressed.
kinase activity associated with the insulin receptor may have an important role in modulating insulin action in muscle. On the other hand, interpretation of these results is confounded somewhat by the fact that normal and dystrophic muscles of turled animals were compared. To circumvent this potentially complicating variable and to examine further the association between insulin action and insulin receptor kinase activity, we have examined insulin binding, receptor phosphorylation, and receptor tyrosine kinase activity in skeletal muscles obtained from the same rat. The present study compares these parameters in red and white muscle.

**EXPERIMENTAL PROCEDURES**

**Materials**—Porcine monocomponent insulin was a gift from Dr. R. Chance, Eli Lilly & Co. [225-Tyr]Thr monoiodoinsulin was obtained from New England Nuclear. [125I]-IGF-I and [125I]-Thr[225]monoiodoinsulin were obtained from Amersham Corp. [γ-32P]ATP was prepared from [82P]orthophosphate (New England Nuclear) using a Gamma-precip kit from Promega Biotech. Unlabeled IGF-I was obtained from AMGEN. All electrophoresis reagents were obtained from Bio-Rad, wheat germ agglutinin (WGA) was obtained from Miles-Ysda Laboratories, Napaville, IL, and disaccinimylidyl suberate from Pierce. All other chemicals were obtained from Sigma.

**Preparation of Insulin Receptors**—Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing between 250 and 300 g and fed ad libitum were used for all studies. Hind limb muscle was obtained from pentobarbital-anesthetized rats and frozen in liquid nitrogen. Red muscle consisted of pooled soles, red portions of the gastrocnemius and quadriceps muscles, and similarly, white portions of the gastrocnemius and quadriceps were pooled as the source of white muscle. On the basis of previous studies documenting fiber composition in different muscles of the rat (15, 16), the red muscle fraction consists primarily of fast-twitch oxidative fibers and the white muscle largely of fast-twitch glycolytic fibers. After powdering in a cooled mortar, 3-4 g of muscle were homogenized using a Polytron homogenizer (setting 8, 30, 4 °C) in a buffer (3.5, v/w) containing 25 mM Hepes, 4 mM EDTA, 1 trypsin inhibitor unit/ml of aprotinin, 2 mM phenylmethylsulfonyl fluoride, 25 mM benzamidine, 1 mM leupeptin, 1 mM pepstatin, and 1 mM bacitracin, pH 7.6. Following homogenization, Triton X-100 (1%) was added, and the homogenate was stirred for 1 h at 4 °C and centrifuged at 150,000 g for 30 min at 4 °C. The 150,000 × g supernatant (6 ml) was recycled three times through a column containing 2 ml of wheat germ agglutinin (WGA) bound to agarose, at 4 °C. The resin was washed with buffer (75 ml) containing 20 mM Hepes, 250 mM NaCl, and 0.1% Triton X-100, pH 7.4. Receptors were eluted from the WGA column with buffer containing 25 mM Hepes, 0.1% Triton X-100, and 0.5 M N-acetyl-d-glucosamine.

**Ligand Binding**—Insulin binding was measured as described by Hollenberg and Cuatrecasas (17). WGA eluate (20 μl) was incubated in 50 mM Hepes, 250 mM NaCl, 0.1% Triton X-100, pH 7.4, containing 100 μg/ml bacitracin (500 cpm 125I insulin (50 pm), and increasing concentrations of unlabeled insulin. Using these conditions, insulin binding was measured at protein concentrations between 25 and 100 μg/ml (data not shown). Insulin binding was measured using conditions with 0.01% Triton X-100. IGF-I binding was assayed using identical conditions with 60,000 cpm 125I-IGF-I and varying concentrations of human IGF-I. Receptors were precipitated with 0.5 ml of bovine γ-globulin (1 mg/ml) and 0.5 ml of polyethylene glycol (25%, v/v). Non-specific binding was estimated as 125I-ligand bound in the presence of 5 μM unlabeled hormone (5-10% of total binding). Binding data are expressed per microgram of protein measured using the Bradford method (18).

**Receptor Cross-linking**—Receptor cross-linking protocols were carried out essentially as described by Pilch and Czech (19). WGA eluate (10 μl) was incubated at 22 °C with 0.5 μM [225I]Thr[225]monoiodoinsulin in the presence or absence of 5 μM unlabeled insulin, final concentration, in a total volume of 100 μl for 60 min. After a further 5-min incubation at 0 °C, disaccinimylidyl suberate in 2 μl of dimethyl sulfoxide was added to achieve a final concentration of 1 mM. Samples were incubated for 15 min at 0 °C. The cross-linking reaction was termi-

1 The abbreviations used are: IGF-I, insulin-like growth factor I; WGA, wheat germ agglutinin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

nated by the addition of 100 μl of Laemmli sample buffer (20.1 M dithiotreitol).

**Autophosphorylation Assay**—WGA eluate (10 μl) was preincubated with increasing insulin concentrations in a buffer containing 36 mM Hepes, 2 mM MgCl2, 10 mM MgCl2, pH 7.6, at 22 °C for 30 min (final volume 200 μl). Phosphorylation was initiated by the addition of 50 μM [γ-32P]ATP (5-10 μCi). Receptor phosphorylation was linear for up to 6 min at 22 °C in the absence and presence of 10 μM insulin (data not shown). All subsequent autophosphorylation reactions were terminated after incubation for 4 min at 22 °C by the addition of an equal volume of Laemmli sample buffer containing 0.1 M dithiotreitol and then incubation for 5 min at 95 °C. Samples were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

**Polyacrylamide Gel Electrophoresis**—Polyacrylamide gel electrophoresis was performed in the presence of SDS described by Laemmli (20). All samples were prepared by incubating with electrophoresis sample buffer for 5 min at 95 °C. The final concentrations of sample buffer constituents were 50 mM Tris, with or without 50 mM dithiothreitol, 1% SDS, pH 6.8. Molecular weight standards were myosin (Mr = 205,000), δ-galactosidase (Mr = 116,000), phosphorylase b (Mr = 92,000), bovine serum albumin (Mr = 68,000), ovalbumin (Mr = 45,000), and carbonic anhydrase (Mr = 31,000). All samples were run on a 3-10% acrylamide gradient resolving gel. Autoradiography of Coomassie Blue-stained, dried gels was performed at −70 °C with Kodak X-Omat AR film using a Cronex Lightning Plus enhancing screen. Radioactivity was determined in the gel by cutting the appropriate regions from the stained, dried gel and counting either Cerenkov or γ radiation. Another portion of the gel, in which there was no obvious radioactive band, was used as background.

**Phosphorylation of an Exogenous Substrate**—To assess phosphorylation of an exogenous substrate, partially purified receptor preparation was preincubated for 30 min as described for autophosphorylation. The substrate (copolymer of Glu/Tyr, 4:1, 0.25 mg/ml) was added, and, after a further 30-min incubation, phosphorylation was initiated with the addition of 50 μM [γ-32P]ATP (2-5 μCi). Preliminary studies indicated that phosphorylation of the exogenous substrate by partially purified receptors was linear for up to 30 min in the absence and presence of 10 μM insulin (data not shown). In addition, phosphorylation of the exogenous substrate was linear with varying concentrations of protein (0-15 μg) in both red and white muscle in the absence and presence of insulin (data not shown). Therefore, all subsequent reactions were carried out using approximately 5 μg of partially purified protein and stopped after 30 min by applying samples to filter paper squares (3 × 3 cm, Whatman No. 3MM) and soaking in 10% trichloroacetic acid containing 10 mM sodium pyrophosphate. Papers were washed at least five times over 3-5 h in acetone, and counted for radioactivity. Non-specific binding of [γ-32P]ATP to the paper accounted for 5-10% of the radioactivity measured associated with the copolymer in the absence of insulin. Furthermore, addition of WGA eluate without copolymer did not result in a significant increase in 32P adsorbed to the papers above background levels. All assays were carried out in triplicate.

**RESULTS**

**Ligand Binding**—Receptors specific for insulin and IGF-I were demonstrated in the wheat germ affinity purified preparation from adult rat skeletal muscle (Figs. 1 and 2). There was approximately 3 times more insulin than IGF-I receptors per microgram of protein eluted from the WGA column, as estimated by Scatchard analysis. Furthermore, insulin had very little affinity for the IGF-I receptor in mixed skeletal muscle, as indicated by the minimal displacement of 125I-IGF-I binding by insulin using concentrations up to 100 nM (Fig. 2). Similar differences in insulin and IGF-I receptor number and affinity of each ligand for the opposing receptor were found in red and white muscle (data not shown). These data are consistent with previous studies in muscle (21). Equilibrium binding of 125I-insulin to partially purified receptors from red and white skeletal muscle yielded identical Scatchard plots for both muscle types (Fig. 1). Linear regression was performed on the initial six binding data points to calculate 

...
Unlabeled insulin in a total volume of 200 pl. See "Experimental Procedures" for further details. The data shown are from a representative Scatchard plot (n = 5, see text). Each point is the mean of duplicate determinations.

**Fig. 1.** Insulin binding in red and white muscle. Insulin receptors were partially purified from red (●—●) and white (O—O) muscle homogenates following solubilization in Triton X-100 and ultracentrifugation using lectin affinity chromatography. The wheat germ agglutinin eluate (20 pl) was incubated for 1 h at 22 °C in a buffer containing 25 mM Hepes, 0.1 mg/ml bovine serum albumin, 100 units/ml bacitracin, 125I-insulin, and varying concentrations of unlabeled insulin (0–100 units/ml) in a total volume of 200 pl. See "Experimental Procedures" for further details. The data shown are from a representative Scatchard plot (n = 5, see text). Each point is the mean of duplicate determinations.

**Fig. 2.** Inhibition of 125I-IGF-I binding to skeletal muscle by IGF-I or insulin. Insulin receptors were partially purified from mixed skeletal muscle as described in the legend to Fig. 1. 125I-IGF-I was determined as described under "Experimental Procedures" in the presence of varying concentrations of unlabeled insulin (O—O) or IGF-I (●—●). Binding is expressed relative to that observed in the presence of tracer concentration alone, which is defined as 100%. Each point is the mean of duplicate determinations.

**Fig. 3.** Affinity cross-linking of 125I-insulin to the insulin receptor in red and white muscle. Partially purified insulin receptors were obtained from red (R) and white (W) muscle as described in the legend to Fig. 1. Partially purified receptor (10 pl) was incubated at 22 °C for 60 min in 30 mM Hepes buffer containing 0.5 nM 125I-insulin in the absence or presence of 5 µM unlabeled insulin. After 5-min incubation at 0 °C, disuccinimidyl suberate was added at a final concentration of 1 mM, and samples were incubated for a further 1 min at 0 °C. The reaction was stopped by the addition of an equal volume of Laemmli sample buffer (0.1 M dithiothreitol). Samples were subjected to electrophoresis in 5–10% polyacrylamide gradient gels as described under "Experimental Procedures."
analysis (Fig. 1) that insulin receptor number per microgram of glycoprotein was similar in red and white muscle. In addition, they further support the observation that the integrity of insulin receptors partially purified from red and white muscle was similar.

Kinase Activity of Muscle Insulin Receptors—Incubation of the partially purified receptor preparation from skeletal muscle with [γ-32P]ATP, cations (MgCl₂, MnCl₂), and insulin resulted in a dose-dependent increase in the incorporation of 32P into a peptide with approximate molecular weight of 95,000 (Figs. 4 and 5). On the basis of its Mr, and numerous previous studies (11-14, 24, 25) this peptide was identified as the β subunit of the insulin receptor. It is unlikely that there is any cross-reactivity with the IGF-I receptor in these studies, since insulin has very little affinity for the IGF-I receptor in muscle (Fig. 2).

Basal and insulin-stimulated 32P incorporation into the β subunit of the insulin receptor partially purified from red muscle was clearly higher than that observed in receptor purified from white muscle (Figs. 4 and 5). This phenomenon was evident when these data were normalized on the basis of insulin receptor number, either estimated via Scatchard analysis (Fig. 5A) or from 125I-insulin affinity cross-linking under reducing conditions (Fig. 5B). It is noteworthy that insulin binding, affinity cross-linking, and kinase activity were studied using identical conditions to establish equilibrium binding. At maximal insulin concentrations (≥10 nM), autophosphorylation of insulin receptors from red muscle was 2.2-fold higher than in white per femtomole of receptor (Fig. 5A). A similar difference in autophosphorylation between red and white muscle was evident in the absence of insulin. Hence, the incremental effect of insulin, above basal, on autophosphorylation was similar in red and white muscle.

The kinase activity of the insulin receptor in red and white muscle was further characterized using an exogenous substrate. The dose-response relationship between insulin and 32P incorporation into a copolymer of Glu/Tyr, in the presence of partially purified insulin receptor, is shown in Fig. 6. The response was virtually identical to that observed for autophosphorylation (Figs. 4 and 5). Insulin receptor, partially purified from red muscle, exhibited an increased ability to phosphorylate the exogenous substrate in the absence of insulin and at all insulin concentrations examined. These data confirm previous observations concerning the specificity of the kinase activity of solubilized insulin receptors for tyrosine. These differences in kinase activity between red and white muscle do not reflect differences in IGF-I receptor kinase activation because IGF-I-dependent phosphorylation of this exogenous substrate could not be demonstrated under the present experimental conditions (data not shown).

One possibility to explain the differences in receptor kinase activity between red and white muscle is that there is a factor (e.g. phosphatase, ATPase) present in the WGA eluate which may act as an activator or an inhibitor of kinase activity under the present experimental conditions. To address this possibility, phosphorylation of an exogenous substrate was examined following incubation for 60 min of equal amounts of insulin receptor from red and white muscle together (Table I). These data indicated that the kinase activity of mixed red and white muscle was additive. To support this finding further, insulin receptors were partially purified from hindquarter skeletal muscle (i.e. equal combination of red and white muscle). A similar number of insulin receptors from this preparation to that used in the in vitro mixing experiment...
In the present study, we have demonstrated that the small difference in insulin action between red and white muscle is not specific for glucose uptake, since a number of functional properties of the kinase were additive. Collectively, these findings rule out the possibility of nonspecific alterations in kinase activity due to the presence of factors in the solubilized receptor preparation.

**DISCUSSION**

The role of the insulin receptor's intrinsic tyrosine-specific protein kinase activity in mediating the complex array of biological responses stimulated by insulin in mammalian cells is not clear. A number of functional properties of the kinase are consistent with it playing a key role (for review, see 7 and 8). Furthermore, studies in several insulin-resistant states (13, 14, 24–26) have found an impairment in insulin action accompanied by decreased insulin receptor kinase activity and little if any change in insulin binding in a variety of tissues. In the present investigation, we have attempted to examine the relationship between the receptor kinase and insulin action under conditions where there is no alteration in the systemic milieu. For this purpose we have taken advantage of previous findings demonstrating that the basal rate of a number of insulin-sensitive processes and the sensitivity and responsiveness of these processes to insulin are greater in red than in white muscle (2–5). The results indicate that differences in the kinase activity of the receptor in vitro, in the presence and absence of insulin, closely parallel these variations in insulin action.

In the present study there was little difference in insulin binding per gram of tissue between red and white muscle, whereas previous studies comparing insulin binding in incubated muscles have shown approximately 3-fold higher insulin binding per unit weight of tissue in the soleus, which contains 90% slow-twitch red fibers, than in the extensor digitorum longus, which contains 70% fast-twitch white and 30% fast-twitch red fibers (9). In the present study, equilibrium insulin binding was studied in a solubilized muscle homogenate following partial purification by wheat germ affinity chromatography. Thus, in comparing our data to that obtained in intact muscle (3), it is possible that the recovery of insulin receptors, using the present purification scheme, may vary in the two muscle types. Furthermore, the ratio of surface-bound to internalized receptor may differ in red and white muscle. Additional studies examining the subcellular distribution of insulin receptors in red and white muscle are required to address these possibilities. Regardless of these possibilities, the differences in receptor kinase activity between red and white muscle is striking.

Using animals of similar weight and age to that employed in the present study, in vivo insulin action has been accurately defined in muscles of different fiber composition (2). In these studies it was demonstrated that insulin sensitivity, as determined by the insulin concentration required for half maximal stimulation of glucose uptake and glycogen synthesis, was 2–3-fold higher in red than in white muscle. Similarly, the maximal response to insulin of these parameters was 3–4-fold higher in red than in white muscle. Therefore, if the subcellular distribution of insulin receptors is similar in both types of muscle, it is unlikely that the small difference in insulin binding between red and white muscle as reported in the present study is adequate to account for the substantial difference in insulin action.

The heterogeneity in insulin action among muscles of different fiber type is not specific for glucose uptake, since a similar heterogeneity has recently been reported for insulin-mediated a-aminoisobutyrate uptake (5). Therefore, we postulated that the differences in insulin action between red and white muscle were related to the mechanism by which insulin binding to the cell surface receptor is coupled to the imple-
mulation of a biological response. Much evidence has been accumulated implicating the tyrosine-specific protein kinase activity of the insulin receptor as playing an integral role in this process. In this regard, the present studies support this concept and suggest that intrinsic alterations in the protein kinase activity of the insulin receptor may contribute to the heterogeneity in insulin action between red and white skeletal muscle. Both basal and insulin-stimulated kinase activity were 2-3-fold higher in red than in white muscle. This phenomenon was evident for autophosphorylation of the β subunit of the receptor (Figs. 4 and 5) and in the ability of the insulin receptor kinase to phosphorylate an exogenous substrate (Fig. 6).

The difference in kinase activity between red and white muscle was evident under basal conditions and in the presence of insulin (Figs. 4-6). Similarly, glucose uptake and [12C]glucose incorporation into glycogen is higher in red than in white muscle in the presence and absence of insulin (2, 4, 6). However, in contrast to insulin-stimulated glucose uptake (2, 4), there was no apparent difference in the ED50 for insulin-stimulated tyrosine kinase activity between red and white muscle (Figs. 4-6). Therefore, it is likely that additional factor(s), such as elevated cell-surface insulin receptor number, may contribute to the greater insulin sensitivity in red compared with white muscle. It is extremely unlikely that the differences in kinase activity between red and white muscle are related to the presence of a nonspecific factor in the wheat germ eluate because the present data were very reproducible among five different muscle preparations. More importantly, when the partially purified receptor preparation from red muscle was incubated with that from white muscle, the ability to phosphorylate the exogenous substrate was additive (Table I). We are also concerned that the structural integrity of the insulin receptor might differ in the two muscle types, and this could explain the difference in kinase activity. However, in studies where [12C]-insulin was affinity cross-linked to the insulin receptor and examined under nonreducing conditions on SDS-PAGE, the pattern of labeling was identical for red and white muscle (Fig. 3). Although small changes in peptide molecular weight may be difficult to discern under these conditions, there was no evidence of differences in the migration profile of the receptor or its subunits between red and white muscle in any of the experiments described. Previous studies have shown that treatment of insulin receptors with neuraminidase may alter the tyrosine kinase activity (27). However, due to the similarity in molecular weight of the insulin receptor isolated from red and white muscle, we believe this to be an unlikely explanation for the differences observed in kinase activity.

Possible explanations for the heterogeneity in kinase activity are changes in the Km of the receptor for cationic cofactors, differential splicing of the insulin receptor gene in red versus white muscle giving rise to subtle sequence changes and subsequent alterations in receptor kinase activity, and in vivo covalent modification of the receptor kinase, such as phosphorylation. Previous studies (28, 29) have demonstrated that phosphorylation of tyrosine residues activates the receptor kinase activity. Also, recent studies indicate that phosphorylation of a serine residue on the insulin receptor causes inhibition of the exogenous kinase activity of the receptor (30).

In a preliminary experiment, alkaline phosphatase treatment of partially purified receptors from red and white muscle equalized their subsequent basal and insulin-stimulated kinase activity toward poly(Glu/Tyr) (data not shown). Further experiments will be necessary to substantiate the hypothesis that differences in receptor phosphorylation states, possibly mediated by serine- and threonine-specific kinases, may account for the findings we report here.

In conclusion, these data indicate that there is a considerable difference in insulin receptor kinase activity between red and white skeletal muscle. These differences complement previously established changes in insulin action among individual muscles of the rat. The finding of altered receptor kinase activity among tissues of functional similarity within the normal laboratory rat suggests that the contributing factors are of cellular origin and not related to systemic changes. These studies lend further support to the hypothesis that the tyrosine-specific protein kinase activity associated with the β subunit of the insulin receptor has a crucial role in receptor coupling transmission.

Acknowledgment—Many thanks to Kerri James for her artistic skills.

REFERENCES
1. Defronzo, R. A., Ferrannini, E., Heidler, R., Felig, P., and Wahren, J. (1983) Diabetes 32, 55-45
2. James, D. E., Jenkins, A. B., and Kraegen, E. W. (1985) Am. J. Physiol. 248, E567-E574
3. Bonen, A., Tan, M. H., and Watson-Wright, W. M. (1981) Diabetes 30, 702-704
4. Richter, E. A., Garetto, L. P., Goodman, M. N., and Ruderman, N. B. (1984) Am. J. Physiol. 246, E476-E482
5. Zorzano, A., Balon, T. W., Garetto, L. P., Goodman, M. N., and Ruderman, N. B. (1985) Am. J. Physiol. 248, E546-E552
6. James, D. E., Barleigh, K. M., Sorlein, L. H., Bennett, S. P., and Kraegen, E. W. (1986) Am. J. Physiol., in press
7. Kahn, C. R., and Crettaz, M. (1985) Diabetes Metab. Rev. 1, 5-32
8. Czech, M. P., Yu, K.-T., Lewis, R. E., Davis, R. J., Mottola, C., MacDonald, R. G., Necessary, P. C., and Corvera, S. (1985) Diabetes Metab. Rev. 1, 33-58
9. Flockhart, D. A., and Corbin, J. D. (1982) Crit. Rev. Biochem. 12, 133-186
10. Houslay, M. D. (1981) Biochi. Rev. 1, 19-34
11. Haring, H. V., Kasuga, M., White, M. F., Crettaz, M., and Kahn, C. R. (1984) Biochemistry 23, 3298-3308
12. Burant, C. F., Treutelaar, M. K., Landreth, G. E., and Buse, M. G. (1984) Diabetes 33, 704-709
13. LeMarchand-Brustel, Y., Areneaux, T., Ballotti, R., and Van Obberghen, E. (1985) Nature 315, 676-679
14. Burant, C. F., Treutelaar, M. K., and Buse, M. G. (1986) J. Clin. Invest. 77, 290-270
15. Azzano, M. A., Armstrong, R. B., and Edgerton V. R. (1972) J. Histochem. Cytochem. 21, 51-55
16. Baldwin, K. M., Klinkerfuss, G. H., Terjung, R. L., Mole, P. A., and Holloszy, J. O. (1972) Am. J. Physiol. 222, 373-378
17. Hollenberg, M. D., and Custerresaca, F. (1976) in Methods in Receptor Research (Blicher, M., ed), pp. 429-477. Marcel Dekker, New York
18. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254
19. Pilch, P. F., and Czech, M. P. (1980) J. Biol. Chem. 255, 1722-1731
20. Laemmli, U. K. (1970) Nature 227, 680-685
21. Kapf, J., Froesch, E. R., and Hummel, R. E. (1981) Curr. Top. Cell. Reg. 19, 257-309
22. Massague, J., Pilch, F. P., and Czech, M. P. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1317-1314
23. Bogé, T. R., Campana, J., Sweet, L. J., and Pessin, J. E. (1985) J. Biol. Chem. 260, 8569-8600
24. Aranis, G., and Livingston, J. N. (1976) J. Biol. Chem. 251, 147-153
25. Kadowaki, T., Kasuga, M., Akanuma, Y., Ezaki, O., and Takaku, F. (1984) J. Biol. Chem. 259, 4208-4216
26. Grigorescu, F., Flier, J. S., and Kahn, C. R. (1984) J. Biol. Chem. 259, 15003-15006
27. Fujita-Yamaguchi, Y., Sato, Y., and Kathuria, S. (1985) Biochem. Biophys. Res. Commun. 129, 739-745
28. Yu, K.-T., and Czech, M. P. (1984) J. Biol. Chem. 259, 5277-5286
29. Rosen, O. M., Herrera, R., Gloew, Y., Petruzelli, L. M., and Cobb, M. (1985) Proc. Natl. Acad. Sci. U.S.A. 80, 2327-2330
30. Stadtmauer, L., and Rosen, O. M. (1986) J. Biol. Chem. 261, 3402-3407