Long-term treatment of isolated rat soleus muscle with phorbol ester leads to loss of contraction-induced glucose transport

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Muscle contraction involves mobilization of intracellular Ca^{2+} and is associated with several metabolic adjustments, including increased glucose transport. In the present study isolated rat soleus muscles were exposed to 12-*O*-tetradecanoylphorbol 13-acetate, and responses to both insulin and contraction in terms of glucose transport were assessed. Muscles treated with this phorbol ester for 12 h showed an increased basal rate of 3-*O*-methylglucose uptake, and responded partially to insulin but did not respond to contraction. Phorbol-ester-treated and non-treated (vehicle-only) muscles were indistinguishable in terms of pre-contraction content of adenine nucleotide, phosphocreatine, lactate and glycogen, as well as contractile performance and contraction-induced glycogenolysis. Phorbol ester treatment of isolated solei for 12 h resulted in the loss of 90 % of protein kinase C activity as determined with histone IIIs as substrate, and 70 % as determined by using phorbol ester binding. It is concluded that treatment of solei with phorbol ester gives rise to a marked loss of contraction-induced glucose transport.

INTRODUCTION

Contraction of skeletal muscle results in an increase in glucose transport (for review see [1]), and until recently this increase was considered to require the presence of a 'permissive' amount of insulin [2]. Such a relationship implies that muscle contraction leads to a markedly increased sensitivity to the prevailing insulin concentration. This now appears not to be the case, and the effects of exercise and insulin on muscle glucose transport appear to be independent [3,4]. However, the mechanism by which contraction stimulates glucose entry into muscle remains to be described [1], although an increase in glucose transporters in the plasma membrane now appears likely [5,6].

Comparisons between the actions of insulin and muscle contraction have been made [1], as both act to increase muscle glucose transport and mechanisms may be shared at least to some extent. Protein kinase C (PKC), a Ca^{2+} - and phospholipid-dependent serine/threonine kinase [7] may be important in some effects of insulin. Experiments have been reported where insulin increases diacylglycerol levels [8–13] and activates PKC [11]. There is also considerable evidence that phorbol esters such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA) have insulin-like effects [14–21], although it is clear that they do not completely mimic the actions of insulin [9,11,17,19,20,22]. Activation of PKC has also been suggested to be involved in modulating responses to insulin [17,21,23–25]. Exposure of intact cells to TPA results in serine phosphorylation of insulin receptors, and incubation of isolated insulin receptors with purified PKC is associated with a decrease in the insulin receptor's basal and insulin-stimulated tyrosine kinase activity [25,26]. In addition, exposure to TPA results in a decreased affinity of the insulin receptor for insulin [17,25].

Attempted down-regulation of PKC by phorbol esters in muscle has led to mixed results. Klip & Ramlal [20] found that treatment of a cultured muscle cell line with TPA resulted in the down-regulation of PKC as assayed by phosphorylation of lysine-rich histone (histone IIIs), but with the retention of full

response to insulin. In another muscle cell line Cooper *et al.* [27] reported that TPA treatment resulted in loss of PKC-directed histone phosphorylation and loss of TPA effects on glucose transport, but PKC-dependent vinculin phosphorylation by type II isoenzyme was retained. This latter observation appeared to explain the continued effectiveness of both insulin and diacylglycerol for stimulating glucose transport in the 'down-regulated' cells [27]. In a third study, involving isolated mouse soleus muscles, Tanti *et al.* [28] reported that down-regulation of PKC, as assayed with lysine-rich histone, inhibited the responses to both TPA and insulin.

Recently we have been exploring the relationship between contraction-induced glucose uptake in skeletal muscle and the involvement of PKC in the control of this process [29]. Our findings indicate that electrical stimulation of skeletal muscle initiates a rapid formation of diacylglycerol and phosphatidate as well as the translocation of PKC, and that these changes precede the increase in glucose uptake [29]. In the present study isolated soleus muscles were incubated with TPA for 12 h and the response of the muscles to electrical stimulation, as well as to insulin, was assessed. PKC activity was monitored by using lysine-rich histone as substrate as well as phorbol ester binding.

MATERIALS AND METHODS

$[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was purchased from Bresatec (Adelaide, Australia). 3-*O*-methyl[1- ^3H]glucose (^3H 3-OMG), [20(n)- ^3H]phorbol 12,13-dibutyrate (^3H PDBu) and $[\text{U-}^{14}\text{C}]\text{sucrose}$ were obtained from Amersham (Bucks., U.K.). Histone IIIs, diolein, phosphatidylserine, TPA, PDD (4 α -phorbol 12,13-didecanoate), as well as coenzymes, enzymes and substrates were obtained from Sigma (St. Louis, MO, U.S.A.). DE-52 DEAE-cellulose and P81 phosphocellulose paper were purchased from Whatman (Maidstone, U.K.). Insulin (Actrapid MC) was from Novo Industri A/S (Copenhagen, Denmark).

Female Hooded Wistar rats (55–65 g) were anaesthetized with

Abbreviations used: PKC, protein kinase C; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; ^3H 3-OMG; 3-*O*-methyl[1- ^3H]glucose; DMSO, dimethyl sulfoxide; ^3H PDBu, [20(n)- ^3H]phorbol 12,13-dibutyrate; PDD, 4 α -phorbol 12,13-didecanoate.

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pentobarbital (5 mg) intraperitoneally. The soleus muscles were removed and attached via the tendons to stainless-steel U-shaped supports (15 mm in width) designed to maintain a tension of approx. 10 g. Muscles were incubated with shaking (100 oscillations/min) in pairs at 30 °C in stoppered 50 ml flasks which contained 5 ml of incubation medium. The incubation medium consisted of modified Krebs–Ringer saline/bicarbonate buffer (93 mM-NaCl, 4.7 mM-KCl, 1.2 mM-KH₂PO₄, 1.2 mM-MgSO₄, 0.05 mM-Na₂EDTA, 1.27 mM-CaCl₂, 5 mM-glucose and 25 mM-NaHCO₃) gassed with O₂/CO₂ (19:1) to give pH 7.4 and contained 0.1 % freeze-dried serum obtained from healthy rats. The total glucose concentration was adjusted to 5 mM; other additions as indicated were 35 mM-mannitol, 100 μ units of insulin/ml, PDD [in dimethyl sulphoxide (DMSO)], and TPA (in DMSO) or DMSO. The flasks were continuously gassed with O₂/CO₂ (19:1) and the medium was changed every 4 h. Muscles were incubated for various times, then removed from the flasks and washed in 25 ml of insulin-free medium before assessment of glucose-uptake rate, metabolite contents, PKC activity and phorbol ester binding.

For determination of glucose uptake, solei were re-mounted by the tendons in a muscle-suspension apparatus, similar to that described by Nesher *et al.* [30]. The incubation medium (4 ml) was modified Krebs–Ringer bicarbonate buffer of the following composition: 93 mM-NaCl, 5 mM-KCl, 1.2 mM-KH₂PO₄, 1.2 mM-MgSO₄, 0.05 mM-Na₂EDTA, 1.27 mM-CaCl₂, 25 mM-NaHCO₃, 5 mM-Hepes, 5 mM-pyruvate, 50 mM-[³H]3-OMG (10 μ Ci/ml) and 5 mM-[U-¹⁴C]sucrose (3.2 μ Ci/ml). The medium was gassed with O₂/CO₂ (19:1) and the pH was adjusted to 7.4. Gas flow was broken into small bubbles to provide adequate mixing of the incubation media. Two muscles were mounted simultaneously, and details for electrical stimulation (Hugo Sachs Elektronik Nerve Muscle Stimulator Type 214) were as follows: 200 ms train of impulses (each impulse 1 ms duration) at a frequency of 50 Hz every 2 s, and the voltage was adjusted (10–20 V) to attain full fibre recruitment. Tension development was recorded with a Harvard Bioscience Isometric Transducer. Solei were frozen (liquid N₂) and then homogenized in 2 ml of water (Ultra-Turrax, 2 $\times$ 30 s), centrifuged (15 min, 220 g), and 1.5 ml of the supernatant was added to 12 ml of ASC II (Amersham) for radioactivity counting. Inclusion of [U-¹⁴C]sucrose in the incubations enabled correction for the extracellular space.

ATP, ADP, AMP, phosphocreatine and lactate were determined in neutralized perchlorate extracts of frozen muscle. The adenine nucleotides were determined by h.p.l.c. (Waters) with a Pharmacia Mono Q anion-exchange column. AMP, ADP and ATP were eluted in order with a linear gradient of 4.5–150 mM-(NH₄)₂SO₄ in 20 mM-Tris/HCl, pH 8.0, and detected at 254 nm. Phosphocreatine, lactate and glycogen were determined by standard methods [31].

For PKC activity determinations, four solei were homogenized in 4 ml of ice-cold 20 mM-Tris/HCl buffer, pH 7.5, containing 250 mM-sucrose and 1 mM-dithiothreitol, with an Ultra-Turrax homogenizer (2 $\times$ 30 s). The homogenate was centrifuged (1 h, 100 000 g) and the resulting pellet (particulate fraction) extracted (1 h) with 6 ml of 20 mM-Tris/HCl buffer, pH 7.5, containing 2.0 % (v/v) Triton X-100, 2 mM-EDTA, 10 mM-EGTA and 1 mM-dithiothreitol. The supernatant was collected by centrifugation (30 min, 100 000 g).

Supernatant extracts containing PKC were applied to columns (4 ml) of Whatman DE-52, which were then washed with 20 ml of 20 mM-Tris/HCl, pH 7.5, containing 2 mM-EDTA, 0.5 mM-EGTA and 1 mM-dithiothreitol. PKC activity was eluted with a linear gradient (36 ml) of 0–0.15 M-NaCl [32]; 4 ml fractions were collected, and typically PKC activity was found in fractions 4–6, corresponding to 30–90 mM-NaCl. Fractions were assayed

for PKC [33] by using histone III_s as the substrate and Whatman phosphocellulose P81 paper to collect the acid-precipitable material. Activity was calculated from the difference in ³²P incorporated into histone in the presence and absence of added phospholipids/Ca²⁺ and was expressed as pmol of ³²P incorporated/min per g fresh wt. of muscle.

Binding of [³H]PDBu was determined by the filtration method [34]. Muscle homogenates were processed as above for the partial purification of PKC. Fractions from the DE-52 column containing PKC activity were pooled from the particulate material and concentrated to approx. 1 ml in an Amicon concentrating system (YM30 membrane). Phorbol-ester-binding reaction mixtures contained 25 mM-Tris/HCl, pH 7.5, 10 mM-magnesium acetate, 1.4 mM-CaCl₂, 0.4 mM-EGTA, 0.4 mM-EDTA, 4 mg of BSA/ml, 100 μ g of phosphatidylserine/ml, 20 nM-[³H]PDBu, with or without 3 μ M-TPA, in a total volume of 400 μ l in glass tubes (10 mm $\times$ 75 mm). After addition of 400 μ l of concentrated sample, the tubes were incubated overnight at 4 °C. Bound [³H]PDBu was separated from free [³H]PDBu by adding 1 ml of 20 mM-Tris/HCl (pH 7.5)/10 mM-magnesium acetate/1 mM-CaCl₂ and filtering the mixture through 2.4 cm-diam. Whatman GF/C filters by suction. The tubes and filters were washed with 5 $\times$ 1 ml of filtering solution, dried in air and then counted for radioactivity. Specific binding was calculated as total binding minus non-specific binding observed in the presence of 3 μ M-TPA.

Data were evaluated with Student's *t* test, and *P* < 0.05 was taken as being statistically significant.

RESULTS

Effect of long-term treatment with TPA on indices of viability

Extended incubation of isolated solei may lead to loss of viability, and agents such as those used in the present study (TPA and DMSO) could exacerbate this effect. Important considerations to minimize loss of function included muscle size [35,36], incubation temperature and medium composition. Several of these were varied, and the following indices of viability determined: water content, ATP, ADP, AMP, phosphocreatine, lactate and glycogen. Indices of incubated muscles were compared with those of freshly excised solei. Components of the incubation medium found to be protective were rat serum, mannitol, insulin and glucose. It was also noted that muscles were best protected when incubated at 30 °C (results not shown). Table 1 compares freshly excised solei with muscles incubated for 12 h at two concentrations of TPA and two concentrations of vehicle. Even the highest concentration of TPA used (5 μ M) did not significantly affect any of the metabolites or the water content of isolated solei (Table 1).

Effect of long-term treatment with TPA on insulin- and contraction-induced transport of 3-OMG

Table 2 shows that freshly isolated solei responded to insulin and electrical stimuli by increasing the rate of 3-O-MG uptake. For insulin (10 munits/ml), the rate increased by 70 %, from 160 to 272 nmol/min per g, and for electrical stimulation the rate increased by 89 %, to 302 nmol/min per g. The magnitude of these responses was less than noted for soleus muscle in perfused hindlimb [37] or *in vivo* [38], but was similar to studies *in vitro* [28,39].

Muscles incubated for 12 h with no additions, 0.06 % DMSO or 0.3 % DMSO each responded to insulin and electrical stimulation to the same extent as freshly excised solei. Treatment with TPA resulted in a partial loss of response to insulin. For 1 μ M-TPA the insulin response decreased from 61 to 40 %, and for 5 μ M from 57 to 24 %. At the higher concentration of TPA the

Table 1. Adenine nucleotides, phosphocreatine, lactate and glycogen contents of isolated solei

Solei either were freshly excised* or had been incubated at 30 °C for 12 h in buffered serum containing TPA in DMSO, or DMSO alone. Muscles were then removed for determination of water content (by drying at 110 °C for 15 h) and metabolites. Glycogen is expressed as μmol of glucose. Means $\pm$ S.E.M. are shown for three observations.

Treatment	Water content ($\mu\text{l/g}$ wet wt.)	Metabolite concn. ($\mu\text{mol/g}$ dry wt.)					
		ATP	ADP	AMP	Phosphocreatine	Lactate	Glycogen
None*	826.4 $\pm$ 8.0	21.6 $\pm$ 1.3	4.1 $\pm$ 1.1	1.0 $\pm$ 0.1	39.2 $\pm$ 2.0	11.5 $\pm$ 0.6	98.1 $\pm$ 21.1
0.06% DMSO	853.6 $\pm$ 8.1	23.6 $\pm$ 3.5	3.1 $\pm$ 0.5	1.9 $\pm$ 0.1	40.6 $\pm$ 2.0	16.0 $\pm$ 2.2	82.6 $\pm$ 7.2
1 μM -TPA	840.2 $\pm$ 11.8	18.1 $\pm$ 0.8	2.4 $\pm$ 0.2	1.2 $\pm$ 0.3	41.0 $\pm$ 3.8	13.2 $\pm$ 2.5	84.7 $\pm$ 4
0.3% DMSO	788.1 $\pm$ 17.3	21.7 $\pm$ 1.1	3.3 $\pm$ 0.4	1.1 $\pm$ 0.1	38.7 $\pm$ 1.4	13.18 $\pm$ 1.7	90.5 $\pm$ 8.6
5 μM -TPA	821.4 $\pm$ 8.5	20.6 $\pm$ 2.4	3.3 $\pm$ 0.5	1.0 $\pm$ 0.2	41.0 $\pm$ 1.2	17.35 $\pm$ 1.5	93.0 $\pm$ 16.1

Table 2. Effect of pretreatment of solei with phorbol esters on insulin- and contraction-induced 3-OMG transport

Solei either were freshly excised† or had been incubated at 30 °C for 12 h in buffered serum containing TPA in DMSO, PDD in DMSO or DMSO alone. Muscles were then transferred to a muscle suspension apparatus containing [^3H]3-OMG and [^{14}C]sucrose for an additional 30 min. Uptake of [^3H]3-OMG was determined at 37 °C as described in the text, and means $\pm$ S.E.M. are shown with the numbers of observations given in parentheses: * $P < 0.05$, [^3H]3-OMG uptake was greater than corresponding 'no addition' control; n.s., not significantly different from corresponding 'no addition' control.

Pretreatment	[^3H]3-OMG uptake (nmol/min per g)		
	No addition	Insulin (10 munits/ml)	Contractions (30 min)
None†	160 $\pm$ 6 (3)	272 $\pm$ 23 (4)*	302 $\pm$ 14 (3)*
Incubated for 12 h	189 $\pm$ 11 (4)	303 $\pm$ 29 (4)*	283 $\pm$ 22 (3)*
0.06% DMSO	187 $\pm$ 14 (4)	301 $\pm$ 19 (4)*	300 $\pm$ 22 (4)*
1 μM -TPA in 0.06% DMSO	198 $\pm$ 10 (5)	278 $\pm$ 7 (5)*	259 $\pm$ 15 (5)*
0.3% DMSO	185 $\pm$ 17 (5)	291 $\pm$ 16 (5)*	288 $\pm$ 13 (5)*
5 μM -TPA in 0.3% DMSO	225 $\pm$ 6 (5)	279 $\pm$ 8 (5)*	211 $\pm$ 10 (5) n.s.
5 μM -PDD in 0.3% DMSO			297 $\pm$ 17 (4)*

apparent loss of insulin response may be exaggerated, owing to the increased basal uptake rate. As shown in Table 2, the basal uptake rate increased from 189 $\pm$ 11 to 198 $\pm$ 10 (1 μM -TPA) and 225 $\pm$ 6 (5 μM -TPA).

Treatment with 5 μM -TPA for 12 h markedly inhibited the response to electrical stimuli (Table 2). At 1 μM -TPA the response decreased from 60% to 31% and at 5 μM -TPA from 56% to zero, as compared with the corresponding DMSO controls. Treatment with 5 μM -PDD, a non-active phorbol ester, had no effect on contraction-induced 3-OMG uptake (Table 2).

Effect of long-term treatment with TPA on contraction-induced twitch tension and glycogenolysis

Since long-term exposure of solei to TPA could produce a variety of changes in muscle biochemistry, some of which could be non-specific, it was considered important to assess the effects on responses related to glucose uptake. Table 3 shows the effect of incubation for 12 h at 30 °C on the twitch tension developed by solei which were subsequently stimulated to contract. Incubation for 12 h without addition showed no loss of contractile performance. TPA had no significant effect as compared with the corresponding DMSO control (Table 3). Thus electrical stimulation of solei previously exposed to a concentration of TPA sufficient to lead to a total loss of glucose transport responded normally in terms of twitch tension development.

Table 1 shows that incubation of muscles for 12 h with or

Table 3. Effect of pretreatment of solei with TPA on electrical-stimulation-induced twitch tension

Solei either were freshly excised† or had been incubated at 30 °C for 12 h in buffered serum containing TPA in DMSO, or DMSO alone. Muscles were then transferred to a muscle suspension apparatus at 37 °C, and twitch tension was recorded during a 30 min period of electrical stimulation (200 ms train of 1 ms impulses at 50 Hz, every 2 s; the voltage was adjusted to attain full fibre recruitment). Means $\pm$ S.E.M. are shown with the numbers of observations given in parentheses: n.s., not significantly different from corresponding DMSO control.

Pretreatment	Twitch tension (g)
None†	10.3 $\pm$ 1.0 (5)
Incubated for 12 h	9.2 $\pm$ 1.5 (4)
0.06% DMSO	8.9 $\pm$ 1.2 (7)
1 μM -TPA in 0.06% DMSO	8.5 $\pm$ 0.5 (5) n.s.
0.3% DMSO	8.0 $\pm$ 1.5 (3)
5 μM -TPA in 0.3% DMSO	8.3 $\pm$ 1.2 (4) n.s.

without TPA did not alter glycogen content, provided that glucose was present in the medium. If the muscles were then stimulated to contract for 30 min, the glycogen content decreased markedly from approx. 88 to 12.1 $\pm$ 4.4 ($n = 3$) and 16.0 $\pm$ 4.8

Fig. 1. Effect of pretreatment with TPA on total PKC activity

Solei attached to metal supports were incubated at 30 °C for 12 h in buffered serum containing TPA in DMSO or DMSO alone, as indicated. The muscles were then homogenized and the total activity (cytosolic+particulate) of PKC was determined. Means $\pm$ S.E.M. (pmol of histone IIIs phosphorylated/min per g wet wt. of muscle) are shown, with the numbers of observations in parentheses. * $P < 0.01$, activity of TPA-treated muscles was less than corresponding DMSO control.

($n = 3$) μ mol/g dry wt. for 0.3 %-DMSO- and 5 μ M-TPA-treated muscles, respectively. Thus loss of glucose transport was not associated with a loss of the glycogenolytic response to muscle contractions.

Effect of long-term treatment with TPA on PKC activity and phorbol ester binding

Long-term treatment of cells has been routinely used to 'down-regulate' PKC (e.g. see [40]). Thus in the present study muscles treated with 1 μ M- and 5 μ M-TPA and which showed loss of contraction-induced glucose transport were assayed for PKC activity. Two methods were used. The first was based on the conventional assay with lysine-rich histone (histone IIIs) as substrate [33]. Fig. 1 shows that exposure of solei to 1 μ M- and 5 μ M-TPA for 12 h decreased total (cytosolic+particulate) activity of PKC by approx. 70 and 90 % respectively. In the second method total binding sites for [3 H]PDBu were determined. Exposure of solei to 5 μ M-TPA for 12 h resulted in a decrease in binding from 2.68 ± 1.11 ($n = 4$; 0.3 % DMSO) to 0.81 ± 0.28 ($n = 4$) pmol/g wet wt.

DISCUSSION

The key finding in the present study was that muscles exposed to TPA for 12 h responded comparably with appropriate controls in terms of tension development and glycogen breakdown, but failed to respond in terms of glucose transport. Since long-term exposure to TPA is now a well-accepted procedure for down-regulation of PKC, it appears likely that the accompanying loss of PKC from the solei was responsible for the loss of contraction-induced glucose transport. Indeed, inspection of the data of Table 2 and Fig. 1 show that down-regulation of PKC by 70 %

coincided with a loss of approx. 50 % of the contraction-induced response, and at higher doses of TPA, when PKC was down-regulated further (by 90 %), a total loss occurred. However, the conclusion that PKC is involved in the metabolic adjustments to muscle contraction, particularly glucose transport, must be treated with caution. There are now several identified isoenzymes of PKC [41] which have different substrate specificities and which may be differentially down-regulated by TPA treatment [18,27,42]. Thus TPA treatment of KM3 cells has been reported to cause translocation and depletion of type II PKC more quickly than for type III enzyme, although both sub-species were totally depleted in 2 h [42]. Perhaps more importantly, when vinculin was used as substrate for the determination of PKC activity [27], TPA treatment of BC3H-1 myocytes did not deplete type II enzyme, even though this sub-species was undetectable with histone in the same TPA-treated cells. Thus in the present study it is possible that the apparent down-regulation of PKC as determined by histone IIIs kinase activity (Fig. 1) did not reflect down-regulation of all sub-species. To address this issue, we have made use of the observations by others that skeletal muscle contains predominantly types II and III PKC [43] and that these types bind [3 H]PDBu with similar affinity [44]. Clearly, the marked loss of total [3 H]PDBu binding which accompanied the 12 h treatment of solei with 5 μ M-TPA would support the view that down-regulation of both types II and III has occurred.

A further consideration is that the loss of contraction-mediated glucose uptake results not from down-regulation of PKC but from the 12 h period of exposure to TPA as a PKC activator. Sowell *et al.* [45] have shown that treatment of solei with TPA leads to a marked inhibition of glycogen synthesis, and there is some indication in the present study and from others [20] that the basal rate of glucose transport is increased by prolonged TPA exposure. In addition, the observation that short-term exposure by phorbol esters induces symptoms of myotonia in skeletal muscle by lowering chloride conductance [46] suggests that ion channels may be modified by PKC activator. Together these changes may be representative of a general metabolic and functional adjustment to chronic PKC activation, which could include loss of contraction-induced glucose transport.

The partial loss of insulin response (Table 2) as a result of long-term treatment with TPA might also suggest that PKC is involved, at least in part, in the action of insulin. This would be consistent with a recent report by Tanti *et al.* [28], but with some difference. In their studies [28] it was reported that 24 h exposure of isolated mouse solei to 1 μ M-TPA led to a 40 % loss of cytosolic PKC activity and a total loss of insulin-mediated 2-deoxyglucose uptake. However, differences between the species may be contributory. For example, 2-deoxyglucose uptake by mouse solei is stimulated by TPA [28]. For rat solei 2-deoxyglucose uptake is not affected by 0.04–1.6 μ M-TPA [45]. Similarly, in our laboratory 3-OMG uptake by isolated rat solei was not stimulated by acute exposure to 1 μ M-TPA [117 ± 10 ($n = 5$) versus 127 ± 21 nmol/min per g ($n = 7$) for control muscles] or 200 μ M-1,2-diocanoyl-*rac*-glycerol [79 ± 2 ($n = 3$) versus 96 ± 16 nmol/min per g ($n = 3$) for matched control muscles].

Comparison of skeletal muscle with other tissues suggests that PKC activity is low (e.g. see ref. [7]) and may be rendered functionally inoperative by the accompanying presence of relatively large amounts of an endogenous inhibitor of PKC [47]. However, when assayed under comparable conditions, the levels of PKC and the cyclic AMP-dependent protein kinase (protein kinase A) are similar in muscle [7], and therefore PKC may have as great a significance to muscle metabolism as does protein kinase A.

Finally, the link between muscle contraction and glucose

transport is still far from clear. Even though the present and our previous studies [29] imply a role for PKC and generally support an association between phosphoinositol metabolism and excitation-contraction coupling [48,49] it will require further studies to establish that PKC is an activator of glucose transport. There have been two recent reports [5,6] indicating that acute exercise increased the number of plasma-membrane glucose transporters in rat skeletal muscle. This may result from translocation of glucose transporters from a microsomal fraction to the muscle sarcolemma, and therefore may be similar to insulin [50], or may result from changes in the intrinsic activity of the glucose transporter. However, it is likely that the mechanisms involved in insulin and exercise differ in some respect, as the effect of a maximal concentration of insulin on glucose transport was further increased by muscle contraction [51].

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