Volume 217, number 2, 232-236 FEB 04831 June 1987

Contraction-associated translocation of protein kinase C in rat skeletal muscle

Erik A. Richter*, Perry J.F. Cleland, Stephen Rattigan and Michael G. Clark

Department of Biochemistry, University of Tasmania, Hobart, Australia and **August Krogh Institute, University of Copenhagen, Copenhagen, Denmark*

Received 22 April 1987

Electrical stimulation of the sciatic nerve of the anaesthetized rat in vivo led to a time-dependent translocation of protein kinase C from the muscle cytosol to the particulate fraction. Maximum activity of protein kinase C in the particulate fraction occurred after 2 min of intermittent short tetanic contractions of the gastrocnemius-plantaris-soleus muscle group and coincided with the loss of activity from the cytosol. Translocation of protein kinase C may imply a role for this kinase in contraction-initiated changes in muscle metabolism.

Ca^{2+} phospholipid-dependent protein kinase; Exercise; Muscle contraction

1. INTRODUCTION

Muscle contraction is associated with changes in the intracellular distribution of Ca^{2+} and changes in several metabolic processes. Recent studies suggest that excitation-contraction coupling in muscle and the resultant mobilization of intracellular Ca^{2+} may involve inositol phosphates [1-4] produced by rapid hydrolysis of phosphatidylinositol. The second product from phosphatidylinositol hydrolysis, diacylglycerol, has been proposed in other tissues to lead to the activation of protein kinase C with translocation of this enzyme from cytosol to the plasma membrane or particulate fraction [5]. Here, we have developed an assay for protein kinase C in skeletal muscle preparations and examined the effect of contraction on the distribution of this enzyme between the cytosol and particulate fractions. A possible role for protein kinase C in exercise-induced Ca^{2+}-dependent metabolic changes is discussed.

Correspondence address: M.G. Clark, Department of Biochemistry, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia

2. EXPERIMENTAL

2.1. *Materials*

[γ-^{32}P]ATP was supplied by Bresa (Adelaide, Australia) and histone IIIs was obtained from Sigma (USA).

2.2. *Methods*

Fed, male hooded Wistar rats (250 ± 10 g) were anaesthetized with pentobarbital (12.5 mg). The skin was removed from the left hindlimb and the sciatic nerve exposed and cut to allow positioning of the distal end in a suction electrode. The gastrocnemius-plantaris-soleus muscle group was exposed by careful removal of overlaying tissue. The knee was secured by the tibiopatellar ligament and the Achilles tendon was attached via a steel wire to a Harvard Apparatus isometric transducer. Tension development was recorded during electrical stimulation (200 ms trains of 100 Hz applied every 2 s) adjusted (10-20 V) to attain full fibre recruitment. Initial tension was 1065 g which decreased to 600 g after 1 min then remained constant for the remaining 4 min. After 0, 1, 2 or 5 min stimulation the gastrocnemius-plantaris-soleus muscle group (approx. 1.3 g), representing

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

mainly fast-twitch red and white muscle [6] was rapidly removed and immediately homogenized in 4 ml ice-cold 20 mM Tris-HCl buffer, pH 7.5, containing 250 mM sucrose and 1 mM dithiothreitol using an Ultra-Turrax homogenizer (2 × 30 s). The homogenate was centrifuged for 1 h at 100 000 × *g* and the pellet (particulare fraction) extracted twice with 2 × 6 ml of 20 mM Tric-HCl buffer, pH 7.5, containing 2.0% (v/v) Triton X-100, 2 mM EDTA, 10 mM EGTA and 1 mM dithiothreitol. To the supernatant was added 1/10 vol. of 20 mM Tris-HCl buffer, pH 7.5, containing 250 mM sucrose, 20 mM EDTA, 5 mM EGTA and 1 mM dithiothreitol. Finely powdered ammonium sulphate was then added to give a concentration of 21% (w/v) and the protein precipitate was removed by centrifugation at 8000 × *g* for 20 min. A further addition of ammonium sulphate was made to the supernatant [final concentration 45% (w/v)] and the protein pellet, recovered after centrifugation, was dissolved in 0.2 ml of 20 mM Tris-HCl buffer, pH 7.5, containing 2 mM EDTA, 0.5 mM EGTA and 1 mM dithiothreitol. After desalting on a 7 ml column of Bio-Gel P6DG (Bio-Rad, USA) all fractions containing protein were applied to a 3.5 ml column of Whatman DE-52 and eluted using a linear gradient of 36 ml of 0–150 mM NaCl [7]. The Triton X-100 extracts of the particulate fraction were applied directly to DE-52 columns and eluted in a similar manner. Fractions were assayed for protein kinase C [8] using histone IIIs as the substrate and Whatman phosphocellulose P81 paper to collect the acid-precipitable material. Activity was expressed in terms of the weight of the gastrocnemius-plantaris-soleus muscle group from non-stimulated animals (1.3 ± 0.1 g).

3. RESULTS

3.1. *Protein kinase C activity in muscle preparations*

Published values for protein kinase C in skeletal muscle (373 pmol/min per g [9]) indicated that the activity was considerably less than brain or spleen (17 000–20 000 pmol/min per g [9]) and therefore suggested that difficulties might be encountered in isolating protein kinase C from muscle extracts by anion-exchange chromatography. To overcome this potential problem and to remove some of the contaminating protein, muscle extracts were fractionated by ammonium sulphate and then desalted. By using this approach recovery of protein kinase C was quantitative (>95%) and total activity approached 3500 pmol/min per g.

Fig.1A shows the DE-52 profile of the ammonium sulphate cut (21–45%, w/v) of the cytosolic fractions of non-contracted and contracted (2 min) skeletal muscle. In fig.1B the data for the first and second 2% Triton/EGTA extraction of

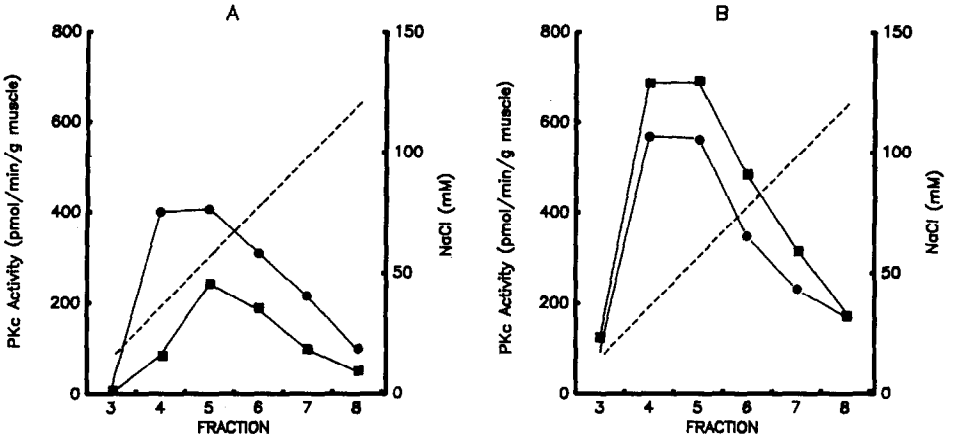

Fig.1. Anion-exchange chromatography of protein kinase C activity from cytosol (A) and particulate (B) preparations of contracting (■) and non-contracting (●) muscle. Details for sample preparation are given in the text. The DE-52 columns were eluted with a linear gradient of NaCl (– – –). Representative data from five contracting (2 min) and nine non-contracting muscles are shown.

the particulate material have been combined. In general the first Triton/EGTA wash extracted 75% of the particulate activity. From fig.1 it can be seen that contraction led to a decrease in protein kinase C activity in the cytosol and a corresponding increase in activity in the particulate fraction. Both cytosolic and particulate activities were eluted at 40–50 mM NaCl and non-Ca^{2+}/phospholipid-dependent protein kinase activities were negligible (not shown).

Contraction is associated with increased blood flow to the gastrocnemius-plantaris-soleus muscle group and the weight of this group was found to increase from 1.3 to 1.5, 1.6 and 1.6 g at 1, 2 and 5 min, respectively. To rule out the possibility that the increased blood content of the muscle group contributed to the observed translocation of protein kinase C, 0.3 g blood from stimulated animals (cardiac puncture) was added to non-contracted muscle during homogenization. This had no effect on either the distribution of protein kinase C between the cytosolic and particulare fractions or on the total activity (not shown). Similarly, homogenization of non-contracted muscle in buffer containing 1 mM $CaCl_2$ also had no effect on these values. It is noteworthy that EGTA could not be included in the initial homogenizing buffers as this displaced the particulate enzyme and diminished

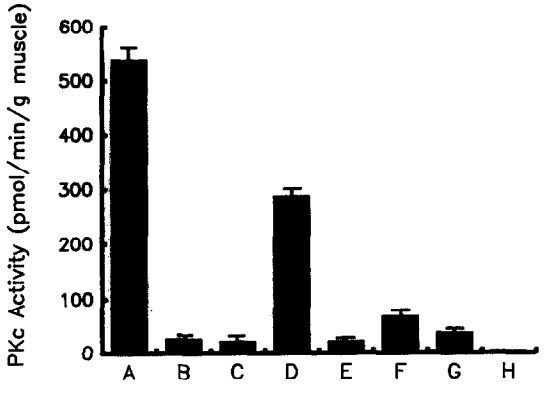

Fig.2. Ligand dependency of protein kinase C from the particulate fraction of contracting muscle. Protein kinase C was partially purified by anion-exchange chromatography of Triton X-100 extracts of the particulate fraction from contracting (2 min) muscles. The complete assay contained 20 mM Tris-HCl, pH 7.5, 0.15 mM EGTA, 0.6 mM EDTA, 7.35 mM Mg acetate, 0.6 mM $CaCl_2$, 42 μg/ml L-α-phosphatidyl-L-serine, 0.84 μg/ml diolein, 0.3 mg/ml histone IIIs, 50 μl protein kinase C sample, and 10 μM [γ-^{32}P]ATP. Changes to the complete assay (A) were: omission of histone (B); omission of phosphatidylserine (C); omission of diolein (D); omission of phosphatidylserine and diolein (E); omission of $CaCl_2$ with the addition of 7.35 mM EGTA (F); omission of $CaCl_2$, phosphatidylserine and diolein with the addition of 7.35 mM EGTA (G); omission of $CaCl_2$, phosphatidylserine, diolein and histone with the addition of 7.35 mM EGTA (H). Values are means ± SE from three experiments.

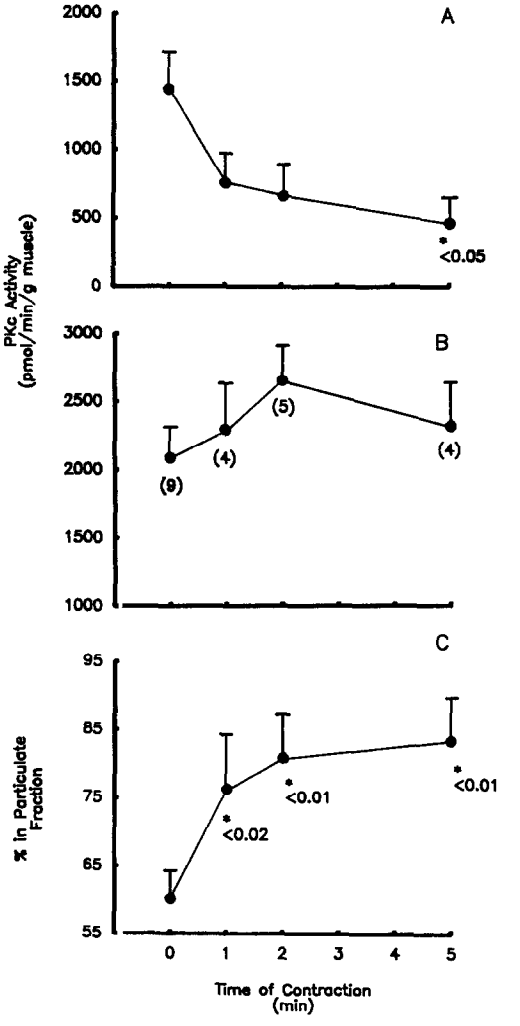

Fig.3. Effect of electrical stimulation-induced contraction of muscle on the distribution of protein kinase C activity in cytosolic (A) and particulate fractions (B). Details are given in the text. Values are means ± SE with the number of experiments given in parentheses. *P values compared to 0 min using Student's t-test.

the translocation. However, when used in conjunction with 2% Triton X-100, 10 mM EGTA improved the extraction of protein kinase C from the 100 000 × *g* particulate material.

3.2. *Ca^{2+} and phospholipid dependency of the translocated particulate enzyme*

Fig.2 shows the ligand dependency of protein kinase C from the particulate fraction of contracted muscle. Full activity for histone phosphorylation was dependent on the presence of diolein, phosphatidylserine and Ca^{2+}. Omission of these substances as well as histone completely inhibited protein phosphorylation. However when histone alone was omitted some autophosphorylation by endogenous kinase(s) was evident. The data of fig.2 are characteristic of protein kinase C [10,11].

3.3. *Time course effects of muscle stimulation*

Fig.3 shows the effect of electrically induced contraction of the gastrocnemius-plantaris-soleus muscle group on the distribution of protein kinase C between the cytosolic and particulate fractions. Approx. 60% of the total protein kinase C activity was located in the particulate fraction prior to contraction. This value increased in a time-dependent manner with contraction to reach a maximum of 83% at 2 min with a corresponding decrease in cytosolic activity. After 2 min the loss of activity from the cytosol continued but was not matched by a corresponding stoichiometric increase in the particulate fraction. Approx. 18% of the total activity remained unaccounted for at 5 min.

4. DISCUSSION

Minor modifications to existing methods have enabled quantitative recovery of cytosolic and particulate activities of protein kinase C from skeletal muscle. Presumably because of the removal of endogenous inhibitors, ATP and ATPases, the total activity found was considerably higher than previous reports [9]. For unstimulated muscle approx. 60% of the enzyme was located in the particulate fraction indicating a potentially high state of activation. Clearly this warrants further study.

The present findings represent the first report of contraction-associated translocation of skeletal muscle protein kinase C from the cytosolic to the particulate fraction. These findings extend the observations of Vergara et al. [1] who reported evidence for inositol 1,4,5-trisphosphate as chemical second messenger between transverse (T)-tubular membrane depolarization and calcium release from the sarcoplasma reticulum of skinned frog muscle. Thus, T-tubular membrane depolarization may involve stimulation of the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C to form inositol 1,4,5-trisphosphate and diacylglycerol. Subsequent activation of protein kinase C by the latter compound may take place in the presence of Ca^{2+} and phospholipid of the membrane. Phosphorylation of membrane-bound substrates could then account for some of the changes in metabolism which occur as a result of increased muscle contraction. Muscle contraction of the magnitude used in the present studies would be expected to lead to increased glucose transport, glycogenolysis, glycolysis as well as increased oxidative metabolism [12,13]. A likely membrane protein candidate for phosphorylation is the glucose transporter protein which from human red-blood cells is a substrate for brain protein kinase C [14]. Furthermore, activation of protein kinase C in 3T3L1 adipocytes by phorbol esters leads to increased glucose transport as well as phosphorylation of the glucose transporter [15]. However, it is as yet not known whether phosphorylation of this protein by protein kinase C in fact alters its transporter properties.

ACKNOWLEDGEMENTS

We thank Geoffrey Appleby for expert technical assistance. This work was supported in part by grants from the National Health and Medical Research Council of Australia, the Ramaciotti Foundations and the Danish Medical Research Council.

REFERENCES

[1] Vergara, J., Tsien, R.Y. and Delay, M. (1985) Proc. Natl. Acad. Sci. USA 82, 6352–6356.
[2] Volpe, P., Salviati, G., Di Virgilio, F. and Pozzan, T. (1985) Nature 316, 347–349.
[3] Thieleczek, R. and Heilmeyer, L.M.G. jr (1986) Biochem. Biophys. Res. Commun. 135, 662–669.
[4] Nosek, T.M., Williams, M.F., Zeigler, S.T. and Godt, R.E. (1986) Am. J. Physiol. 250, C807–C811.

[5] Nishizuka, Y. (1984) Nature 308, 693–698.
[6] Ariano, M.A., Armstrong, R.B. and Edgerton, V.R. (1973) J. Histochem. Cytochem. 21, 51–55.
[7] Averdunk, R. and Gunther, T. (1986) FEBS Lett. 195, 357–361.
[8] Kikkawa, U., Takai, Y., Minakuchi, R., Inohara, S. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 13341–13348.
[9] Kuo, J.F., Andersson, R.G.G., Wise, B.C., Mackerlova, L., Salomonsson, L., Brackett, N.L., Katoh, N., Shoji, M. and Wrenn, R.W. (1980) Proc. Natl. Acad. Sci. USA 77, 7039–7043.
[10] Inoue, M., Kishimoto, A., Takai, Y. and Nishizuka, Y. (1977) J. Biol. Chem. 252, 7610–7616.
[11] Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T. and Nishizuka, Y. (1979) Biochem. Biophys. Res. Commun. 91, 1218–1224.
[12] Richter, E.A., Ruderman, N.B., Gavras, H., Belur, E.R. and Galbo, H. (1982) Am. J. Physiol. 242, E25–E32.
[13] Richter, E.A., Ploug, T. and Galbo, H. (1985) Diabetes 34, 1041–1048.
[14] Witters, L.A., Vater, C.A. and Lienhard, G.E. (1985) Nature 315, 777–778.
[15] Gibbs, E-M., Allard, W.J. and Lienhard, G.E. (1986) J. Biol. Chem. 261, 16597–16603.