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Protein kinase C activity in rat skeletal muscle

Apparent relation to body weight and muscle growth

Erik A. Richter and Niss B.S. Nielsen

August Krogh Institute, University of Copenhagen, Copenhagen, Denmark

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Protein kinase C (PKC) may be involved in growth regulation. In the present study the relationship between body weight, and thereby age, and the activity of PKC in muscle as well as in rapidly growing overloaded muscle were investigated. PKC activity in music was linearly inversely correlated to rat weight in both soleus ($r = -0.59$, $P < 0.05$) and in plantaris ($r = -0.74$, $P < 0.01$) muscles. During compensatory hypertrophy, PKC activity per muscle was maximally increased compared with the contralateral control muscles after 4 days in both soleus (126%) and in plantaris (105%) but had returned to basal levels by the 9th day. The data are in agreement with a role for PKC in muscle growth.

Compensatory hypertrophy; Aping; Growth

1. INTRODUCTION

The regulation of muscle growth is not completely understood. Recently, however, protein kinase C (PKC) has been implicated in growth regulation of several tissues [1]. For instance, PKC phosphorylates the insulin receptor [2,3], the EGF receptor [4], the Na^+/H^+ antitransporter [3] and the somatomedin-C receptor [2], all of which are believed to take part in the promotion of growth. Furthermore, PKC is activated by tumor promoting phorbol esters [5,6]. In vitro experiments have shown that rapidly growing cells exhibit high levels of PKC in the membrane fraction and little activity in the cytosol, whereas growth-arrested cells exhibit low membrane associated PKC activity and high cytosolic activity [1]. Because protein kinase C is phospholipid-dependent, translocation from the cytosol to the membrane fraction is considered as the activation of the enzyme [7].

Skeletal muscle grows in the maturing organism and as a function of increased usage. Since protein kinase C is abundant in skeletal muscle [8,9] and furthermore is translocated from the cytosol to the membrane fraction during muscle contractions [8,9], protein kinase C might be involved in muscle growth. As a first approach to this question, protein kinase C activity and its subcellular distribution were studied in muscles of rats of various body weights and thereby ages, and in muscles undergoing rapid growth during overloading.

Correspondence address: E.A. Richter, August Krogh Institute, 13 Universitetsparken, DK-2100 Copenhagen, Denmark.

2. MATERIALS AND METHODS

2.1. *Muscle protein kinase C and rat weight*

Fed, female Wistar rats between 53 g and 431 g were anaesthetised with pentobarbital (0.1 g/100 g rat weight). The soleus muscle (consisting mainly of slow-twitch fibers [10]) and the plantaris muscle (consisting of a mixture of fast-twitch red and fast-twitch white fibers [10]) were removed from both hindlimbs and homogenized on ice in 3 ml 20 mM Tris buffer, pH 7.5, containing 250 mM sucrose, 1 mM dithiothreitol and 50 μM C-AMP using a polytron homogeniser for 2×30 s. The homogenate was centrifuged for 1 h at $100\,000 \times g$. The resulting supernatants (cytosolic fractions) were applied to columns containing 3 ml Whatman DE-52 cellulose anion-exchange resin, equilibrated with buffer (20 mM Tris-HCl, 2 mM EDTA, 0.5 mM EGTA, 1 mM dithiothreitol, 50 μM C-AMP). The pellets (particulate fractions) were extracted twice with 3 ml ice-cold extraction buffer,pH 7.5, which contained 20 mM Tris-HCl, 2% Triton X-100, 10 mM EGTA, 1 mM dithiothreitol, and then centrifuged for 30 min at $100\,000 \times g$. The resulting supernatants were combined and applied to the columns and eluted. The fractions were then assayed for PKC by the method described by Kikkawa et al. [11]. Whatman phosphocellulose P81 paper was used to collect the acid precipitable material.

2.2. *Compensatory hypertrophy experiment*

Fed, female Wistar rats between 235 g and 270 g were anaesthetised with pentobarbital and the distal half of the gastrocnemius muscle was carefully removed. Care was taken to leave nerve and vascular supply to the soleus and plantaris muscles intact. The contralateral leg had the same skin incision but was otherwise left intact. The incisions were then sutured. The rats were then subjected to a program of treadmill exercise consisting of running 18 m/min, 13° uphill, 5 min/day. The rats were sacrificed 2, 4, 6, 10 and 34 days after surgery and the soleus and plantaris muscles from both hindlegs were assayed for PKC activity as described above. In additional experiments, muscles of operated as well as sham operated legs were weighed before and after drying for 24 h at 180°C to establish the relative content of dry matter and water.

3. RESULTS

3.1. *Relationship between rat weight and protein kinase C activity*

Total activity of protein kinase C (PKC) in both the soleus and plantaris muscles were linearly and inversely related to rat weight (Fig. 1). The subcellular distribution of PKC also indicated higher activity in younger rats: in the plantaris the fraction of PKC in the cytosol was significantly correlated to rat weight ($r = 0.72$, $P < 0.01$), whereas the correlation was not significant in the soleus muscle.

3.2. *Protein kinase C and compensatory hypertrophy*

After ablation of the distal half of the gastrocnemius muscle, a rapid increase in plantaris and soleus muscle weight occurred (Table I). However, part of the increase in muscle mass was due to an increased muscle water content but even when expressed as dry muscle weight a rapid increase in muscle mass occurred (Table I). Interestingly, in the plantaris, dry muscle mass was already increased by 22% on the second postoperative day whereas in the soleus, dry weight was not significantly increased before the 6th day after surgery (Table I). After 34 days of overload, the plantaris had increased its wet and dry weight more than the soleus (Table I).

After surgery a rapid increase in muscle PKC activity occurred. In the plantaris the total muscle activity was increased by 62% already after 2 days and by 105% after 4 days compared to the contralateral muscle (Fig. 2). However, due to changes in muscle mass the maximal increase was only 64% when expressed per g dry muscle mass (Fig. 2). Also in the soleus, PKC activity was highest on the 4th postoperative day whether expressed as total activity or per unit wet or dry weight. In both muscles, PKC activity was not different from the activity in the contralateral muscles on the 9th and 34th postoperative day (Fig. 2).

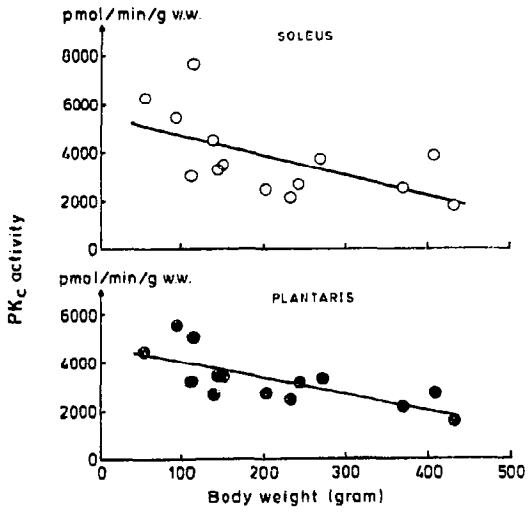

Fig. 1. Muscle protein kinase C activity in the soleus (top panel) and in the plantaris (bottom panel) muscles in relatin to body weight. For soleus, $r = -0.59$, $P < 0.05$, and for plantaris $r = -0.74$, $P < 0.05$.

Table I

Percentage increase in wet and dry weight in plantaris and soleus muscle after overload

Days post surgery	Plantaris		Soleus	
	Wet weight	Dry weight	Wet weight	Dry weight
2	50 ± 15	22 ± 10	25 ± 6	− 3 ± 9
4	26 ± 8	11 ± 6	21 ± 5	4 ± 4
6	26 ± 6	14 ± 4	33 ± 11	22 ± 10
10	21 ± 7	13 ± 5	15 ± 9	11 ± 11
34	39 ± 5	30 ± 4	14 ± 4	6 ± 4

Values (means ± SE) are expressed as percentage increase compared to the contralateral side. $n = 8$ in all groups.

The subcellular distribution of PKC was not markedly altered during compensatory hypertrophy in neither muscle.

4. DISCUSSION

The linear negative correlation between muscle PKC activity and rat weight represents the first report of weight, and thereby age-associated changes in PKC activity in skeletal muscle. Furthermore, in fast-twitch muscle (plantaris) we show that with an increase in body weight the subcellular distribution of PKC is changed towards less activity in the particulate fraction suggesting a lesser state of activation [7]. Thus, our data are in agreement with findings in other tissues in which the subcellular distribution of PKC is dependent upon growth rate [1]. Our data do not allow us to conclude that PKC has a causal role in muscle growth but they are compatible with the notion that PKC may play a role in growth regulation in muscle.

During compensatory hypertrophy, PKC activity increased markedly during the first 4 days of overload but returned to control levels within 6–10 days of overload

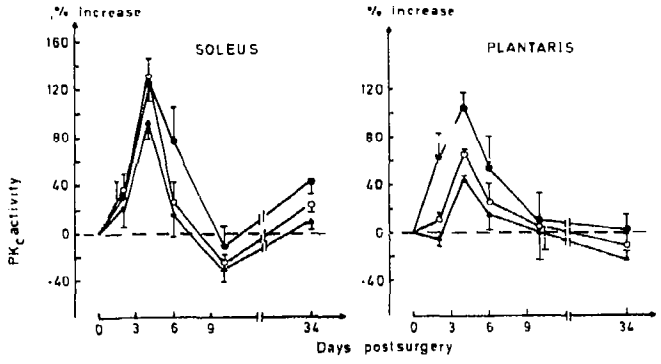

Fig. 2. Protein kinase C activity in overloaded muscles expressed as percent of activity in the contralateral muscles. Muscles were subjected to overload on day 0. Percent increase is based on total activity in muscle (filled circles), on activity per g wet muscle weight (triangles) and on activity per g dry muscle weight (open circles). Values are means and SE of three observations.

(Fig. 2). It is apparent from the data in Table I that most of the total growth over the 34 day observation period occurred early-on after overload, both when expressed as increase in wet and in dry weight. However, compensatory hypertrophy results not only in enlargement of the muscles but also in some remodelling. For instance the myosin isoforms change dramatically in fast muscles with a threefold increase in slow myosin and a reciprocal decrease in fast myosin [12]. In the slow-twitch soleus the changes in myosin isoforms are minor [12]. Since these changes require 11 weeks to be fully established [12] it is questionable if PKC is involved in this remodelling but an increase in PKC activity could be an important initial signal.

A final point is that compensatory hypertrophy after ablation of synergist muscles results in an initial inflammatory response characterised by infiltration of the muscles with leucocytes [13]. Since leucocytes apparently have a markedly higher PKC activity than muscle [14], part of the increase in PKC activity in muscle during overload could be due to leucocyte infiltration. However, the inflammatory response is maximal 1–2 days after surgery and lasts for 16 days [13]. Taken together with our findings of a maximal PKC activity after 4 days and a return to baseline after 6–9 days, it is unlikely that leucocyte infiltration markedly affected the PKC activity measured in the present experiment.

It is concluded that the present data are compatible with a role for PKC in the growth of rat skeletal muscle.

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