Acute treatment with tumour necrosis factor-α induces changes in protein metabolism in rat skeletal muscle

Cèlia García-Martínez, Francisco J. López-Soriano and Josep M. Argilés

Unitat de Bioquímica B, Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08071-Barcelona, Spain

Received 2 February 1993; accepted 6 May 1993

Abstract

Acute treatment of rats with recombinant tumour necrosis factor (TNF-α) caused an enhanced proteolytic rate – measured as tyrosine released in the presence of cycloheximide – in soleus muscle (34%). The cytokine treatment also decreased the rate of protein synthesis in this muscle (22%) while it had no effect upon the same parameter in extensor digitorum longus (EDL) (26%) muscle. In addition, treatment of rats with TNF-α increased amino acid uptake by transport system A in the incubated muscles both in soleus (45%) and EDL (99%) in the presence of insulin in the incubating medium. This effect was not associated with a direct action of TNF on muscle since the addition of different concentrations of the cytokine to the preparations did not alter the uptake of α-(methyl)-aminoisobutyric acid by the incubated muscles. It can be concluded that acute TNF-α treatment causes changes in protein metabolism in red-type muscles – such soleus – while little effects are seen in white-type muscles – such as EDL. The results presented may, to some extent, be related to the cachectic response associated with cancer and inflammation. (Mol Cell Biochem 125: 11-18, 1993)

Key words: tumor necrosis factor, cytokines, protein metabolism, skeletal muscle

Introduction

The development of cachexia is associated with cancer and several other chronic and inflammatory diseases [1, 2]. In response to neoplastic and infectious stimuli, a variety of cells, including stimulated macrophages and lymphocytes, secrete cytokines which can alter the host’s metabolism. It has been suggested that two of these cytokines, interleukin-1 (IL-1) and tumour necrosis factor (TNF-α) mediate the metabolic changes associated with the cachectic process [3]. Weight loss, enhanced urinary nitrogen excretion, acute-phase protein synthesis and muscle wasting are among the most important changes associated with this pathological state. While the effects of TNF-α on lipid metabolism have been well documented, several studies have failed to
consistently reveal any changes in protein metabolism in skeletal muscle tissue incubated in vitro with recombinant TNF-α [4]. On the other hand, studies involving administration of TNF-α in vivo have shown an increase in nitrogen efflux from skeletal muscle in non-weight losing humans with disseminated cancer [5, 6], loss of body protein in growing rats [7] and a reduced rate of protein synthesis in rat muscle [8]. Flores et al. [9] infused 14C-leucine to rats and calculated protein breakdown in muscle from the relation of the tissue to circulating radioactive amino acid. In their study infusion of recombinant TNF-α significantly enhanced protein degradation, this effect being synergistically augmented by recombinant IL-1 treatment. Conversely, the administration of an acute TNF-α dose to rats did not induce an enhanced proteolysis or PGE₂ production in soleus or EDL muscles subsequently incubated [10]. Moldawer et al. [11] administered recombinant IL-1 to mice and observed, after incubating their EDL muscles, that there was an increase in PGE₂ production although the rates of both protein synthesis and degradation were unaffected, thus concluding that the rise in PGE₂ was not associated with a rise in protein turnover. Very recently, Goodman [12], measuring both tyrosine and 3-methylhistidine release by incubated EDL muscles from recombinant TNF-treated rats, concluded that TNF-α does indeed activate skeletal muscle protein degradation in the rat. The reasons for these conflicting results are not clear. It is for this reason that the aim of the present investigation was to study the effects of acute TNF-α treatment on rat skeletal muscle protein metabolism. Bearing this in mind, the effects of an acute TNF-α treatment on the rates of protein synthesis and degradation together with the capacity for amino acid uptake by the A system, have been investigated.

Experimental

Animals

Female Wistar rats from our own colony were used. Their body mass (60–70 g) was chosen so that their muscles could be incubated whole, without being under anoxia. Soleus and extensor digitorum longus (EDL), were chosen because they were representative of red and white muscles, respectively. The animals were housed in collective polypropylene cages (5 animals per cage) maintained at 22–23°C with a 12 hr-light/12 hr-dark cycle. They were fed Purina Laboratory chow ad libitum.

Biochemicals

All enzymes and coenzymes were either obtained from Boehringer Mannheim S.A. (Barcelona, Spain) or from Sigma Chemical, St. Louis, U.S.A. Recombinant-derived TNF-α was generously given by BASF/Knoll A.G., Ludwigshafen, Germany.

Cytokine administration

The animals were injected with 6 nmol of recombinant-derived TNF-α in 0.5 ml of Krebs-Henseleit saline intravenously through the dorsal tail vein under light diethyl ether anaesthesia; control animals received 0.5 ml of vehicle. All injections were administered between 09:00 and 10:00 h on the day of the experiment, with the animals being used 1 h later; food was available during this period.

Muscle preparations and incubations

The dissection and isolation of the extensor digitorum longus (EDL) and soleus were carried out under pentobarbital anaesthesia as previously described [13]. Their weights were 25.6 ± 1.9 and 28.5 ± 2.2 mg respectively. The isolated muscles were fixed to a stainless-steel clip in order to maintain the muscle under slight tension (making it comparable to resting length) during the incubation. Such muscles are able to maintain normal ATP and phosphocreatine concentrations during a 3 hr incubation period. The muscles were incubated in a shaking-thermostatised water bath at 37°C for 3 hr in 3 ml of Krebs-Henseleit physiological saline pH 7.4, containing 5 mM glucose, bovine serum albumin (2 mg/ml) and 20 mM HEPES. After the addition of the muscles to the vials, these were stoppered and the incubation started at a shaking rate of 70 cycles/min. Vials were gassed with O₂/CO₂ (19 : 1) during the whole incubation period. The incubation medium was kept for no longer than 90 min and was renewed thereafter with fresh medium with the same composition as described above but with insulin (200 nM) where stated.