BRIEF REPORT / RAPPORT BREF

Citrulline malate limits increase in muscle fatigue induced by bacterial endotoxins

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Abstract: Citrulline malate is known to improve performance in weakened muscles.The present experiment was designed to test the hypothesis that citrulline malate can limit the effect of endotoxins on muscle fatigability. Endotoxemia was induced in rats by injection of lipopolysaccharides from *Klebsiella pneumoniae*. Resistance to fatigue was quantified by measuring tension production during repetitive electrical stimulation of the isolated epitrochlearis muscle. Oral treatment by citrulline malate was found to increase resistance to fatigue in infected rats, whereas twitch tension was not modified. This demonstrates the efficacy of citrulline malate for limiting an increase in muscle fatigue elicited with bacterial endotoxins.

Key words: citrulline malate, muscle fatigue, endotoxins, infection.

Résumé : Le malate de citrulline est connu pour améliorer la performance d'un muscle affaibli. La présente expérimentation a été réalisée afin de vérifier l'hypothèse d'une limitation de l'effet des endotoxines sur la fatigue musculaire par le malate de citrulline. L'endotoxémie a été induite par injection de lipopolysaccharides provenant de *Klebsiella pneumoniae*. La résistance à la fatigue a été quantifiée en mesurant la production de force au cours d'une stimulation électrique répétitive du muscle épitrochlearis isolé. Un accroissement de la résistance à la fatigue a été observé chez les rats infectés et traités par le malate de citrulline alors que l'amplitude de la secousse musculaire n'était pas modifiée. Ces résultats démontrent l'efficacité du malate de citrulline pour limiter l'accroissement de la fatigue musculaire induit par des endotoxines bactériennes.

Mots clés : malate de citrulline, fatigue musculaire, endotoxines, infection.

Introduction

Previous studies in humans and rats have indicated that citrulline malate (CM) may be used for improving performance in weakened muscles. The beneficial effect of CM was first described in terms of more rapid recovery of physical activity in postinfectious human diseases (Creff 1982). More recently, CM was found to improve muscular performance in treadmill running tests of rats treated with endotoxins (Verleye et al. 1994, 1995). Despite several pharmacological approaches, the mechanism by which CM restores muscle alterations is far from clear (Callis et al. 1991). However, the interactions between production of NO from L-arginine by macrophages in response to endotoxins and the coproduct citrulline could explain the effects of exogenous CM. The aim of the present study was to demonstrate that CM can improve performance in a weakened skeletal muscle by modifying its fatigue characteristics. For this purpose, a technique of repetitive stimulation of an isolated muscle of the rat was utilized. As in a previous experiment (Goubel et al. 1995), injection of endotoxins as lipopolysaccharides (LPS) was performed in order to mimic infection, leading to an increase in muscle fatigue. Thus, an attempt was made to prove that oral administration of CM limits the increase in muscle fatigue induced by LPS.

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Methods

Animals and muscle preparation

Male Wistar rats initially weighing about 300 g were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care with approval by the Ethics Committee of the Université de technologie de Compiègne. As in a previous experiment (Goubel et al. 1995) the muscle of interest was the epitrochlearis muscle. This forelimb muscle is very thin and flat (length 15 mm; width 4 mm; thickness 0.2 mm).Of the fibres, 70–80% are type IIb, 10% are type I, and 10–20% are type IIa (Wallberg-Henriksson 1987). Such a preparation is currently used as an in vitro model for metabolic studies since it allows adequate diffusion of substrates to the entire muscle. After anesthesia of the animal (intraperitoneal injection of pentobarbital sodium, 5 mg/100 g body mass), the muscle was dissected and immediately placed in a chamber for mechanical analysis. The chamber was perfused with a buffered physiological salt solution (composition in mM: NaCl, 118; $NaHCO_3$, 28; $CaCl_2$, 2.5; $MgSO_4$, 3.1; KCl, 3.5; KH_2PO_4, 1.2; glucose, 11.1). The physiological solution (pH 7.3) was maintained at 25°C and continuously gassed with 95% O_2 and 5% CO_2.

Fatigue test

The muscle was mounted horizontally, with its distal part fixed to a force transducer. Its proximal extremity was connected to an electromagnetic ergometer as previously described (Lensel-Corbeil and Goubel 1989). Stimulation was delivered to two parallel silver electrodes located on each side of the muscle and connected as alternate anode and cathode. The muscle was adjusted to a reference length (L_o) defined as the length at which maximum isometric twitch tension was elicited. Three to five twitches were recorded, and maximal twitch tension (P_t) and associated contraction time (CT) were measured. Then, a fatigue test was performed using a procedure similar to that first described by Burke et al. (1973). For this purpose, 330-ms trains of 40 Hz were delivered once per second for 2 min. Resistance to fatigue was quantified by measuring tension production (P_{40}) every 5 s, using appropriate software. Relative P_{40} (percentage of maximum tension during the fatigue test) was then calculated and used as an index of fatigability (FI) of the muscle.

Experimental protocols

Experiment 1 was designed to evaluate the effect of CM on normal muscle. For this purpose 12 rats were assigned randomly to two groups. The first group ($n = 6$) received, by gavage, doses of CM at 1 g/kg three times a day during 48 h. The second group ($n = 6$) was given an equivalent volume of vehicle (flavoured purified water, pH 3.3) and served as control. For each group 12 muscles were analysed.

Experiment 2 was designed to evaluate the effect of CM on weakened muscle. For this purpose 24 rats were assigned randomly to three groups. The first group (LPS: $n = 8$) was treated with LPS. For this purpose, LPS from *Klebsiella pneumoniae* (Sigma Chemical, St. Louis, Mo.) was dissolved in physiological saline and injected intraperitoneally at 3 mg/kg body weight at times 0 and 24 h. Muscle analyses were performed 48 h after the first injection of LPS. The second group (LPS + CM: $n = 8$) was also treated with LPS but received CM as in experiment 1. The third group (CON: $n = 8$) received physiological saline and served as control. For each group of rats 16 muscles were analysed.

Statistical analysis

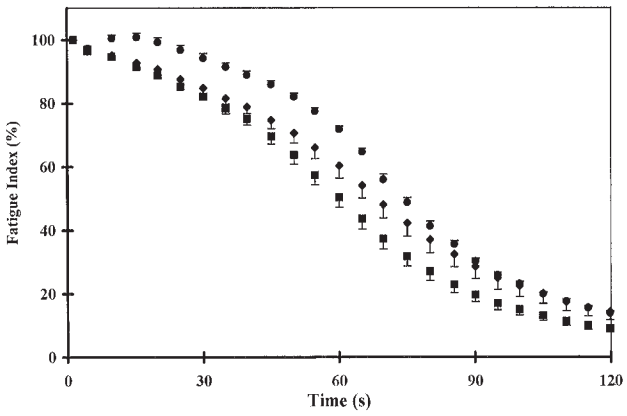
Differences in FI between groups were analysed by ANOVA (analysis of variance) at five selected times, mainly during the second part of the fatigue test. Pairwise comparisons were made post hoc using a Student–Newman–Keuls test. Significance was assumed for $p < 0.05$. Differences between mechanical characteristics were tested using Cochran's test. For each group of rats, each parameter was expressed as mean ± SEM.

Results

Results of experiment 1 indicated an absence of effect of CM on normal muscle. As a matter of fact both groups of muscles exhibited similar values for mechanical characteristics (maximum P_{40}: 0.522 ± 0.050 vs. 0.428 ± 0.035 N, ns) as well as for FI kinetics. FI at 2 min in the presence of CM was 22.7 ± 3.1%, not significantly different from values found for controls (19.7 ± 2.6%). Furthermore, for both groups, twitches did not differ in terms of P_t and CT.

In experiment 2, the peripheral injection of LPS produced lethargy, hypomotility, and behavioral depression in rats. However, in the absence of fatigue, the three groups of muscles had equivalent mechanical characteristics. Maximal isometric twitches had an amplitude of 0.192 ± 0.016 N for the CON group. Similar values were obtained for the LPS group (0.222 ± 0.015 N) and for the LPS + CM group (0.204 ± 0.017 N). An absence of difference between groups was also found for CT (35.1 ± 0.9 ms in CON, 36.2 ± 0.8 ms in LPS, 38.1 ± 0.9 ms in LPS + CM).The same holds for maximum P_{40} (0.561 ± 0.025 N in CON, 0.592 ± 0.023 N in LPS, 0.522 ± 0.036 N in LPS + CM). On the other hand, with respect to FI kinetics (Fig. 1), ANOVA revealed a significant effect of the treatment and time factor and their interaction. The pairwise multiple comparison procedure showed significant differences in FI values between LPS and CON, and LPS and LPS + CM. More precisely, FI was significantly lower for LPS compared with CON whatever the time. In the same way, CM was found to modify FI kinetics, since FI was significantly higher for LPS + CM compared with LPS at 60, 80, 90, and 100 s. FI at 2 min was 14.1 ± 0.8% for CON; it decreased to 8.6 ± 1.1% for LPS, whereas it reached 13.4 ± 2.6% for LPS + CM.

Fig. 1. Changes in relative tension during the fatigue test of experiment 2. Results for the CON (●) group, the LPS (■) group, and the LPS + CM (◆) group. For each group, $n = 16$. Data points are means with SEM.



Discussion

The present results are in close agreement with those of a previous study (Goubel et al. 1995) which demonstrated a significant sensitivity to fatigue of the epitrochlearis muscle. This is consistent with the fact that the rat epitrochlearis muscle consists predominantly of type II fibres (Nesher et al. 1980; Allaf et al. 1994). FI at 2 min below 40% are usually reported for fast muscles of the rat (Winiarski et al. 1987). The increase in muscle fatigability as a result of LPS injection is also confirmed. Moreover LPS treatment did not significantly modify the mechanism of force generation itself since twitches and initial P_{40} were similar in all groups of rats. Thus present results confirm that the in vitro preparation of the epitrochlearis muscle of the rat is a suitable model for studying muscle fatigue induced by bacterial endotoxins. Furthermore, it is demonstrated that CM does not modify mechanical characteristics and fatigability of normal muscle. It cannot be argued that problems with regard to the diffusion of substrates and oxygen give an artifactual character to the observed fall in tension during fatigue tests: the epitrochlearis muscle is sufficiently thin to avoid this kind of problem.

The most striking result of the current study is however that, in terms of FI kinetics, the LPS + CM group was different from the LPS group, indicating a relative increase in resistance to fatigue as a result of CM supplementation. This demonstrates

that CM treatment is able to limit the negative effect of endotoxemia on muscle fatigability tested by repetitive stimulation. A discussion about the mechanism by which CM limits muscle fatigue is beyond the scope of this paper. However, it should be recalled that the development of fatigue in an isolated muscle is multifactorial, including metabolic events and changes in excitation–contraction coupling. Moreover, the production of citrulline in the pathway of nitric oxide synthesis associated with the presence of malate from the Krebs cycle suggests that both compounds might be involved in the elimination of by-products of muscle metabolism, contributing to the improvement of muscle fatigue (Vanuxem et al. 1990). It is known that LPS has various biological activities including macrophage activation with release of NO and cytokines. Such an excess is probably implicated in the expression of the weakness behavior (Bluthé et al. 1992) The production of NO is arginine dependent, and one of the main renal synthetic pathways of arginine involves citrulline (Dhanakoti et al. 1990). The renal cycle activity, altered by LPS, could be improved with exogenous citrulline. Finally, the ability of citrulline to feed back negatively on the intracellular production of NO and (or) arginine can be hypothesized.

In conclusion, the efficacy of CM for limiting an increase in muscle fatigue induced by bacterial endotoxins is proved in terms of changes in muscle mechanical response. Additional experiments including biochemical studies are needed to elucidate the genuine mechanism of action of CM.

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