In Vivo Rates of Skeletal Muscle Protein Synthesis in Rats Are Decreased by Acute Ethanol Treatment but Are Not Ameliorated by Supplemental α-Tocopherol1

Matthew E. Reilly,*† Vinood B. Patel,† Timothy J. Peters† and Victor R. Preedy*†2

Departments of *Nutrition and Dietetics and †Clinical Biochemistry, King’s College, London, UK

ABSTRACT Some studies have shown that reductions in tissue protein synthesis, under a variety of cytotoxic conditions, are ameliorated by α-tocopherol (ATC) supplementation. We have also shown evidence of increased oxidative stress and reduced protein synthesis rates in alcohol-exposed muscle. Serum levels of ATC fall and rates of muscle protein synthesis are reduced in patients with alcoholic myopathy. We therefore tested the hypothesis that treatment with ATC could ameliorate the ethanol-induced changes in muscle protein synthesis, a contributory event in the pathogenesis of alcoholic muscle disease. Studies were carried out on gastrocnemius (Type II fiber-predominant and usually considered representative of the musculature as a whole), soleus (Type I fiber-predominant) and plantaris (Type II fiber-predominant) muscles. For comparative purposes, we also investigated the liver. Young male Wistar rats (90 g body weight) were injected intraperitoneally (i.p.) daily with ATC (30 mg/kg body weight) in Intralipid fat emulsion (0.1 mL/100 g body, i.p.) for 5 d. Controls were similarly injected with the Intralipid vehicle alone. After ATC supplementation, rats were given ethanol (75 mmol/kg body weight, i.p., 2.5 h) or saline (0.15 mol/L NaCl, i.p.). Fractional rates of tissue protein synthesis (i.e., the percentage of the tissue protein pool renewed each day, kR, %/d) and RNA activities (i.e., the amount of protein synthesis each day per unit RNA, kRNA, mg protein/d/mg RNA) were then measured. Supplementation increased ATC concentrations in plasma, gastrocnemius and liver. There was no effect of ATC supplementation alone on kR in any of the tissues. ATC supplementation in the absence of alcohol increased kRNA in the plantaris muscle. In nonsupplemented groups, acute ethanol treatment reduced skeletal muscle (soleus, plantaris and gastrocnemius) kR. Hepatic kR was not altered by ethanol, although ATC concentrations in this tissue increased due to ethanol. However, none of the changes in muscle kR or kRNA due to ethanol were significantly affected by ATC supplementation. In conclusion, ATC supplementation does not appear beneficial in ameliorating acute alcohol toxicity in skeletal muscle as defined by reductions in protein synthesis. J. Nutr. 130: 3045–3049, 2000.

KEY WORDS: • α-tocopherol • muscle • liver • protein synthesis • rats • alcohol

Alcohol toxicity induces a variety of pathogenic reactions and affects a diverse range of tissues such as the salivary glands (Smith et al. 1996), gastrointestinal tract (Seitz and Poschl 1997), skeletal muscle (Xu et al. 1996), heart (Fernandez Sola et al. 1997) and brain (Zimakin and Deitrich 1997). A reduction in the rate of protein synthesis is a central feature of acute ethanol toxicity affecting all of the aforementioned tissues [see, for example Bonner et al. (2000), Marway et al. (1997), Preedy and Peters (1988), Proctor et al. (1993) and Siddiq et al. (1997)]. These reductions are reported to be related to changes in the activation of translation initiation factors involved in the binding of met-tRNA to the 40S ribosomal subunit, i.e., eukaryotic initiation factor 2B (eIF2B)3 and the initiation factors that are involved in the binding of mRNA to the 43S preinitiation complex eIF4E (Lang et al. 1999). Muscle and liver respond differently with respect to these subcellular changes (Lang et al. 1999). In skeletal muscle, reductions in protein synthesis may be an initiating factor in the pathogenesis of the disease entity alcoholic myopathy (Preedy et al. 1990, Reilly et al. 1995). However, cellular factors for initiating the reductions in tissue protein synthesis in alcohol toxicity are unknown, although consideration must be given to the excessive generation of reactive oxygen species (ROS), leading to enhanced lipid peroxidation and/or membrane damage (Adachi et al. 2000b; Preedy et al. 1998a and 1998b). We found that two cholesterol-derived hydroperoxides, 7a-hydroperoxysterol-5-en-3β-ol (7a-OOH) and 7β-hydroperoxycholesterol-5-en-3β-ol (7β-OOH), were significantly elevated in both soleus and plantaris muscle of rats 24 h after acute ethanol treatment (75 mmol/kg body as used in this study). This reflects greater oxidative stress in the pathology of muscle of rats treated acutely with ethanol and, together with the observations of perturbations in membrane lipids in response to ethanol, has important implications for the pathogenesis of

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2 To whom correspondence should be addressed.
3 Abbreviations used: 7a-OOH, 7α-hydroperoxycholesterol-5-en-3β-ol; ATC, α-tocopherol; eIF-2B, eukaryotic initiation factor 2B; kR, fractional rate of protein synthesis; kRNA, RNA activity; ROS, reactive oxidative species; S, specific radioactivity of protein-bound amino acid; S, specific radioactivity of free amino acid in intracellular pools; S, specific radioactivity of free plasma amino acid.

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alcohol-induced muscle disorders. Although increased hydroperoxides were shown to be elevated at 24 h (Adachi et al. 2000b), we have also shown significant increases in plantaris 2.5 h after ethanol treatment (Adachi et al. 2000a). A number of studies have implicated oxidant and antioxidant factors in the pathogenesis of other alcohol-related disorders such as alcoholic pancreatic (Van Gossum et al. 1996) and liver diseases (Chen et al. 1995).

Numerous studies have also implicated the generation of ROS and/or enhanced lipid peroxidation in the pathogenesis of reduced tissue protein synthesis. The shelf life toxin okadacid acid reduces protein synthesis in cultures of vero cells, by a mechanism involving increased lipid peroxidation (Matias et al. 1999). Similarly, an increase in intestinal lipid peroxidation in Salmonella typhimurium infection perturbs amino acid transport, thereby leading to a reduction in enterocyte protein synthesis (Mehta et al. 1998). In metabolically degenerated neuronal tissue in vitro and liver tissue slices, there are also decreases in protein synthesis in response to enhanced lipid peroxidation (Fraga et al. 1989, Uto et al. 1995). These reductions in protein synthesis can be prevented by α-tocopherol (ATC) (Fraga et al. 1989, Matias et al. 1999, Uto et al. 1995).

From the above, we suggest that ATC supplementation may also have an ameliorative effect on ethanol-induced reductions in skeletal muscle protein synthesis. We tested this hypothesis by investigating changes in tissue ATC concentration and indices of protein metabolism in response to acute ethanol treatment in vivo, with or without ATC supplementation. Comparative reference was also made to the response of the liver because of its central role in both ethanol and ATC metabolism.

MATERIALS AND METHODS

Animals and reagents. Male Wistar rats were obtained from Charles River (Margate, Kent, UK) and consumed ad libitum a commercial pelleted diet containing crude protein, 17.9%; crude fiber 3.6%; carbohydrate, 57%; 13.3 MJ/kg (CRM diet, Special Diets Services, Essex, UK). They were housed in cages in an air-conditioned (20–25°C), humidified (40–60%) animal house with a 12-h light-dark cycle starting at 0800 h. Sterile α- (−) -tocopherol (950 µg/L) for injections was obtained from Sigma Chemical (Leuwes, Sussex, UK) and Intralipid intravenous fat emulsion from KabiPharma (Ting, Buckinghamshire, UK). Pyrogallol, HPLC-grade ammonium acetate and HPLC-grade methanol were from Merck/BDH (Poole, Dorset, UK). All other materials and chemicals were from either Sigma Chemical or Merck/BDH.

ATC supplementation and ethanol dosage. Four groups of rats (60–70 g body weight) were weight-ranked into groups of equivalent mean body weights and allocated for treatment with either vehicle (Intralipid) or ATC (supplemented) and saline or ethanol as listed below. They were then fed and housed as described above for an 25-d period in the proportion of 0.1 mL/100 g body, after which time they weighed ~150 g. This regimen ensured a daily dose of 30 mg ATC/kg body weight, in addition to the small amount of mixed tocopherols present in the Intralipid vehicle (mass/L Intralipid: 0.024 g RRR-α-tocopherol, 0.184 g RRR-γ-tocopherol and 0.088 g RRR-δ-tocopherol) which totaled 0.296 g. Although control (i.e., vehicle-injected) rats also received the small amount of mixed tocopherols in the Intralipid vehicle, the dose of ATC received represented ~1% of that administered to the ATC-supplemented rats. In addition, the biological activities of γ-tocopherol and δ-tocopherol in protection against ROS are far less important than that of ATC (Chow 1985). After supplementation, rats were allowed a 1-d clearance period before i.p. ethanol or saline treatment. This was to ensure that uptake of i.p. injected saline or ethanol was not influenced by previous intraperitoneal ATC administration.

Ethanol-dosed rats were injected with a single bolus of ethanol [75 mmol/kg body weight as 1.0 mL/kg body, i.p. (Peevy et al. 1996)]. Saline-injected controls were similarly injected with isovolumetric 0.15 mol/L NaCl (sterile). Treatment of saline-injected rats was identical to that of ethanol-injected rats.

At 140 min after ethanol or saline injection, rats were injected intravenously with a "flooding dose" of [4-3H]-phenylalanine and killed by decapitation at 2.5 h after injection of isotope. Rats were then allowed to clear for 1-d. At 24 h (Adachi et al. 2000a). A number of studies have implicated oxidant and antioxidant factors in the pathogenesis of studies have implicated oxidant and antioxidant factors in the pathogenesis of liver diseases (Chen et al. 1995). From the above, we suggest that ATC supplementation may also have an ameliorative effect on ethanol-induced reductions in skeletal muscle protein synthesis. We tested this hypothesis by investigating changes in tissue ATC concentration and indices of protein metabolism in response to acute ethanol treatment in vivo, with or without ATC supplementation. Comparative reference was also made to the response of the liver because of its central role in both ethanol and ATC metabolism.

RESULTS

Effects of α-ATC supplementation. ATC-supplementation significantly increased plasma concentrations of ATC (P < 0.001; Table 1). There were also large increases in ATC concentrations in gastrocnemius muscle (P < 0.025) and liver (P < 0.001). There were no significant differences in protein or RNA contents in any of the tissues due to ATC treatment.
(data not presented). ATC supplementation per se did not affect fractional rates of protein synthesis ($k_s$) in any of the tissues examined (Table 2). The RNA activity ($k_{RNA}$) in the plantaris but not in other tissues increased significantly ($P < 0.02$; Table 3) in response to ATC supplementation. The meaning of the increase in plantaris $k_{RNA}$ is unclear because $k_s$ values (Table 2) and protein and RNA contents (not shown) were not significantly raised, but this may be due to Type II fiber-specific response because this effect was not observed in the soleus, a Type I fiber–predominant muscle.

**Effects of acute alcohol toxicity.** The i.p. ethanol treatment produced high plasma ethanol concentrations (for example, $275 \pm 12$ mg/100 mL; $60$ mmol/L), similar to clinically determined values arising during alcoholic binge drinking in humans, i.e., $61$ mmol/L (Donovan et al. 1999).

ATC concentration was not significantly affected in either plasma or muscle due to ethanol treatment (Table 1). However, acute ethanol treatment significantly increased liver ATC concentrations ($P < 0.05$; Table 1). Ethanol treatment reduced $k_s$ in all skeletal muscles studied ($P < 0.001$), but the fractional synthesis rates in the liver were unaffected (Table 2). Ethanol decreased the $k_{RNA}$ in all muscles studied ($P < 0.001$; Table 3).

**Effects of ATC-supplementation on ethanol-induced differences in protein synthesis.** No significant interactions were observed between ATC and ethanol on RNA and protein contents (data not shown), ATC concentrations (Table 1), $k_s$ (Table 2) or $k_{RNA}$ (Table 3) in any of the tissues studied.

**DISCUSSION**

The excessive generation of ROS leading to enhanced lipid peroxidation and/or membrane damage is an important feature of alcohol toxicity (Preedy et al. 1998a and 1998b). There are four areas of evidence to support the hypothesis that ATC-α-tocopherol supplementation may be beneficial in ameliorating alcohol-induced biochemical lesions in muscle. The first pertains to studies (with the same treatment protocol as that used in this study, i.e., $75$ mmol/kg body) that show increased oxidative stress as evidenced by elevations in skeletal muscle cholesterol-derived hydroperoxides, $7\alpha$-OOH and $7\beta$-OOH (Adachi et al. 2000a and 2000b). This is suggestive of pertur...
A number of processes have been proposed as causative mechanisms in alcohol-induced muscle damage, including excessive generation of ROS or impaired antioxidant systems (Reilly et al. 1995). Increased generation of ROS has been reported after acute ethanol administration [see, for example, Preedy et al. (1999) and Reinke et al. (1997)]. Increased skeletal muscle production of ROS has been suggested to arise from intramuscular xanthine oxidase activities causing raised lipid peroxidation of fatty acid moieties within the cellular and subcellular membranes (Reinke and McCay, 1996). The involvement of ROS in the pathogenesis of alcohol-related disorders has previously been assessed by attempts to reverse or ameliorate pathologic changes using antioxidant therapies. Beneficial effects of ATC supplementation on the extent of chronic ethanol-induced hepatic injury have been observed (Nanji et al. 1996). However, there are comparatively few studies on the protective effects of antioxidants on skeletal muscle. In skeletal muscle, the efficacy of ATC supplementation in ameliorating ROS-mediated protein oxidation has been shown after exercise (Meydani et al. 1993), glucocorticoid-induced myopathy (Ohtsuka et al. 1998) and immobilization atrophy (Appell et al. 1997). In ischemia or ischemia-reperfusion injury, α-tocopherol can ameliorate the reductions in muscle ATP, neutrophil infiltration and increased lipid peroxidation; some of the beneficial effects of α-tocopherol may be related to its ability to impede the expression of adhesion molecules such as ICAM-1 (Punz et al. 1998). However, in our studies, ATC supplementation did not significantly attenuate the ethanol-induced reductions in protein synthesis. The reason for this relative lack of effect is unknown but may pertain to the complex etiology of protein synthetic perturbations.

We used a 5-d protocol of ATC supplementation, which was sufficient to raise circulating, liver and muscle levels of ATC. Such acute regimens have proved efficacious in past studies on ROS-associated tissue or metabolic damage in animals (Bauersachs et al. 1993). For example, a single dose given before the onset of ischemia can be effective in ameliorating reductions in muscle ATP (Punz et al. 1998) or reduce malondialdehyde after hypoxia (Varskeviciene et al. 1984).

It is possible that the failure to observe a protective effect of ATC was due to a protocol that allowed 1-d clearance before ethanol administration. However, we do not think this is likely because muscle, liver and plasma ATC concentrations were still raised 1 d after the last dose.

The presence of raised oxidative challenge (i.e., increased ROS) after ethanol administration is now well documented, as
is the role of ATC as an important antioxidant for membrane lipid moieties (Reinke and McCay 1996). It is possible that the oxidative stress induced by ethanol may have been insufficient to cause changes in membrane oxidation or that this membrane has a higher antioxidant capacity. This can be refuted because ethanol induces oxidative damage to muscle membrane lipids, an effect that is not seen in liver (Adachi et al. 2000a). However, it is important to realize that not all muscle pathologies or indices of muscle damage can be ameliorated by ATC supplementation. For example, raising muscle ATC concentrations by dietary supplementation produced observable improvements in muscle antioxidant capacity without improvement in indices of cellular injury induced by eccentric exercise (Siciliano et al. 1997) or myotonic dystrophy (Omdahl et al. 1994). It seems then that alcohol-induced muscle damage, as reflected by changes in tissue protein synthesis, may fall into this "nonresponsive" category. The inability of skeletal muscle to respond to ATC may reflect the fact that other processes may be involved in ethanol-induced reductions in protein synthesis. Candidate mechanisms include the formation of acetaldehyde-protein adducts, which will render inoperative cytoskeletal or other proteins involved in translation, by a process involving covalent binding (Niemela 1999).

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LITERATURE CITED

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