Responses of tissue protein synthesis to nutrient intake in rats exposed to interleukin-1β or turpentine

Peter E. BALLMER, Margaret A. McNURLAN, Ian GRANT and Peter J. GARLICK
Rowett Research Institute, Aberdeen, U.K.

(Received 18 November 1992/8 April 1993; accepted 11 May 1993)

INTRODUCTION

Catabolic losses from various body compartments, and in particular losses of lean body mass with substantial wasting of skeletal muscle, are important characteristics of the acute-phase reaction (APR). In addition, the APR is characterized by rapid onset of fever and an increase in the synthesis of the acute-phase proteins (APP), e.g. fibrinogen and α1-acid glycoprotein. Several hormones, e.g. glucocorticoids and insulin [1, 2], are involved in the regulation of the changes in the APR. However, the cytokines, in particular interleukin-1 [3], interleukin-6 [4] and tumour necrosis factor-α [5], emerge as important regulators of metabolism in the APR. Thus, interleukin-1 induces fever via hypothalamic production of prostaglandin E2 [6] and increases hepatic protein synthesis rates [7], whereas interleukin-6 has been shown to regulate APP synthesis [4, 8]. Recently, we were able to demonstrate that subcutaneous injection of interleukin-1β (IL-1β) into rats induced a marked decrease in muscle protein synthesis [7]. By 9 h after injection of IL-1β, the fractional synthesis rate (FSR) fell significantly in gastrocnemius and soleus muscle. Comparison of the changes in skeletal muscles after IL-1β injection with a classical APR induced by turpentine showed a similar and in part synchronous depression in muscle protein synthesis, suggesting that IL-1β may be an important mediator of the decrease in muscle protein synthesis in the APR. As an expression of the production of APP, total liver protein synthesis increased significantly in both the IL-1β- and the turpentine-injected animals [7].

The question of whether during an APR the normal responses to nutrition might remain intact and thus might mitigate nitrogen losses is important. Provision of nutrients in the APR might increase total liver protein synthesis including the APP and moderate the APR-induced decrease in muscle protein synthesis. However, amino acids might alternatively be used as fuel and diverted to gluconeogenesis. Consequently, we have investigated whether protein synthesis rates in various tissues in rats exposed to IL-1β or turpentine were able to respond to the provision of nutrients [9].

MATERIALS AND METHODS

Human recombinant IL-1β (molecular mass 17 kDa) was a generous gift of Ciba-Geigy, Basle, Switzerland, and was stored at −70°C in 100 mmol/l Tris buffer. It was prepared for injection in sterile pyrogen-free 0.9% NaCl with 5 mg of BSA (Sigma, Poole, Dorset, U.K.)/ml as a carrier protein for injection.

Key words: acute-phase reaction, amino acids, fever, glucose, inflammation, interleukin-1β, liver, muscle, nutrition, protein synthesis, turpentine.

Abbreviations: APP, acute-phase proteins; APR, acute-phase reaction; FSR, fractional synthesis rate; IL-1β, interleukin-1β; PN, parenteral nutrition; RL, Ringer's lactate.

Correspondence: Dr Peter E. Ballmer, Department of Internal Medicine, University of Berne, Inselspital, CH-3010 Berne, Switzerland.
Experimental design

Young male rats (Rowett Hooded Lister strain, n=6 per group) were maintained on a 12 h dark/12 h light cycle at room temperature and were fed a standard chow (Biosure; S.D.S., Manea, Cambridgeshire, U.K.) After an overnight fast, the rats (average body weight 100 g) were subcutaneously injected with (a) 6.25 μg of IL-1β in 0.2 ml of 0.9% NaCl [7], or (b) 0.5 ml of turpentine (British Pharmacopoeia grade, Thornton and Ross, Huddersfield, U.K.) and 0.2 ml of 0.9% NaCl containing 5 mg/ml BSA, or (c) 0.2 ml of pyrogen-free NaCl with 5 mg/ml BSA. The rats had also no access to food thereafter.

Two hours before measurement of protein synthesis rates the animals were infused with Ringer's lactate (R.L.; Hartmann's solution, Baxter Health Care Limited, Egham, Surrey, U.K.) or a solution of nutrients consisting of 3.3% glucose (Glucosteril 10%; Fresenius, Stans, Switzerland) and 8.3% amino acid mixture (Proteinsteril 10%; 100 g of amino acids and 16 g of nitrogen/l; Fresenius). Proteinsteril 10% contains all major amino acids in a balanced composition for total parenteral nutrition in humans. It contains the following amino acids: leucine (7.1 g/l), isoleucine (4.7 g/l), valine (5.9 g/l), lysine (6.0 g/l), methionine (4.1 g/l), phenylalanine (4.8 g/l), threonine (4.2 g/l), tryptophan (1.8 g/l), arginine (10.6 g/l), histidine (2.9 g/l), alanine (15.0 g/l) and proline (15.0 g/l). The parenteral nutrition (PN) regimen was an attempt to imitate a single meal, in order to study the short-term response of protein synthesis to the provision of nutrients [10-12].

Infusion of RL or PN. One millilitre of a 150 mmol/l amino acid mixture (Proteinsteril 10%; 100 g of amino acids and 16 g of nitrogen/l; Fresenius) was mixed in a sterile bottle, and the solution was checked for bacterial contamination (aerobic and anaerobic) at the end of the experiment. The infusion rate was 1.5 ml/h for 2 h containing 5.6 kJ plus 249 mg of amino acids/ml, and was given by a lateral tail vein as described previously [10]. The 5.6 kJ infused per 2 h would amount to roughly 70 kJ/day, which is close to the resting energy expenditure of a 100 g rat per day. The chosen regimen was an attempt to imitate a single meal, in order to study the short-term response of protein synthesis to the provision of nutrients [10-12].

Body temperature was measured with a rectal probe at time-points 0, 1, 3, 5, 7, 9, 13 and 24 h after injection. The probe was inserted 3.5 cm into the rectum and the temperature was recorded with an electronic device.

Measurement of protein synthesis

Protein synthesis was measured as the FSR, which is the percentage of the protein pool synthesized per day (in % /day). FSR was measured in vivo by a flooding dose of phenylalanine [13] at time-points 9 and 24 h after injection of IL-1β or turpentine corresponding to 2 h after the beginning of the infusion of RL or PN. One millilitre of a 150 mmol/l phenylalanine solution (Sigma) containing 60 μCi of L-[2,6-3H]phenylalanine (Amersham, Little Chalfont, Bucks, U.K.) was injected into the tail vein cannula and the infusion was continued. After an incorporation time of 10 min the rats were killed by decapitation. The liver, heart, gastrocnemius muscle and soleus muscle were removed, immediately frozen in liquid nitrogen and stored at −20°C. Subsequently, frozen tissue samples were pulverized between CO2-cooled aluminium blocks. The specific radioactivities of the free and the protein-bound phenylalanine were determined as described previously [7, 13, 14]. In brief, after precipitation with 2 mol/l HClO4 the protein was washed several times and hydrolysed for 24 h in 6 mol/l HCl at 110°C. Phenylalanine was converted to β-phenethylamine with tyrosine decarboxylase (Sigma). β-Phenethylamine was isolated by sequential extraction into n-heptane (BDH, Poole, Dorset, U.K.) and 0.01 mol/l H2SO4, and the concentration was measured fluorimetrically (model 8-9; Locarte, London, U.K.) with ninhydrin (Sigma). The radioactivity was counted on a scintillation counter (Packard 1900; Packard Instruments, Downersgrove, IL, U.S.A.). The concentrations of the tissue protein were measured by the method of Lowry et al. [15].

FSR was calculated as described previously [7] by the formula:  
FSR = \left( \frac{S_p \times 100}{S_i \times t} \right)

where Sp is the specific radioactivity in the protein pool at time t in days, and Si is the specific radioactivity of the tissue free phenylalanine pool.

Other analytical procedures

Plasma insulin concentration was measured by radioimmunoassay with a pig anti-insulin antibody (Miles Scientific, Slough, Berks., U.K.) and 125I-insulin as the tracer.

Statistics

The time-courses of temperature and protein synthesis rates were analysed by analysis of variance using the program Genstat 5 [16]. All values from a single tissue were examined together, first for differences between treatments (i.e. saline, IL-1β or turpentine injection) and then for interactions between time and treatment. The responses to nutrition were then examined in relation to treatment and finally for interactions between time and nutrition.

RESULTS

Rectal temperature

Fig. 1 summarizes the changes in body temperature over the whole time-course. IL-1β injection and turpentine injection produced significant changes in
Protein synthesis during inflammation

Fig. 1. Time course of body temperature in control (a), IL-1β-injected (b) and turpentine-injected (c) animals. Values are means ±SEM. ○, RL; ●, PN.

body temperature when compared with control animals (P < 0.001, split-plot type of analysis of variance) [16]. As described previously [7], the control animals (NaCl-injected) showed a circadian rhythm of rectal temperature with the highest values during the night, the active part of the day for the rat. No differences occurred between the RL- and the PN-infused animals except at 24 h, when the temperatures in the RL group fell, but those in the PN group were maintained. The IL-1β-injected rats showed a sharp rise in rectal temperature by 3 h, with a short plateau up to 5 h, which was followed by a continuous fall up to 24 h. By then both the RL group and the PN group showed a hypothermic response compared with control animals, as observed previously [7]. As in control animals, PN was effective in maintaining body temperature at a higher level at 24 h. The turpentine-injected animals showed a delayed response in rectal temperature, with a gradual increase up to 13 h and only a slight decrease by 24 h. There were no differences between the RL- and the PN-infused animals with turpentine.

<table>
<thead>
<tr>
<th>Time after injection…</th>
<th>FSR (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 h</td>
</tr>
<tr>
<td></td>
<td>RL</td>
</tr>
<tr>
<td>Control</td>
<td>77.8±3.9</td>
</tr>
<tr>
<td>IL-1β</td>
<td>91.1±3.0</td>
</tr>
<tr>
<td>Turpentine</td>
<td>94.9±4.9</td>
</tr>
</tbody>
</table>

Liver protein synthesis

FSRs of liver protein were measured at time-points 9 and 24 h after injection of saline, IL-1β or turpentine (Table 1). Analysis of variance showed that the responses to IL-1β and turpentine were statistically significant (P < 0.001), but that their time-courses were different (P < 0.001). Thus, IL-1β-injected animals showed a stimulation of protein synthesis at 9 h and 24 h, whereas in the turpentine-injected animals the stimulation was greater at 24 h. PN infusion resulted in higher rates of protein synthesis in all cases, but whereas these effects were similar at the two time points in control animals, they appeared to be more pronounced at 9 h in IL-1β-injected animals and at 24 h in turpentine-injected animals. However, the analysis of variance showed that although the effect of PN was highly significant overall (P < 0.001), these responses did not differ significantly with treatment (i.e. saline, IL-1β or turpentine injection) or with the time after injection.

Skeletal muscle protein synthesis

Protein synthesis rates in gastrocnemius and soleus muscles are shown in Tables 2 and 3. In the gastrocnemius muscle the continued period of fasting between 9 and 24 h caused a significant fall in FSR, irrespective of intravenous feeding. IL-1 injection caused a significant reduction in protein synthesis in the gastrocnemius muscle at 9 h, but this effect was reversed at 24 h, as reported previously [7]. The difference in response at the two times was significant (P < 0.001). By contrast, turpentine injec-
tion had no apparent effect on protein synthesis at either time point. Analysis of variance showed that overall the effect of PN was highly significant ($P < 0.001$) but, despite the apparently larger responses in the IL-1 and turpentine groups, differences between responses to PN with different treatments and different times were not significant. Hence, both IL-1/β- and turpentine-injected animals were able to respond normally to the provision of PN.

As the response to turpentine was smaller than shown previously, an additional experiment comparing FSR in the gastrocnemius muscle with and without RL infusion was performed. Non-infused rats showed a decrease in FSR from $9.4 \pm 0.3\%/day$ ($n = 8$) to $8.4 \pm 0.4\%/day$ ($n = 7$) ($P < 0.005$) $9\ h$ after turpentine injection. Animals infused with RL solution had lower rates of synthesis but responded similarly to turpentine ($8.4 \pm 0.2\%/day$, $n = 7$), falling to $7.0 \pm 0.3\%/day$ ($n = 7$, $P < 0.005$) after turpentine. The equivalent values for liver were $82.7 \pm 3.0\%/day$ ($n = 8$) versus $110.1 \pm 5.9\%/day$ ($n = 8$) for non-infused animals and $88.2 \pm 3.8\%/day$ ($n = 8$) versus $109.4 \pm 4.3\%/day$ ($n = 7$) for infused rats. The stimulation by turpentine was significant ($P < 0.005$) and was not affected by infusion.

The soleus muscle appeared to respond similarly to the gastrocnemius muscle (Table 3). However, the values for FSR were much higher and the responses to fasting and PN were smaller. IL-1/β caused a fall in FSR in the RL-infused animals at $9\ h$, but this effect was not apparent at $24\ h$ or in fed animals. Also, turpentine had no significant effect. There were significant increases due to PN ($P < 0.001$), which appeared to be smaller in the control rats than in IL-1/β- or turpentine-injected rats at $9\ h$. However, analysis of variance showed that these differences in response were not significant, suggesting that responses at $9\ h$ to PN were normal during an inflammatory reaction.

### Heart muscle protein synthesis

Heart muscle responded differently to the injection of IL-1/β than the skeletal muscles (Table 4). Analysis of variance showed a significant increase in FSR of heart overall ($P < 0.001$). Although the response to feeding was more pronounced in the IL-1 group than the controls at $9\ h$, analysis of variance showed that the responses to PN in the IL-1 group did not significantly differ from those of controls at either time-point. As with the other muscles, turpentine injection did not influence FSR. It was also noted that the variation within the groups given turpentine was much smaller than in the other groups, so a separate analysis of variance $9\ h$ after turpentine showed that the responses to PN in the IL-1 group were significant ($P < 0.005$), which was similar to that of controls at both time points.

### Plasma insulin concentration

PN induced an overall significant, but small, increase in plasma insulin concentration ($P < 0.001$ by analysis of variance) (Table 5). IL-1/β produced a significant decrease in plasma insulin concentration ($P < 0.001$ by analysis of variance), but there was little change with turpentine.

### DISCUSSION

We have previously shown that both IL-1/β and turpentine produce an APR in the fasted rat, characterized by alterations in protein synthesis rates in various tissues [7, 17]. The present study was designed to see whether the animals' ability to respond to the provision of nutrients was altered by these treatments. Nutrients were given intravenously, to avoid possible differences resulting from altered gut absorption, and for a short period (2 h),
as it has previously been shown that a short period of infusion of a mixture of glucose plus amino acids into healthy postabsorptive rats is sufficient to restore protein synthesis in skeletal muscle to a rate close to its fed value [10]. This feeding procedure was not an attempt to imitate total PN, which would require much longer infusion periods and a more nutritionally complete mixture (the nitrogen intake was adequate, but energy given as glucose was too low). This regimen was chosen to investigate short-term regulatory effects of feeding on protein synthesis rates, and to clarify whether the APR modifies the response to an amount of nutrients which is known to stimulate protein synthesis in healthy animals [11, 12]. Two different time periods after injection of the IL-1β or turpentine were investigated, 9 and 24 h, as these were found in our previous study to show the most pronounced responses to IL-1β and turpentine, respectively. All animals, however, were fasted from 23.00 hours the previous evening (i.e. about 10 h before the time of injection) and throughout the whole experiment, so that differences in feeding behaviour or gut absorption would not confound the results. Hence, the decline in the rate of muscle protein synthesis in the control animals reflects the progression of starvation. In the liver there was no decline in the rate of protein synthesis between 9 and 24 h (i.e. 19–24 h after food withdrawal) in control animals. However, our previous work has shown that there is a decline in protein synthesis due to fasting before 9 h [7]. This is consistent with the stimulation produced by PN in controls (Table 1). As observed previously, both IL-1β and turpentine stimulated synthesis at two time periods studied [7]. Although analysis of variance showed that the stimulation by PN in the IL-1β- and turpentine-treated animals was not significantly different from that in controls, there was a suggestion that the feeding effect was enhanced at the peak of the APR (9 h for IL-1β and 24 h for turpentine).

The gastrocnemius muscle of growing rats has previously been shown to be responsive to fasting and refeeding [10, 18]. This is manifest in the present work by the continued fall in protein synthesis between 9 and 24 h in the controls. Analysis of variance showed that rates were significantly higher in the intravenously fed control animals (P < 0.02) as shown previously [10, 12]. IL-1β inhibited protein synthesis in this muscle at 9 h, but at 24 h there was a slight increase, as shown previously [7]. However, the stimulation due to feeding was larger than that in controls at both time points. Although this difference in response between IL-1β-injected and control groups was not significant, it does suggest that the response to feeding might be enhanced, rather than diminished, by the APR. By contrast, Ash and Griffin [19] showed no response of protein synthesis in the extensor digitorum longus muscle (similar to the gastrocnemius muscle [18]) to intravenous feeding in endotoxemic rats, even though the response they observed in control rats was greater than that in the present study and the effect they observed in liver was similar to that reported here. The reason for the difference in muscle is not clear, but the mechanisms involved might be different. Ash and Griffin [19] infused both endotoxin and nutrients for 18 h. Moreover, their animals showed no febrile reaction. However, in the present study, although hypocaloric, the short-term feeding regimen was effective in stimulating muscle protein synthesis under inflammatory conditions.

Although the effect of IL-1β on the gastrocnemius muscle was similar to that reported previously [7], the effect of turpentine was different, in that no decrease in protein synthesis occurred at either time point in the RL-infused animals. Also there was little effect in soleus muscle and the response to feeding was the same as in control animals. Responses to turpentine in the other tissues were also less pronounced than those in the previous study, although the changes in body temperature were exactly the same as those seen before. By contrast to the previous study, when the animals were fasted, all animals were infused with either RL or PN, which might have modulated the response to turpentine injection. In accord with our observation, Wusteman and Elia [20] showed an inhibition of muscle protein synthesis in turpentine-injected animals, which was absent when the animals were infused with saline and anaesthetics (fentanyl and fluanisone) at a rate of 1 ml h⁻¹ 100 g⁻¹ body weight, which was similar to our infusion rate. However, a separate experiment (see the Results section) showed an inhibition by turpentine which was the same whether or not RL was infused, although rates of muscle protein synthesis were somewhat lower in the infused animals. This is consistent with the generally lower rates of muscle synthesis observed in the present, compared with the previous, study [7]. The magnitude of the response to turpentine was intermediate between that in the present (Table 2) and past [7] studies. However, this does not explain the low sensitivity to turpentine shown in Tables 1–4, but suggests that there might be variations in the potency of different batches of turpentine.

The soleus muscle has been shown before to be rather insensitive to short periods of starvation [18]. In the present study the decline with starvation was less pronounced than in the gastrocnemius muscle, as was the response to PN. The fall in protein synthesis with IL-1β injection was also smaller in the soleus muscle than in the gastrocnemius muscle, but the response to feeding was similar. The heart, like the soleus muscle, was relatively insensitive to starvation, but showed a contrasting effect of IL-1β, a substantial stimulation being apparent, particularly at 9 h. We suggested previously that this elevation might have resulted from increased work as a consequence of hyperthermia and hyper-
metabolism [7]. At this time, subsequent decline to a submaximal rate by 24 h was also stimulated by nutrition. As with the soleus muscle, there was little effect of turpentine in the heart and the response to feeding was similar to that of controls.

The conclusion from these experiments is that the provision of nutrients effectively influences muscle and liver protein synthesis in rats suffering from an inflammatory insult. The responses to feeding were not diminished in IL-1β- or turpentine-treated animals in comparison with healthy control animals, and might even have been enhanced, showing that the inflammatory response does not counteract stimulatory effects of nutrition.

ACKNOWLEDGMENTS

We are grateful for financial support from the Swiss National Science Foundation, Nestec Limited, Lausanne, Switzerland. The Scottish Office Agriculture and Fisheries Department and Grampian Research into Intestinal Disorders, Scotland, U.K. We thank Dr M. Franklin of the Scottish Agricultural Statistics Service for performing the statistical analysis.

REFERENCES