Effects of human recombinant interleukin-1 β on protein synthesis in rat tissues compared with a classical acute-phase reaction induced by turpentine

Rapid response of muscle to interleukin-1 β

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The early time course (1, 3, 9, 24 h) of changes in rates of protein synthesis (k_s) in liver and three different muscles (gastrocnemius, soleus and heart) was investigated after injection of saline, interleukin-1 β (IL-1) or turpentine in rats. IL-1 injection induced a consistent increase in body temperature of about 3 °C between 3 and 5 h, but thereafter a hypothermic response occurred. With turpentine, a delayed fever response with a peak value by 9 h was observed. Both IL-1 and turpentine had no effect on protein synthesis in the small intestine, but produced a significant increase in k_s in the liver at 9 h. By 24 h in IL-1-treated animals, liver k_s had returned back to control values, whereas the turpentine-treated group showed a progressive rise in k_s . Gastrocnemius and soleus muscles exhibited a significant fall in k_s at 9 h after IL-1 and turpentine injection compared with the control. In contrast, the k_s of heart muscle increased at 3–9 h after IL-1 injection, but there was no effect of turpentine. Thus for the first time a marked decrease of protein synthesis in skeletal muscle in response to IL-1 could be demonstrated.

INTRODUCTION

An acute-phase reaction such as infection or injury is characterized by a typical response of the immune system, fever and miscellaneous metabolic changes. Whereas various hormones, e.g. glucocorticoids and insulin, direct some of the metabolic changes (Wilmore, 1976; Frayn, 1986), an independent system of hormone-like substances, the cytokines, which direct these alterations from the onset of inflammatory disease, have now been identified (Dinarello, 1984). Of these, interleukin-1 (IL-1) induces fever, increases the hepatic synthesis of acute-phase proteins and enhances protein breakdown in skeletal muscle (Baracos *et al.*, 1983; Flores *et al.*, 1989). However, whereas the response of protein metabolism to chronic administration of IL-1 over the first few days had been well studied (Moldawer *et al.*, 1988), there is little information about the early time course after a single injection of IL-1.

Because of substantial wasting of body protein and muscle mass in experimental (Fong *et al.*, 1989) and clinical acute-phase reactions, the pattern of protein metabolism is a major concern in acute and chronic disease. The changes in protein synthesis in tissues such as liver have been reported, but the situation in skeletal muscle is less clear. Negative nitrogen balance and wasting of skeletal muscle could be caused by an increased breakdown of skeletal muscle and/or a lowered protein synthesis. However, initial evidence for an increase in protein degradation in isolated rat muscles incubated with partially purified human IL-1 *in vitro* (Baracos *et al.*, 1983) could not be confirmed in later studies with human recombinant IL-1 (Goldberg *et al.*, 1988). Despite this, other investigators have recently demonstrated alterations in muscle protein breakdown after administration of IL-1 to the intact animal (Flores *et al.*, 1989). In most of these studies muscle protein synthesis was also investigated, but in no case could any change be detected. This contrasts with the effects

of tumour necrosis factor- α (TNF- α), which has many properties in common with IL-1 (Dinarello, 1987; Argiles *et al.*, 1989), and was shown to inhibit protein synthesis in the skeletal muscle of rats (Charters & Grimble, 1989). After a single dose of TNF- α , protein synthesis fell by 20 % in 8 h and had returned to normal by 24 h, suggesting that effects of IL-1 on protein synthesis might also occur within this time frame.

We have therefore investigated the early time course of protein synthesis in liver, small intestine, two different skeletal muscles (gastrocnemius and soleus) and heart muscle after subcutaneous injection of human recombinant IL-1 β . By comparing the response to IL-1 β with that in a classical acute-phase reaction, induced by subcutaneous turpentine injection, a more complete pattern of the IL-1-mediated effects can be gained (Ballmer *et al.*, 1990a,b).

Part of this study was presented in abstract form at the FASEB meeting, Washington, 1990, and the Nutrition Society, Edinburgh, 1989.

MATERIALS AND METHODS

Human recombinant IL-1 β of molecular mass 17 kDa was generously provided by Ciba-Geigy, Basel, Switzerland, and was stored at –70 °C in 100 mM-Tris buffer. For injection, IL-1 was freshly prepared in sterile pyrogen-free 0.9 % NaCl with 5 mg of BSA/ml as a carrier protein (Sigma, Poole, Dorset, U.K.).

Experimental design

Young male rats (Rowett Lister Hooded strain) were individually caged for 1 week before the experiment, fed on a standard chow (CRM diet; Biosure, S.D.S. Mill, Manea, Cambs., U.K.) and maintained on a 12 h-dark/12 h-light cycle in a temperature-controlled room (21 °C). Their average body weight was 100 g on the day of the experiment. After an overnight fast

Abbreviations used: IL-1, interleukin-1; TNF, tumour necrosis factor; k_s , fractional protein synthesis rate; k_{RNA} , protein synthesis rate related to RNA.

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(free access to water), the rats were subcutaneously injected between 08:00 and 09:00 h with (a) 6.25 μ g (Dubuis *et al.*, 1989) of IL-1 β in 0.2 ml of 0.9% NaCl, (b) 0.5 ml of turpentine (British Pharmacopoeia grade; Thornton and Ross, Huddersfield, U.K.) plus 0.2 ml of 0.9% NaCl containing 5 mg of BSA/ml, or (c) 0.2 ml of pyrogen-free NaCl containing BSA. Rectal temperature was measured at intervals up to 24 h in all rats with a plastic-coated electronic probe inserted 3.5 cm into the rectum.

Measurement of protein synthesis rates

The protein synthesis rate (k_s) was measured *in vivo* by injection of a flooding dose of phenylalanine at 1 h, 3 h, 9 h and 24 h after IL-1 injection (Garlick *et al.*, 1980). A 1 ml portion of a 150 mM-phenylalanine solution (Sigma) containing 30 μ Ci of L-[2,6- 3 H]phenylalanine (Amersham) was injected via a lateral tail vein and the animals were killed after an incorporation time of exactly 10 min. The liver, gastrocnemius, soleus and heart muscle were dissected on ice, weighed and immediately frozen in liquid nitrogen and stored at -20°C . A 30 cm segment of the upper intestine was rinsed thoroughly and frozen as a whole. Samples of tissue were pulverized between aluminium blocks cooled by solid CO_2 and the specific radioactivity of the free and protein-bound phenylalanine was determined as described previously (Garlick *et al.*, 1980; McNurlan *et al.*, 1982). In brief, protein was precipitated with 2 M- HClO_4 , repeatedly washed and then hydrolysed for 24 h in 6 M-HCl at 110°C . Phenylalanine was enzymically converted with tyrosine decarboxylase (Sigma) into β -phenethylamine, which was separated by sequential extraction into n-heptane (BDH Ltd., Poole, Dorset, U.K.) and then 0.01 M- H_2SO_4 . Radioactivity was counted using a scintillation counter (Minaxi Tri-Carb 4000 Series; Packard Instrument Co., Downersgrove, IL, U.S.A.) with Opti-Fluor scintillation fluid (Packard). The β -phenethylamine concentration was measured with ninhydrin (Sigma) on a fluorimeter (Model 8-9; Locarte, London, U.K.). The fractional synthesis rates, k_s , expressed as %/day, were calculated according to the equation:

$$k_s = (S_p \times 100) / (S_f \times t)$$

where S_p is the specific radioactivity in the protein pool at time t (days) and S_f is the specific radioactivity of free phenylalanine in the tissue.

Tissue protein concentrations were determined by the method of Lowry *et al.* (1951), and RNA concentrations were measured spectrometrically at 232 and 260 nm as described previously (McNurlan *et al.*, 1982; Ashford & Pain, 1986).

Other analytical procedures

Insulin concentration was measured with a radioimmunoassay using a pig anti-insulin antibody (Miles Scientific, Slough, Berks., U.K.), ^{125}I -insulin (Amersham) as a tracer and rat insulin standards (Sigma).

Statistical analysis

Statistical analysis was performed by two-way analysis of variance (ANOVA), using the program Genstat 5 (Genstat, 1987). Differences between individual groups at a specific time point were calculated as the least significant difference (Parker, 1979).

RESULTS

Body temperature

Fig. 1 shows the time course of changes in body temperature in the saline-, IL-1- and turpentine-treated animals from 1 h to 24 h after injection. The saline-injected control animals showed an increase in body temperature during the hours of darkness,

their most active time, and this elevation persisted until 24 h. To see whether the elevation resulted from continued fasting or whether it was part of a circadian rhythm, temperature was measured from time point -1 h until 25 h in a separate experiment using six fasted control rats (Fig. 2). There was a sharp drop in temperature between -1 h and 1 h and between 24 h and 25 h, thus confirming the circadian rhythm (Fig. 2).

The IL-1-injected animals exhibited a very consistent increase in rectal temperature of about 3°C by 3 h ($P < 0.001$ versus control). The temperature maintained a short plateau up to 5 h, and decreased rapidly thereafter. At the end of the observation period (24 h), a hypothermic reaction of IL-1 was observed ($P < 0.005$) compared with control animals. In contrast, the body temperature of turpentine-treated rats did not peak until 9 h after injection, when a difference of about 1.5°C (versus control) was observed. However, the temperatures returned to values similar to those in controls by 24 h.

Liver protein synthesis

Synthesis rates of total liver protein were determined at 1, 3, 9 and 24 h after IL-1 injection (Table 1). Fractional synthesis rates

Fig. 1. Time course of changes in rectal temperature in control animals (a) and after IL-1 (b) or turpentine (c) injection

Fig. 2. Time course of circadian changes in rectal temperature

The experiment was carried out using six control rats.

Table 1. Effects of IL-1 and turpentine on the k_s and k_{RNA} of liver

Changes in liver protein synthesis, expressed as k_s (%/day) and k_{RNA} (mg of protein/day per mg of RNA), are given after subcutaneous injection of saline, IL-1 or turpentine (means $\pm$ S.E.M.). Significance of differences: * $P < 0.05$, ** $P < 0.01$ versus control; † $P < 0.05$; †† $P < 0.01$ versus IL-1-treated.

Treatment		Time after injection ...	1 h	3 h	9 h	24 h
Control	k_s		125.6 $\pm$ 4.6	131.0 $\pm$ 4.5	118.9 $\pm$ 3.2	117.5 $\pm$ 3.5
	k_{RNA}		22.7 $\pm$ 3.9	21.0 $\pm$ 0.6	16.2 $\pm$ 1.0	17.2 $\pm$ 1.4
IL-1	k_s		127.3 $\pm$ 2.9	124.9 $\pm$ 4.3	147.4 $\pm$ 3.3**	131.4 $\pm$ 3.8*
	k_{RNA}		19.7 $\pm$ 0.8	19.2 $\pm$ 0.8	21.5 $\pm$ 1.1*	17.4 $\pm$ 1.1
Turpentine	k_s		126.5 $\pm$ 5.2	138.8 $\pm$ 4.8†	144.6 $\pm$ 9.3**	183.2 $\pm$ 6.2**††
	k_{RNA}		19.1 $\pm$ 1.0	22.0 $\pm$ 1.0	20.1 $\pm$ 2.6	24.4 $\pm$ 1.6*†

Fig. 3. k_s of total liver protein

(a) Control, (b) IL-1 injection, (c) turpentine injection.

Table 2. Effects of IL-1 and turpentine on the k_s and k_{RNA} of gastrocnemius muscle

Changes in gastrocnemius muscle protein synthesis, expressed as k_s (%/day) and k_{RNA} (mg of protein/day per mg of RNA), are given after subcutaneous injection of saline, IL-1 or turpentine (means $\pm$ S.E.M.). Significance of differences: * $P < 0.05$, ** $P < 0.01$ versus control; † $P < 0.05$; †† $P < 0.01$ versus IL-1-treated.

Treatment		Time after injection ...	1 h	3 h	9 h	24 h
Control	k_s		11.3 $\pm$ 0.5	10.1 $\pm$ 0.7	8.8 $\pm$ 0.5	6.6 $\pm$ 0.3
	k_{RNA}		8.7 $\pm$ 0.4	8.3 $\pm$ 0.6	7.5 $\pm$ 0.3	6.6 $\pm$ 0.3
IL-1	k_s		11.1 $\pm$ 0.6	8.7 $\pm$ 0.3	6.3 $\pm$ 0.5**	7.5 $\pm$ 0.4
	k_{RNA}		8.4 $\pm$ 0.5	6.9 $\pm$ 0.2*	5.4 $\pm$ 0.4**	7.3 $\pm$ 0.5
Turpentine	k_s		9.7 $\pm$ 1.0*	9.1 $\pm$ 0.6	6.4 $\pm$ 0.8**	5.2 $\pm$ 0.3**††
	k_{RNA}		7.3 $\pm$ 0.5*	7.7 $\pm$ 0.4	5.3 $\pm$ 0.8**	5.1 $\pm$ 0.4*††

indicated significant variations with time after injection and between the three treatment groups (ANOVA, $P < 0.001$). Hence specific differences between groups were assessed by least significant differences.

The k_s in control animals decreased over the 24 h period due to continued fasting. IL-1 injection resulted in a significantly higher k_s by 9 h compared with controls ($P < 0.001$). By 24 h the IL-1 group showed a decline in k_s , but the values were still higher than in the control group ($P < 0.05$).

In contrast to IL-1 treatment, turpentine injection resulted in a trend towards an earlier increase in k_s , as shown by the significantly higher value than in the IL-1 group at 3 h. However, the k_s was not significantly increased compared with the control ($0.05 < P < 0.1$). After 3 h, a progressive increase in k_s continued throughout the 24 h period (Fig. 3).

Protein synthesis rates were also related to the tissue RNA content and are expressed as k_{RNA} (mg of protein/day per mg of RNA). This has been referred to as the efficiency of protein synthesis and is a measure of the rate of protein synthesis per ribosome (Millward *et al.*, 1973). Fasting alone (control group) resulted in a drop of k_{RNA} between 3 and 9 h ($P < 0.05$). The changes in k_{RNA} were in general smaller than the changes in k_s , and were only significant for the 9 h IL-1 versus control and the 24 h turpentine versus control groups (Table 1).

Muscle protein synthesis

The data for the three muscles, gastrocnemius, soleus and heart, are summarized in Tables 2–4. Continued fasting during the 24 h study period resulted in a progressive decline in k_s in all muscles. Changes in k_s due to both treatment ($P < 0.001$) and

Table 3. Effects of IL-1 and turpentine on the k_s and k_{RNA} of soleus muscle

Changes in soleus muscle protein synthesis, expressed as k_s (%/day) and k_{RNA} (mg of protein/day per mg of RNA), are given after subcutaneous injection of saline, IL-1 or turpentine (means $\pm$ S.E.M.). Significance of differences: * $P < 0.05$, ** $P < 0.01$ versus control; † $P < 0.05$; †† $P < 0.01$ versus IL-1-treated.

Treatment		Time after injection ...	1 h	3 h	9 h	24 h
Control	k_s		16.0 $\pm$ 0.4	17.2 $\pm$ 1.0	14.3 $\pm$ 0.3	13.5 $\pm$ 0.3
	k_{RNA}		10.7 $\pm$ 0.2	11.6 $\pm$ 0.6	9.9 $\pm$ 0.3	10.9 $\pm$ 0.4
IL-1	k_s		17.5 $\pm$ 1.0	14.8 $\pm$ 0.6**	11.7 $\pm$ 0.7**	11.2 $\pm$ 0.5*
	k_{RNA}		11.6 $\pm$ 0.6	10.2 $\pm$ 0.4*	8.2 $\pm$ 0.5**	8.9 $\pm$ 0.4**
Turpentine	k_s		16.1 $\pm$ 0.4	15.3 $\pm$ 0.8*	13.9 $\pm$ 0.6†	10.0 $\pm$ 0.5**
	k_{RNA}		10.6 $\pm$ 0.1	10.1 $\pm$ 0.5*	9.9 $\pm$ 0.4††	7.9 $\pm$ 0.4**

Table 4. Effects of IL-1 and turpentine on the k_s and k_{RNA} of heart muscle

Changes in heart muscle protein synthesis, expressed as k_s (%/day) and k_{RNA} (mg of protein/day per mg of RNA), are given after subcutaneous injection of saline, IL-1 or turpentine (means $\pm$ S.E.M.). Significance of differences: * $P < 0.05$, ** $P < 0.01$ versus control; †† $P < 0.01$ versus IL-1-treated.

Treatment		Time after injection ...	1 h	3 h	9 h	24 h
Control	k_s		18.4 $\pm$ 0.7	17.1 $\pm$ 0.7	16.5 $\pm$ 0.8	14.8 $\pm$ 1.0
	k_{RNA}		10.9 $\pm$ 0.5	10.4 $\pm$ 0.4	10.1 $\pm$ 0.5	9.9 $\pm$ 0.6
IL-1	k_s		17.5 $\pm$ 0.7	20.4 $\pm$ 0.8**	19.7 $\pm$ 1.0**	15.1 $\pm$ 0.7
	k_{RNA}		10.3 $\pm$ 0.6	12.4 $\pm$ 0.6**	11.8 $\pm$ 0.5*	9.3 $\pm$ 0.4
Turpentine	k_s		16.9 $\pm$ 0.9	17.0 $\pm$ 0.4††	14.6 $\pm$ 0.3††	14.0 $\pm$ 0.7
	k_{RNA}		9.7 $\pm$ 0.4	10.0 $\pm$ 0.3††	8.9 $\pm$ 0.3††	9.4 $\pm$ 0.5

Fig. 4. k_s of gastrocnemius muscle

(a) Control, (b) IL-1 injection, (c) turpentine injection.

Fig. 5. k_s of soleus muscle

(a) Control, (b) IL-1 injection, (c) turpentine injection.

time were significant for all three muscles ($P < 0.001$ by ANOVA). In addition, the changes in k_{RNA} due to treatment and time were highly significant for all muscles ($P < 0.001$).

Gastrocnemius muscle showed an early decrease in k_s which was significant by 9 h after injection of IL-1, but there was a recovery to control values by 24 h (Fig. 4). The turpentine-treated group exhibited an ongoing fall in k_s which was significant at 9 h and 24 h compared with the control. The trend in values for k_{RNA} was slightly different from that for k_s in turpentine-treated animals, indicating a rapid drop in k_{RNA} at 3 h and a continued depression of k_{RNA} until the end of the observation period (24 h).

The changes in protein synthesis in soleus muscle were broadly similar to those in the gastrocnemius muscle, although the absolute values for k_s were higher (Fig. 5). Also, changes in k_s occurred more rapidly, becoming statistically significant by 3 h after injection of IL-1 or turpentine. With both treatments the k_s remained lower than control values until 24 h, there being no recovery similar to that in the gastrocnemius at 24 h after IL-1 injection. The early (3 h) and late (24 h) responses of k_{RNA} were similar for both the IL-1-treated and the turpentine-treated groups. At 9 h the turpentine group maintained a plateau, whereas IL-1-injected animals showed a drop in k_{RNA} .

The alterations in protein synthesis rates in the heart differed from those in the skeletal muscles (Table 4). Turpentine had no effect compared with control rats, but IL-1 produced a stimulation in k_s at 3 and 9 h, with a return to control values by 24 h. The pattern of changes in k_{RNA} was the same as for k_s .

Protein synthesis rates in the small intestine revealed no effects of either time ($P = 0.17$) or treatment ($P = 0.47$). However, smooth muscle and mucosa were not separately examined (results not shown).

Plasma insulin concentrations

Insulin concentrations did not show significant fluctuations, and were low at all time points in all groups. The peak plasma insulin concentration occurred in all three groups at 3 h, with a value of 0.188 ± 0.040 ng/ml for the control and IL-1-treated groups, and of 0.153 ± 0.034 ng/ml for the turpentine-injected animals.

DISCUSSION

In this study we have observed an early response of protein synthesis in rat liver and skeletal muscle to a single injection of human recombinant IL-1 β , which contrasts with some earlier findings, where a delayed hepatic response (Moldawer *et al.*, 1988), and no effect on muscle protein synthesis (Baracos *et al.*, 1983; Moldawer *et al.*, 1987) were found.

The most obvious difference when comparing our results with those of other workers is the different time schedule for measuring protein synthesis rates. Baracos *et al.* (1983) studied synthesis and degradation of muscle protein *in vitro* with up to 2 h of IL-1 exposure and was not able to demonstrate any effect. Moldawer *et al.* (1987) investigated protein synthesis both at 2 h after a single IL-1 injection and at 5 days after repeated daily IL-1 injections. According to the time sequence seen in our experiment, both groups would have missed the important time period between 3 and 24 h. Similarly, Flores *et al.* (1989) could not demonstrate changes in either muscle or liver protein synthesis after injection of IL-1 or TNF alone or in combination. They used a lower dose and a higher dose than ours (i.e. 2 μ g and 10 μ g per 100 g rat respectively) and measured protein synthesis during the following 6 h by constant infusion of [14 C]leucine. Even if

changes in synthesis had developed by 6 h, the measured rate would be an average over the entire 6 h infusion, including several hours when, according to our data, no change would have occurred.

A very rapid effect of cytokine administration was also observed by Charters & Grimble (1989), who reported changes in protein synthesis in both liver and muscle strikingly similar to those shown here with IL-1. They investigated the effect of TNF- α injection into rats and observed an increased k_s in the liver and a decreased k_s in skeletal muscle by 8 h after TNF injection. This similarity is not surprising, as IL-1 and TNF are known to share many properties (Dinarello, 1987).

The temporal inter-relationship between changes in protein synthesis and body temperature is important. IL-1 produced a consistent peak of rectal temperature by 3 h (Fig. 1). In contrast, protein synthesis in the liver had increased only after 9 h, suggesting that another mediator might contribute substantially to changes in liver protein synthesis. For example, IL-6 has been shown to be the main modulator of the production of acute-phase reactants (Ramadori *et al.*, 1988). Protein synthesis in muscle, in particular soleus muscle, declined earlier in the time course (as early as 3 h), thus indicating a synchrony with body temperature. A change in body temperature itself might influence protein synthesis rates. Thus Sayegh & Lajtha (1989) have shown that protein synthesis rates in lizards increased in both muscle and liver when the body temperature was raised. In fish there is also a rise in protein synthesis with increasing environmental temperature, with a Q_{10} measured both *in vivo* and *in vitro* between 2.5 and 3.0 (Haschemeyer, 1978). However, the delay in the increase in liver protein synthesis compared with the rise in body temperature, and the fall in muscle protein synthesis rates at the time of the highest fever, indicate that body temperature itself had no detectable regulatory action on protein synthesis in the present study. The hypothermic action of IL-1 from 9 h onwards (Fig. 1) has not been reported previously. Long *et al.* (1990) showed an enhanced endotoxin fever after administration of antiserum to TNF, thus suggesting an endogenous antipyretic effect of TNF. It is therefore possible that injection of IL-1 induced the release of TNF, which caused a delayed hypothermic action.

We have chosen the comparison between IL-1 and turpentine injection as a means of investigating whether IL-1 can mimic a real acute-phase reaction. Overall, the patterns of changes in protein synthesis were rather similar after the two treatments. However, the effects of turpentine persisted for at least 24 h, whereas those of IL-1 were not apparent after 9 h. This is not surprising, since IL-1 would be rapidly cleared from the body, whereas the abscess resulting from turpentine would produce a chronic inflammatory response. Paradoxically, there was also a trend towards a faster change in protein synthesis after turpentine injection (see liver and gastrocnemius muscle), which cannot be conclusively explained. It is possible that a direct toxic or neural effect of turpentine, e.g. via pain generation and the sympathetic nervous system, might have occurred. A similarly rapid effect of surgery on muscle protein synthesis has been demonstrated between 1.5 and 3 h after the first incision (Essen *et al.*, 1990).

Similar effects to those of turpentine have been observed when endotoxin was injected (Jepson *et al.*, 1986), but the onset of the reaction was delayed in comparison with our injection of turpentine or IL-1. After subcutaneous injection of endotoxin, liver protein synthesis increased and gastrocnemius muscle protein synthesis fell, but only at 24 h (Jepson *et al.*, 1986). The earlier responses seen with IL-1 and turpentine were not observed. This suggests that endotoxin injection does not act directly but via release of other secondary factors, e.g. cytokines or hormones such as corticosterone.

The changes in the rates of protein synthesis are the net result of both the action of IL-1 or turpentine and progressive starvation. Continued fasting caused a larger decline in hepatic k_{RNA} than in k_s , thus indicating a decline in the efficiency of protein synthesis. In contrast, k_{RNA} tended to be maintained during the inflammatory insult.

Gastrocnemius muscle, as a representative of most skeletal muscles with a mixed fibre type (Maltin *et al.*, 1989), exhibited a loss of efficiency in protein synthesis, reflected by a depression of k_{RNA} . By contrast, soleus muscle, composed of slow fibres, was resistant to fasting (Baillie & Garlick, 1991). k_{RNA} also fell in the acute-phase reaction.

Heart muscle displayed a rather different pattern of changes in protein synthesis when compared with skeletal muscle. The increase in k_s paralleled the rise in body temperature. It is possible that the high fever or the IL-1 itself was responsible for an increase in cardiac workload, resulting in the initiation of cardiac hypertrophy and an associated increase in protein synthesis. This is supported by earlier studies *in vivo* and *in vitro* showing changes in cardiac output and regional blood flow after injection of IL-1 or TNF (Flores *et al.*, 1989). It has also been shown that severely ill rats infected by *Plasmodium* still show an increase in protein synthesis in heart muscle when animals are near to death, presumably in response to the increased cardiac workload resulting from anaemia (Fern *et al.*, 1985). The inhibition of cardiac contractile responsiveness and the reaction to β -adrenergic stimulation is also inhibited by IL-1 and TNF- α (Gulick *et al.*, 1989), thus possibly resulting in hypertrophy of heart muscle in order to meet the demands of the organism. It is surprising, therefore, that the stimulation of cardiac protein synthesis does not occur in turpentine-treated animals. However, the fever response was not as pronounced as with IL-1, and therefore the demand for an increased cardiac output may have been more limited. Moreover, the effect of turpentine might be two-fold, inducing a decrease in protein synthesis, as in skeletal muscle, and at the same time increasing protein synthesis due to fever and a greater cardiac workload. In addition, turpentine might initiate some compensatory mechanisms, e.g. neural or humoral, counteracting the greater cardiac workload.

The dose of IL-1 injected in this study was relatively high in comparison with that used by some other workers. However, Flores *et al.* (1989) compared the effects of IL-1 injections of 2 and 10 μg (per 100 g rat) on rates of protein synthesis. They injected half of the dose as a single bolus intravenously, and the rest as a constant infusion over 6 h. Our single dose of 6.25 μg , given subcutaneously, is comparable with their latter bolus dose. Certainly this amount of IL-1 is not in the physiological dose range, but the rats tolerated the IL-1 well and had completely recovered before 24 h. Moreover, even with high systemic IL-1 doses, the local tissue concentration, which is relevant for the biological effects, might still be in the physiological range.

Finally, this study cannot differentiate between the direct and indirect effects of IL-1 on protein synthesis rates. The rapid onset of the changes and some synchrony with the development of fever might be an argument for an immediate effect of IL-1. However, the generation of fever, a very early sign after IL-1 injection, is known to be mediated indirectly by prostaglandins. The similarity of the actions of IL-1 and TNF is an argument for the activation by IL-1 of a cytokine cascade involving TNF, thereby effecting protein synthesis. Thus an acute-phase reaction may never be mediated by a sole reactant, but is the result of the combined action of cytokines, hormones and nervous stimuli.

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