Inhibition of liver RNA breakdown during acute inflammation in the rat

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Liver RNA- and protein-degradation rates were measured after the induction of acute inflammation in the rat. A preliminary study determined changes in hepatic RNA and protein content 12, 18 and 24 h after a turpentine oil injection. The RNA content in turpentine-treated rats compared with pair-fed animals increased significantly and sharply from 12 h (+11%) to 18 h (+32%) and slightly thereafter (+37% at 24 h). The liver protein content was significantly enhanced only at 24 h (+11%) in response to inflammation. RNA-degradation rates were determined in livers perfused cyclically *in situ* for 15 min by measuring the accumulation of radioactive cytidine in the medium 60 h after *in vivo* labelling of RNA by [5-³H]cytidine instead of [6-¹⁴C]orotic acid, the most commonly used radioactive marker. Several validation procedures showed that the method employed was a valid alternative to the use of radioactive orotic acid. RNA-degradation rates, which mainly reflect rRNA break-

down, were significantly lower in the turpentine-treated rats than in respective pair-fed animals at 18 and 24 h (57 and 45% decrease respectively). Proteolysis rates measured at 24 h together with RNA breakdown by valine accumulation in the perfusion medium were not modified after turpentine treatment. The main factors known to regulate RNA degradation (amino acids, insulin/glucagon ratio) were measured in the portal blood 24 h after induction of acute inflammation. Of the known regulatory amino acids, only glutamine and to a lesser extent methionine were increased in the turpentine-treated rats as compared with their pair-fed counterparts. The insulin/glucagon molar ratio was similar in both groups. In conclusion, the reduced breakdown of RNA, especially rRNA, is largely responsible for the accumulation of hepatic RNA during acute inflammation. This inhibition of RNA degradation could possibly be related to the increase in glutamine.

INTRODUCTION

Both inflammatory and infectious stimuli are associated with marked changes in RNA and protein metabolism especially in muscle and liver [1–6]. A loss of RNA and protein is observed in muscle whereas both macromolecules accumulate in liver.

Cellular RNA and protein accumulation may be the result of enhanced synthesis, reduced degradation or a combination of the two. Inflammation and sepsis induce an increase in the hepatic production of numerous plasma proteins called acute-phase reactant proteins as well as intracellular proteins [1,5,7]. These anabolic processes are usually preceded by increased synthesis of rRNA reflecting a greater protein-synthetic capacity [6]. Although numerous studies have focused on the synthesis of both macromolecules in inflammation and sepsis, data relevant to the degradation of rRNA are lacking and those dealing with proteolysis are scarce and conflicting. Rosenblatt et al. [8] reported reduced proteolysis in liver slices from septic patients compared with those from non-infected patients whereas Pederesen et al. [2] observed no alteration in protein degradation in rat liver slices 24 h after induction of peritonitis. Similarly, Vaubour-dolle et al. [9] observed no change in proteolysis in rat liver lobes 2 days after a burn injury.

The present study was designed to determine rates of hepatic RNA, specifically rRNA, degradation as well as proteolysis in rat livers perfused *in situ* after induction of acute inflammation. This experimental model allows accurate measurement of breakdown rates that occur *in vivo* at the beginning of the perfusion [10,11]. In experiments, liver RNA is usually labelled with radioactive orotic acid. In the present work, we tested the suitability of using [5-³H]cytidine, a more readily available compound, for the measurement of RNA breakdown. As several studies performed in the perfused liver and in isolated or cultured

hepatocytes have shown that liver RNA and protein degradation are mainly regulated by amino acids, glucagon and insulin [12–14], these factors were also investigated.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Charles River, Saint Aubin-Les-Elbeuf, France) weighing 180–200 g were used. They were maintained under controlled conditions of temperature (22±2 °C) and light (12 h on/off cycle) on a commercial diet (UAR, Villemoisson-sur-Orge, France) and were allowed water *ad libitum*. Three groups of rats were used in the different experiments: (1) control rats fed *ad libitum*; (2) rats in which acute inflammation was induced by a subcutaneous injection of sterile turpentine oil (0.5 ml/100 g body weight); their food intake was recorded for 24 h; (3) pair-fed rats matched to each turpentine-injected rat for initial body weight and food intake; these rats were injected with saline.

The protocols were approved by the French Agriculture Office in conformity with European legislation on laboratory animals.

Experimental design

The study was divided into two main parts: (1) validation of the use of prelabelling of RNA with [5-³H]cytidine to determine RNA breakdown; (2) measurement of rates of RNA and protein degradation during acute inflammation.

(1) The principle of measurement of RNA degradation in the perfused rat liver is based on the accumulation of radioactive

Abbreviations used: IL, interleukin; TNF-α, tumour necrosis factor α.

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cytidine in the perfusion medium as demonstrated using [6-¹⁴C]orotic acid [10,15]. Although the use of [5-³H]cytidine to label RNA should be consistent with this principle, it was necessary to check that radioactive cytidine accumulating in the perfusion medium originated from CMP residues in liver RNA and to re-evaluate factors used to calculate RNA-degradation rates. In addition, the validation of the technique included the comparison between RNA-degradation rates obtained using [6-¹⁴C]orotic acid and [5-³H]cytidine.

(2) The second part of the study was divided into three experiments: experiment 1, the time course (12, 18 and 24 h) of accumulation of liver RNA and protein was studied after the induction of acute inflammation in order to select the most appropriate time point(s) for the measurement of RNA and protein breakdown; experiment 2, fractional rates of liver RNA and protein degradation were determined 18 and 24 h after the induction of acute inflammation; experiment 3: factors known to regulate RNA and protein breakdown (portal amino acid concentrations and insulin/glucagon ratio) were assayed at 24 h.

RNA labelling

RNA was labelled *in vivo* 60 h before liver perfusions by two intraperitoneal injections of [5-³H]cytidine (100 μ Ci per rat) in 0.9 % NaCl. RNA labelling using [6-¹⁴C]orotic acid (20 μ Ci per rat) in 0.9 % NaCl was only carried out to compare rates of RNA degradation using the two tracers.

Liver perfusion

Perfusions were started at 11:00 h. The rats were anaesthetized with pentobarbital (100 μ l/100 g body weight). Livers were cyclically perfused *in situ* at a flow rate of 6–7 ml/min as described by Mortimore and Mondon [16]. The perfusion medium was composed of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 3 % (w/v) BSA (fraction V), 27 % (v/v) washed bovine erythrocytes, 0.5 mM cytidine and 18 μ M cycloheximide. After a wash-out period of 45–60 s, the perfusate was recycled and five 3 ml samples were collected between 5 and 15 min for the measurement of plasma [5-³H]cytidine or [¹⁴C]cytidine and valine accumulation. At the end of the perfusion, the livers were rapidly excised, weighed and frozen in liquid nitrogen.

Determination of fractional RNA-degradation rates

RNA breakdown

This was calculated from the accumulation of radioactive cytidine in the perfusate plasma plus liver extracellular water space between 5 and 15 min in the presence of unlabelled cytidine to prevent the reutilization of radioactive cytidine [10]. Rates of release (d.p.m./min) were computed by least-squares regression. Cytidine accumulation, expressed as μ mol/min per liver, was then computed by dividing the rate of label released by the specific radioactivity of CMP in liver RNA. When RNA was labelled with [6-¹⁴C]orotic acid, it was shown that the specific radioactivity of CMP could be calculated from the specific radioactivity of RNA measured in each liver sample divided by 1.737 [17]. This value represents the specific radioactivities, RNA/CMP for the livers of rats submitted to *in vivo* labelling procedures. Absolute rates of RNA degradation were obtained by dividing the rate of cytidine release by the proportion of CMP residues in RNA (0.87 μ mol/mg of RNA) [15]. Fractional rates of RNA degradation were obtained by dividing rates of RNA degradation by the hepatic RNA content at the end of the perfusion. When RNA was labelled with [5-³H]cytidine, the same principles were used for the calculation except that a new specific

radioactivity ratio RNA/CMP was determined and a correction factor was applied to the accumulation of radioactive cytidine. The details are given in the Results section.

Radioactive cytidine in the plasma perfusate was measured by Dowex 50 chromatography as described by Lardeux et al. [15].

Liver RNA content was determined as described by Lardeux et al. [15].

Specific radioactivity of cytidine

RNA was labelled *in vivo* using [5-³H]cytidine as previously described; livers were perfused *in situ* for 15 min with medium containing no radioactive cytidine. Medium samples were collected 5, 10 and 15 min after the beginning of the perfusion and centrifuged. At 15 min, the liver was removed, rapidly frozen in liquid nitrogen and kept at -20°C until measurement of RNA and CMP specific radioactivities. Plasma was deproteinized with trichloroacetic acid (10 % final concn.) and passed through Dowex 50 [15]. The separation of cytidine from Dowex 50 eluates was performed by reverse-phase chromatography in a Beckman HPLC system using C-18 Ultrasphere columns (4.6 mm $\times$ 250 mm; 5 μ m particles). Eluents were as follows: A, 10 mM ammonium phosphate (pH 5.3); B, 10 mM ammonium phosphate (pH 4.9) containing 35 % acetonitrile. After sample injection, the column was eluted at room temperature, at a flow rate of 1.4 ml/min, using the following gradient: 100 % A for 1 min, 40 % B between 1 and 15 min, 100 % B between 16 and 21 min. Eluent B was maintained at 100 % for 5 min, followed by a linear decrease to 0 % B (or 100 % A) in 3 min. The run was completed within 34 min. A_{254} was recorded. Fractions of one drop corresponding to the cytidine peak were collected and radioactivity was counted in a liquid-scintillation counter (LKB 1214). The concentration of cytidine was computed by linear regression from a standard curve consisting of pure cytidine.

Specific radioactivity of CMP residues in RNA

Nucleotide residues from RNA prelabelled with [5-³H]cytidine were obtained after 24 h of alkaline hydrolysis of liver homogenate samples in 0.3 M KOH at 37°C . The acid-soluble nucleotides were separated by precipitation with 1.2 M perchloric acid [15]. The supernatant was stored overnight at -20°C in order to precipitate substantial amounts of potassium perchlorate. The supernatant was then passed twice through small columns (5.5 mm $\times$ 25 mm) of Dowex AG 1 (X4; OH⁻ form; 100–200 mesh). The columns were washed with water and eluted with 5 ml of 0.1 M HCl. The eluates were air-dried, and the dried nucleotide extracts were dissolved in 20 mM KH₂PO₄, pH 3. The nucleotides were separated by reverse-phase ion-pairing chromatography using the system described above. Eluents were as follows: A, 20 mM KH₂PO₄, pH 3, containing 10^{-5} M tetrabutylammonium dihydrogen phosphate; B, 100 % methanol. After sample injection, the column was eluted at room temperature, at a flow rate of 1 ml/min with the following gradient: 100 % A at 0 min to 50 % B between 7 and 15 min; eluent B was maintained at 50 % for 2 min, followed by a linear decrease to 100 % A in 1 min. The run was completed within 33 min. A_{270} was recorded. Specific radioactivity of CMP residues was determined from the peak corresponding to 2'-CMP. Fractions of one drop corresponding to that peak were collected and radioactivity was counted by liquid scintillation. The concentration of 2'-CMP residues in the samples was computed by linear regression from 2'-CMP standards.

Determination of fractional protein-degradation rates

Proteolysis rates were determined together with RNA-breakdown rates from the accumulation of valine in the perfusate volume (plasma plus 60% of red-cell volume) and liver extracellular space in the presence of cycloheximide to prevent valine reutilization [18]. Proteolytic rates were computed by least-squares regression from changes in total valine release. Valine accumulation was then converted into protein equivalent, 1 mg of protein containing approx. 0.591 μ mol of valine [19]. Fractional rates of proteolysis were computed by dividing rates of protein breakdown by the hepatic protein content at the end of the perfusion.

Valine in the perfusate plasma was determined by reverse-phase chromatography as previously described [20].

Liver protein content was measured by the method of Lowry et al. [21].

Other analytical procedures

Liver DNA content was assessed by the method of Munro and Fleck [22].

Portal amino acid, glucagon and insulin levels were assayed in portal blood samples collected from controls, turpentine-treated rats 24 h after induction of inflammation and pair-fed rats. Insulin and glucagon concentrations were determined as previously described [20]. After deproteinization of an aliquot of serum with 50 mg/ml sulphosalicylic acid, amino acids were quantified by ion-exchange chromatography using an amino acid analyser (Hitachi L 8500A, B. Braun Scientec, Les Ulis, France).

Statistical analysis

Results are means $\pm$ S.E.M. Comparisons between two independent groups and between the turpentine-treated rats and their pair-fed partners were performed using Student's *t* test and the paired *t* test respectively. Comparisons between the controls on the one hand and the turpentine-treated rats or the pair-fed animals on the other were carried out by using one-way analysis of variance followed by the least-significant-difference test.

Chemicals

[5-³H]Cytidine (20–30 mCi/mmol) was purchased from ICN Pharmaceuticals and [6-¹⁴C]orotic acid (45–60 mCi/mmol) from Isotopchim, Ganagobie-Peyruis, France. Dowex 50W (X4) and AG 1 (X4) were obtained from Bio-Rad Laboratories, Richmond, CA, U.S.A. Methanol and acetonitrile came from Merck, Darmstadt, Germany. Tetrabutylammonium dihydrogen phosphate was from Fluka A.G., Buchs, Switzerland, and turpentine oil from Prolabo, Paris, France. Cytidine, 2'-CMP, cycloheximide, BSA, ammonium phosphate and KH₂PO₄ were obtained from Sigma Chemical Co., St Louis, MO, U.S.A.

RESULTS

Measurement of RNA degradation using [5-³H]cytidine: validation of the technique

As previously observed with [6-¹⁴C]orotic acid [10], radioactive cytidine accumulation in the perfusion medium was linear between 5 and 15 min after the beginning of the cyclic perfusion (results not shown).

When the specific radioactivity of CMP residues in RNA was measured after *in vivo* RNA labelling with [5-³H]cytidine, radioactivity was mainly associated with 2'- and 3'-CMP (> 85%) but

was also found associated with 2'- and 3'-UMP. Although Camiener and Smith [23] did not detect cytidine deaminase activity in tissues other than the liver in the rat, we cannot exclude the possibility that some activity might have contributed to the conversion of cytidine into uridine. The specific radioactivities of 2'-CMP and 3'-CMP were similar. The specific radioactivity ratio RNA/CMP was 1.11 ± 0.02 ($n = 13$).

Cytidine specific radioactivity was measured at 5, 10 and 15 min after the beginning of the perfusion. The average value for the three time points was computed for each rat. The specific radioactivity ratio cytidine/CMP was 0.93 ± 0.07 ($n = 4$). The fact that the specific radioactivity of cytidine was close to that of CMP in RNA and constant over the 15 min of perfusion (results not shown) suggests that cytidine is randomly released from RNA during the degradation process as for [¹⁴C]orotic acid [15]. Unexpectedly, the radioactivity recovered after Dowex elution amounted to only $4.4 \pm 0.6\%$ ($n = 5$) of the total measured in the plasma perfusate. As ³H exchange was likely to occur during long-term *in vivo* labelling, part of this radioactivity might have consisted of tritiated water. A pool of plasma was lyophilized and the remaining radioactivity amounted to 7.6% of the initial radioactivity. Therefore more than 90% of ³H in the perfusate plasma corresponded to ³H₂O. In addition, the radioactivity in Dowex 50 eluates was not completely associated with cytidine but was also distributed in two other peaks. None of them corresponded to cytosine, the only potential degradation product of cytidine. As ³H could have exchanged with various macromolecules during *in vivo* labelling, it is difficult to specify the nature of the molecules that bound to Dowex. Some might have been amino acids. In order to determine accurately RNA-degradation rates, it was necessary to quantify the fraction of radioactivity corresponding exclusively to cytidine. The radioactivity associated with the cytidine peak was determined by HPLC and found to be $65.7 \pm 2.1\%$ ($n = 5$). We have checked that the percentage recovery of radioactivity in the cytidine peak did not vary in different experimental conditions. For this purpose, measurements were made on a pool of samples from five turpentine-treated and five pair-fed rats. The percentage recovery of radioactivity was similar in the two groups, 64.4 and 63.8 respectively, and very close to the average value obtained for control animals. Consequently, the slope of radioactive cytidine accumulation (d.p.m./min per liver) computed for each sample after Dowex 50 elution of the plasma collected between 5 and 15 min of perfusion corresponds to the slope of radioactivity accumulation (d.p.m./min per liver) $\times 0.657$.

Fractional rates of RNA degradation were significantly higher when livers were labelled with [5-³H]cytidine instead of [6-¹⁴C]orotic acid (0.91 ± 0.04 compared with $0.70 \pm 0.05\%/h$ respectively; $P < 0.05$). This difference could not be explained on the basis of a different recycling of the label released from RNA since the addition of 0.5 mM non-radioactive cytidine to the perfusion medium inhibited to the same extent the reutilization of the labelled cytidine whatever the tracer used (results not shown). Yet, we cannot exclude the possibility that a greater percentage of mRNA was labelled when [³H]cytidine was used instead of [¹⁴C]orotic acid. As the turnover rate of mRNA is much faster than that of long-lived RNA (rRNA and tRNA), this might have contributed to the increase in RNA-degradation rates. However, the RNA-degradation rates obtained with [³H]cytidine remained in the physiological range [20] and comparison between the controls, the turpentine-treated rats and the pair-fed animals were valid since the percentage recovery of radioactivity in the cytidine peak was similar in the three groups. Therefore, despite ³H exchange during *in vivo* labelling with [5-³H]cytidine, the method presented in this study is a suitable

Table 1 Effects of inflammation on food intake, body weight, liver weight and hepatic RNA and protein content

The parameters were measured in control rats fed *ad libitum*, in turpentine-injected rats (I), 12, 18 and 24 h after induction of acute inflammation and in the respective pair-fed animals (PF). The results are means $\pm$ S.E.M. for nine rats per group except for the controls ($n = 11$) and for the I and PF rats at 12 h ($n = 4$). ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ compared with PF; ^d $P < 0.05$, ^e $P < 0.01$ compared with controls.

	Food intake (g)	Body weight (g)	Liver weight (g)	Liver RNA (mg/liver)	Liver protein (mg/liver)
Control	23.0 $\pm$ 0.8	189 $\pm$ 4	8.88 $\pm$ 0.30	71.59 $\pm$ 2.49	1439 $\pm$ 80
I (12 h)	17.0 $\pm$ 1.7 ^e	192 $\pm$ 3 ^a	7.75 $\pm$ 0.36	71.18 $\pm$ 1.82 ^a	1379 $\pm$ 66
PF (12 h)	—	186 $\pm$ 2	7.80 $\pm$ 0.41	63.76 $\pm$ 0.57	1481 $\pm$ 38
I (18 h)	15.9 $\pm$ 0.6 ^e	182 $\pm$ 3	8.04 $\pm$ 0.13 ^d	85.30 $\pm$ 1.58 ^{ce}	1506 $\pm$ 45
PF (18 h)	—	184 $\pm$ 3	7.61 $\pm$ 0.30 ^e	64.59 $\pm$ 2.13	1386 $\pm$ 53
I (24 h)	15.1 $\pm$ 1.1 ^e	176 $\pm$ 4 ^d	8.12 $\pm$ 0.34 ^b	82.38 $\pm$ 2.87 ^{cd}	1439 $\pm$ 50 ^a
PF (24 h)	—	176 $\pm$ 4 ^d	6.92 $\pm$ 0.34 ^e	60.14 $\pm$ 1.50 ^e	1299 $\pm$ 51

Table 2 Effects of inflammation on RNA and protein fractional degradation rates

The liver RNA and protein fractional degradation rates were measured during a 15 min cyclic perfusion in control rats, in turpentine-treated rats, 18 and/or 24 h after induction of acute inflammation and in their respective pair-fed controls. The results are means $\pm$ S.E.M. for five rats in each group: ^a $P < 0.0001$ compared with the respective pair-fed groups, ^b $P < 0.001$ compared with the controls.

Group	RNA breakdown (%/h)		Proteolysis at 24 h (%/h)
	18 h	24 h	
Controls	—	0.91 $\pm$ 0.04	2.11 $\pm$ 0.18
Turpentine-treated	0.54 $\pm$ 0.03 ^{ab}	0.56 $\pm$ 0.1 ^{ab}	1.66 $\pm$ 0.07
Pair-fed	1.25 $\pm$ 0.15	1.03 $\pm$ 0.14	1.82 $\pm$ 0.10

alternative to the use of [¹⁴C]orotic acid if the radioactivity associated with cytidine is accurately determined.

Determination of rates of RNA and protein breakdown

Experiment 1: time course of accumulation of liver RNA and protein in response to acute inflammation (Table 1)

Food intake was significantly reduced as early as 12 h after turpentine treatment and continued to decrease until 24 h (37% decrease compared with control values).

Initial body weight was similar in all groups. Body weight was significantly lower in the turpentine-treated rats and in their pair-fed counterparts at 24 h than in the control group. It did not differ between the turpentine-treated rats and their respective pair-fed controls, except at 12 h.

Liver weight was lower in the pair-fed rats at 18 and 24 h than in the controls. It was significantly increased (17%) only 24 h after turpentine injection as compared with the pair-fed group. This increase was not due to cell proliferation as liver DNA content was similar in both groups (results not shown).

Liver RNA content was significantly lower in the pair-fed rats at 24 h than in the controls. By contrast, it was significantly higher in the turpentine-treated rats than in the respective pair-fed animals at all time points. The difference in RNA content between the two groups peaked between 18 and 24 h (32 and

Table 3 Portal amino acid concentrations (mmol/l) during acute inflammation

Portal blood was collected from anaesthetized rats 24 h after a subcutaneous turpentine oil injection. The results are means $\pm$ S.E.M. for six rats per group. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ compared with the pair-fed group; ^d $P < 0.05$, ^e $P < 0.01$ compared with the controls. The eight amino acids printed in bold are called regulatory amino acids and are considered to be the most potent inhibitors of autophagy [24].

	Concentration (mmol/l)		
	Control	Turpentine-treated	Pair-fed
Leu	117.5 $\pm$ 9.3	102.9 $\pm$ 9.4	111.8 $\pm$ 5.0
Pro	311.8 $\pm$ 47.0	213.6 $\pm$ 22.9	213.3 $\pm$ 17.4
Gln	474.8 $\pm$ 15.3	602.3 $\pm$ 73.5 ^b	458.4 $\pm$ 36.9
His	76.2 $\pm$ 2.8	67.1 $\pm$ 6.8	61.9 $\pm$ 2.7
Phe	54.2 $\pm$ 3.6	58.4 $\pm$ 2.8	53.2 $\pm$ 2.7
Tyr	70.8 $\pm$ 5.2	68.4 $\pm$ 4.8 ^a	85.6 $\pm$ 2.0
Met	51.9 $\pm$ 1.8	67.7 $\pm$ 5.3 ^{bd}	47.3 $\pm$ 1.7
Trp	61.3 $\pm$ 3.9	34.0 $\pm$ 3.4 ^{ce}	58.5 $\pm$ 2.3
Asn	100.1 $\pm$ 9.5	85.5 $\pm$ 16.3 ^a	109.5 $\pm$ 13.4
Ala	909.6 $\pm$ 58.8	731.8 $\pm$ 79.7	790.6 $\pm$ 52.9
Ser	329.0 $\pm$ 9.4	317.1 $\pm$ 30.2	307.5 $\pm$ 11.5
Glu	166.8 $\pm$ 20.3	246.4 $\pm$ 21.8 ^{cd}	151.4 $\pm$ 13.5
Gly	493.7 $\pm$ 25.7	410.3 $\pm$ 44.9 ^a	509.0 $\pm$ 15.6
Val	183.6 $\pm$ 12.8	138.3 $\pm$ 11.6 ^{bd}	166.7 $\pm$ 5.3
Ile	84.1 $\pm$ 7.2	66.3 $\pm$ 5.5 ^b	81.7 $\pm$ 2.4
Thr	234.0 $\pm$ 14.2	174.7 $\pm$ 18.1	208.7 $\pm$ 16.6
Lys	356.6 $\pm$ 34.9	412.7 $\pm$ 55.2	456.6 $\pm$ 47.2
Arg	111.4 $\pm$ 27.3	180.0 $\pm$ 36.7	149.5 $\pm$ 4.2
Asp	16.6 $\pm$ 3.2	21.4 $\pm$ 1.7 ^c	12.2 $\pm$ 0.5

37% increase in the turpentine-treated rats at 18 and 24 h respectively).

Acute inflammation resulted in significant but moderate protein accumulation as compared with pair-feeding only at 24 h (11% increase).

Experiment 2: fractional rates of RNA and protein degradation 18 and 24 h after turpentine treatment (Table 2)

RNA degradation was measured at 18 and 24 h since these time points corresponded to the peak phase of liver RNA accumulation. Fractional rates of RNA degradation in the turpentine-treated rats were significantly lower than those of the controls at 18 and 24 h (40% decrease) whereas there was no significant difference between both pair-fed groups and the controls. RNA-breakdown rates were dramatically depressed in the turpentine-treated rats compared with the pair-fed animals at both time points. The percentage inhibition amounted to 57 (range 34–70) and 45 (range 29–63) at 18 and 24 h respectively but was not significantly different between the two time points.

Fractional proteolysis rates were determined only at 24 h when the liver protein content was significantly enhanced. They were not modified by turpentine treatment or pair-feeding.

Experiment 3: portal amino acid, insulin and glucagon concentrations 24 h after induction of acute inflammation

The effects of turpentine treatment on portal amino acid concentrations are shown in Table 3. No difference was noticed between the pair-fed rats and the controls. The main difference between turpentine administration and pair-feeding was the increase in

Table 4 Portal insulin and glucagon levels 24 h after induction of acute inflammation

Portal blood was collected from anaesthetized rats 24 h after subcutaneous turpentine injection. The results are means $\pm$ S.E.M. for six rats per group. The glucagon/insulin molar ratio was computed by using molecular masses of 5734 and 3550 Da for insulin and glucagon respectively.

Group	Insulin (ng/ml)	Glucagon (ng/ml)	Insulin/ glucagon molar ratio
Controls	2.65 $\pm$ 0.11	0.64 $\pm$ 0.04	2.47 $\pm$ 0.18
Turpentine-treated	2.58 $\pm$ 0.14	0.55 $\pm$ 0.08	3.14 $\pm$ 0.55
Pair-fed	2.88 $\pm$ 0.33	0.59 $\pm$ 0.05	3.05 $\pm$ 0.55

glutamine and glutamate levels in the turpentine-treated rats. In this last group, methionine and aspartate concentrations were also enhanced whereas those of tyrosine, asparagine, tryptophan, glycine, valine and isoleucine levels were depressed.

Portal insulin and glucagon levels as well as insulin/glucagon molar ratio were not significantly different in the controls, the turpentine-treated rats and the pair-fed group (Table 4).

DISCUSSION

The large increase in liver RNA content observed 18 and 24 h after turpentine treatment (32–38 %) agrees with results obtained using other inflammatory and infectious stimuli, namely between 6 and 24 h after lipopolysaccharide injection, in chronic inflammation and after chronic lipopolysaccharide, interleukin (IL) 1 or tumour necrosis factor α (TNF- α) administration [4,5,25]. An increase in liver protein content mainly resulting from the accumulation of long-lived or resident proteins also occurred at 24 h but was moderate (11 %). As Wusteman et al. [3] reported a 33 % increase in liver protein mass in turpentine-injected rats compared with pair-fed animals 48 h after turpentine administration, it is possible that the peak of protein accumulation was not yet reached at 24 h.

Our results clearly showed a dramatic inhibition of RNA degradation in response to acute inflammation. The accumulation of radioactive cytidine in the perfusion medium 60 h after RNA labelling *in vivo* has been shown to reflect mainly the breakdown of long-lived RNA, in particular rRNA and tRNA, which have similar turnover rates and make up over 90 % of cytoplasmic RNA [10,26]. As rRNA amounts to 85–90 % of cytoplasmic RNA [26], the technique used provides a good estimate of rRNA degradation. We can thus assume that the inhibition of hepatic RNA breakdown reflects mainly that of rRNA. Since Piccoletti et al. [6] reported significantly enhanced rates of rRNA synthesis in liver nucleoli isolated from rats 12 and 24 h after turpentine treatment, we can thus assume that RNA accumulation 18–24 h after turpentine administration resulted from the combination of increased synthesis and reduced breakdown.

By contrast, liver protein breakdown was not significantly modified. However, the measurement of valine accumulation in the perfusate without previous protein labelling reflects total hepatic proteolysis corresponding to the combined degradation of long-lived, short-lived and endocytosed proteins [27]. As the synthetic rate of secreted proteins is enhanced in inflammatory states [5], the turnover of short-lived proteins mainly consisting of signal peptides and other nascent products of protein synthesis [12] should be accelerated. Thus, even if the breakdown rates of

long-lived or resident proteins is reduced, this effect may be counterbalanced by stimulation of the degradation of short-lived proteins. Consequently, the technique used does not allow us to exclude completely a reduction of the breakdown of long-lived proteins.

Ribosomal RNA, like long-lived intracellular protein, is mainly degraded in the lysosomal system by macroautophagy [12]. In order to identify the mechanisms responsible for suppressing RNA degradation during acute inflammation, the factors regulating macroautophagy need first to be examined. Amino acids are considered to be the most potent inhibitors of macroautophagy as well as of breakdown of long-lived protein and RNA [13,24,28]. Results obtained in liver perfused *in situ* showed that, of the 20 amino acids that constitute the complete amino acid mixture, only eight (leucine, phenylalanine, tyrosine, proline, histidine, tryptophan, methionine, glutamine) appear to be really effective in suppressing macroautophagy and proteolysis, leucine being the most important [24,28]. The effects of amino acids on RNA breakdown were studied in the perfused liver and in cultured hepatocytes [13,14]. Although it was not possible to define clearly a group of amino acids exhibiting inhibitory activities evoking those of a complete mixture, leucine and phenylalanine seemed to be the most effective. The flux of amino acids to the liver seems to be a determining factor in regulation of autophagy. Indeed, the use of a leucine analogue [29] or of the non-transportable multiple antigen peptide, [Leu8] MAP [30], strongly suggested that autophagic inhibition was initiated from site(s) located on the plasma membrane or close to it. We first determined the portal concentration of amino acids. The results showed that of the eight regulatory amino acids, only glutamine and to a lesser extent methionine displayed increased levels 24 h after turpentine treatment. The increase in glutamine in the portal circulation might be explained on the basis of its particular use by the gut. Thus Souba and Wilmore [31] have shown in dogs that, 24 h after operation, glutamine was taken up by the gut in greater amounts than in controls and that it was released from the gastrointestinal tract only in the operated animals. However, since the concentrations of glutamine and methionine remained in the physiological range, it seemed unlikely that their increase could fully account for the large extent of inhibition of RNA breakdown.

In addition to alterations in portal amino acid levels, other factors can enhance the flux of amino acids to the liver, e.g. accelerated uptake and increased blood flow. Stimulated muscle proteolysis concomitant with increased uptake of amino acids by the liver is a characteristic of trauma and sepsis [1]. In endotoxaemia, increased glutamine, alanine and arginine transport has been observed as well as increased liver blood flow [32,33]. The changes in these factors during acute inflammation have been poorly documented. However, Powanda et al. [34] reported enhanced movement of [14 C]cycloleucine from muscle to liver as early as 2 h after turpentine administration. It is thus possible that, in addition to the elevation of portal glutamine concentration, accelerated amino acid uptake may have contributed to the inhibition of RNA degradation.

Insulin and glucagon modulate the action of amino acids on macroautophagy. Studies in the perfused rat liver have shown that insulin inhibits while glucagon stimulates macroautophagy in the presence of physiological concentrations of amino acids [12]. As the two hormones exhibit antagonistic effects, the determination of the insulin/glucagon molar ratio is more informative than the absolute concentration of either hormone. However, as this ratio was not altered in response to acute inflammation, these hormones could not have contributed to the reduction in RNA breakdown.

Besides amino acids, insulin and glucagon, other factors may influence RNA degradation in response to inflammation, in particular cytokines and glucocorticoids. Although it is known that IL-1, TNF- α and to a much lesser extent IL-6 contribute to muscle proteolysis [35,36], their effects on liver protein breakdown are poorly documented. Two studies reported that the chronic (6 h or 7 days) combined infusion of IL-1- α and TNF- α reduced hepatic proteolysis [7,37]. In the present study TNF- α cannot be involved in the inhibition of rRNA degradation since turpentine administration unlike endotoxaemia does not elicit the production of this cytokine by non-parenchymal liver cells [38]. Moreover, the circulating levels of TNF- α are undetectable in this experimental model of inflammation [39]. Further studies will be needed to examine the putative inhibitory effect of IL-1 and IL-6 alone and in combination with other regulatory factors (amino acids, hormones).

Although glucocorticoids have long been known to increase liver protein and RNA content, data relevant to their effects on proteolysis in different *in vivo* and *in vitro* experimental models are conflicting [40–42]. However, these hormones by themselves do not affect or even stimulate protein catabolism.

In conclusion, our results show that the reduced breakdown of RNA, particularly rRNA, is an important mechanism contributing to hepatic RNA accumulation and therefore to the increased protein-synthesis capacity of hepatocytes in response to acute inflammation. The strong inhibition of RNA degradation cannot be explained by the portal levels of the main physiological regulatory factors (regulatory amino acids, insulin and glucagon). The effects on RNA degradation of cytokines in combination with glucocorticoids and amino acids and of changes in liver sensitivity to amino acids are at present under investigation.

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