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EFFECT OF ETHANOL ON TURPENTINE-INDUCED ACUTE PHASE RESPONSE IN RATS

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Abstract—Turpentine-induced acute-phase response and its modulation by ethanol in rats at 48 hr has been studied. There was more than 2.3–5.1 fold increase in fibrinogen and seromucoids concentrations in plasma, accompanied by 28% decline in albumin concentration in turpentine-stimulated rats. The fractional synthesis rate of these two acute-phase proteins was increased by 4.1–6.4 fold, while that of albumin (non acute-phase protein) was reduced by 32.6%. Ethanol inhibited this induction of acute-phase protein synthesis at 48 hr. The inhibition of acute-phase response by ethanol was significantly more pronounced for seromucoids than for fibrinogen and appeared to be dependent on the carbohydrate content of the acute-phase glycoprotein.

The acute-phase reactants can be defined as trauma-inducible glycoproteins of hepatic origin [1]. These include several plasma proteins, enzymes, protease inhibitors, etc., some of whose functions are still not clear [1–3]. The known functions of a few proteins appear to be beneficial to animal or man. The acute-phase response has been studied under typical conditions which include local inflammation, thermal or mechanical injury, major surgery, bacterial or endotoxin injection and neoplastic growth [1].

Ethanol has specific acute and chronic effects on plasma protein metabolism in the liver [4, 5]. In addition, ethanol modifies the effects of many compounds whose target organ happens to be the liver [6–8]. Under these conditions it is interesting to investigate the modulatory effects of ethanol on turpentine-induced acute-phase response. Local subcutaneous inflammation caused by turpentine may not have direct effects on the liver—unlike hepatotoxins. Turpentine itself does not reach the liver from subcutaneous route [9], but it does have secondary effects in the form of induction of acute-phase reactants in the liver. The purpose of the present investigation is to study the effect of ethanol on turpentine-induced acute-phase response in rats in the light of recent available information.

MATERIALS AND METHODS

Treatment. Acute local inflammation was induced in male Wistar rats (250–300 g) by subcutaneous injection of turpentine oil (1.0 ml/rat). Control rats received equivalent volume of sterile saline by the same route. Within 5 min after the subcutaneous injection, the experimental group received ethanol (3.2 g/kg, 20% in saline) and the control group received isocaloric dextrose–saline (25%) by intraperitoneal route. Twenty-four hours after these injections, all animals received a second dose of ethanol or dextrose-saline as before and were sacrificed 24 hr later (48 hr after the first injections). Food (16% protein) and water were available till the time of sacrifice.

Incorporation of ^{14}C-amino acids into liver and plasma proteins. All the animals were injected intraperitoneally (10 μCi or 370 kBq/100 g) with ^{14}C-labelled chlorella protein hydrolyzate (26 mCi or 962 MBq per m Atom C., obtained from Bhabha Atomic Research Centre, Bombay, India). Under ether anesthesia blood was withdrawn from the heart 2 hr after the tracer administration. This time interval has been shown to achieve near maximum labelling of all plasma proteins [10]. The liver was perfused *in situ* through portal vein with ice-cold saline. Liver pieces were removed immediately for total protein and free amino acids pool estimations. Albumin, fibrinogen and seromucoids from plasma, and total proteins from liver, were isolated by well-established chemical procedures which have been described recently [8]. The respective protein precipitates were washed thoroughly with carrier amino acids to eliminate free labelled amino acids. The specific activities (cpm/mg) of proteins and amino acids were determined [8]. The fractional synthesis rates of individual proteins were computed by the following formula [8, 11].

$$\text{Fractional Synthesis Rate (F.S.R.)} = \frac{\text{sp. act. of protein} \times \text{protein conc. (mg/ml or mg/g)}}{\text{sp. act of liver amino acid pool}}$$

For comparison this fractional synthesis rate was expressed in a relative manner as:

$$\text{Relative synthesis rate} = \frac{\text{F.S.R. in exptl group}}{\text{F.S.R. in control group}} \times 100.$$

Albumin, which is not an acute-phase reactant, was studied for comparison with the other two acute-phase proteins. Liver total proteins were evaluated for studying the general effects of treatments at the site of protein synthesis

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RESULTS

In this study the acute-phase response has been monitored at 48 hr after the stimulation by following the plasma levels and synthesis rates of proteins. Further the modulation, if any, of this response by ethanol has also been studied at this time interval. For this purpose the experimental groups and their respective controls received the treatments as described above.

As shown in Table 1, at 48 hr the acute-phase proteins were elevated 2.3–5.1 fold in stimulated rats. Ethanol alone had only a marginal effect on the concentrations of albumin, fibrinogen and liver total proteins at this time-interval. As would be expected, turpentine-induced inflammation resulted in significantly reduced (32.6%) fractional synthesis rate of albumin and 4.1–6.4-fold increase in acute-phase protein synthesis (Table 1). In liver total proteins the effect was discernible in the form of significantly elevated synthesis. This increased synthesis was perhaps the reflection of highly stimulated acute-phase protein-synthesis. Ethanol significantly inhibited the induction of plasma protein synthesis in turpentine-stimulated rats at 48 hr. This inhibition was not uniform but was more pronounced for albumin and seromucoids. Thus ethanol enhanced the negative effects of turpentine on albumin synthesis. In control rats ethanol alone had inhibitory effects only on seromucoids synthesis at 48 hr, while all the other proteins studied were uninfluenced by ethanol.

DISCUSSION

Although the incorporation of labelled precursors into specific proteins can be regarded as a direct proof of net protein synthesis, the expression of data merely as specific activities can lead to errors, especially when the protein pool is augmented. Such a situation arises in turpentine-induced acute-phase response where large scale fluctuations occur in plasma concentrations of several proteins [1, 11]. These fluctuations are many orders of magnitude more (Table 1) than possible alterations in plasma volume. Hence the method adopted here for computation should give a reliable estimate of protein synthesis. It is also true that any change in the process of secretion of proteins from the hepatocytes to intravascular compartment may influence these results. In such a case one would expect large variations in the liver total protein concentrations. In fact liver total protein concentrations were more stable than plasma concentrations of specific acute-phase proteins and albumin (Table 1). Further, it is important to note here that Jamieson *et al.* [12] have demonstrated, by using liver slice technique, that there are no significant changes in the pathway of secretion of α_1-acid glycoprotein (about 91% of seromucoid fraction) in normal rats in comparison to turpentine-stimulated rats. In parallel with the increase in the hepatic synthesis of acute-phase proteins, there is a rise in the polymerized tubulin in inflammation [13]. Recently Bernuau *et al.* [14] have documented evidence to show increased fibrinogen secretion by single hepatocytes from rats with turpentine-induced acute inflammatory reaction.

In the present investigation the acute-phase response has been monitored at 48 hr. The alterations in plasma levels of different proteins in turpentine-stimulated group are generally in agreement with the observations of others [1]. The pattern of changes in the *in vivo* synthesis rates of proteins are also comparable to the recent findings of Schreiber *et al.* [10]. This induction of acute-phase protein synthesis was significantly inhibited by ethanol. In spite of this the levels of seromucoids remained elevated and were higher in ethanol-treated inflamed rats. This may be due to increased levels of circulating asialo-glycoproteins as a result of reduced binding capacities of hepatic plasma membranes in rats with induced inflammation [15]. Thus, increased synthesis of acute-phase proteins in turpentine-stimulated rats is likely to be accompanied by diminished catabolism [9].

Among the two acute-phase reactants studied here, seromucoids appears to be more sensitive (than

Table 1. Effect of ethanol on turpentine-induced acute-phase response in rats: concentrations and relative synthesis rates of liver and plasma proteins* (48 hr after stimulation)

	I. Control	II. Turpentine	III. Turpentine + Ethanol	IV. Ethanol
Albumin				
Conc. (g/dl)	2.83 ± 0.12	2.04 ± 0.16†	2.13 ± 0.20†	2.94 ± 0.05
Synthesis	100 ± 17	67 ± 18†	43 ± 7†‡	86 ± 5
Fibrinogen				
Conc. (mg/dl)	387 ± 84	884 ± 39†	998 ± 32†‡	391 ± 52
Synthesis	100 ± 12	414 ± 154†	376 ± 92†	92 ± 10
Seromucoids				
Conc. (mg/dl)	131 ± 34	664 ± 81†	712 ± 84†	167 ± 36
Synthesis	100 ± 19	636 ± 9†	481 ± 36†‡	66 ± 3†
Liver Total Proteins				
Conc. (mg/g)	152.0 ± 15.2	160.7 ± 11.4	148.7 ± 7.6	151.2 ± 7.9
Synthesis	100 ± 12	146 ± 32†	110 ± 18†	92 ± 7

* Values are expressed as mean ± S.D. for 4–5 rats. For details of computation of relative synthesis rates refer to text.
† $P < 0.05$ (vs control).
‡ $P < 0.05$ (vs turpentine).

fibrinogen) to turpentine-stimulation and inhibition by ethanol. The α_1-acid glycoprotein (main constituent of seromucoids) has the highest carbohydrate content compared to other acute-phase glycoproteins, with fibrinogen having the lowest content [16]. The increased glycosylation potential of the liver in turpentine-induced inflammation [17] would favour the synthesis and secretion of glycoproteins with high carbohydrate content. At the same time such glycoproteins would be more susceptible to the inhibitory effects of ethanol [5, 8]. In fact there appears to be a direct relationship between the carbohydrate content of a protein and inhibitory effect of ethanol on its synthesis. Isolated rat liver perfusion studies with 22 mmol/l. of ethanol have shown that the extent of inhibition of net synthesis of plasma glycoproteins is directly proportional to the carbohydrate contents of these proteins; the order of inhibition being: α_1—acid glycoprotein > haptoglobin > fibrinogen (Nadkarni and Miller, unpublished observations). Incidentally this happens to be the precise decreasing order of carbohydrate content of these proteins [16].

Some of the acute-phase reactants have specific beneficial functions in the body during inflammatory reactions [1–3]. These proteins may get biological priority for their biosynthesis compared to some other proteins. One such protein whose synthesis is compromised during inflammation, perhaps as an adaptive measure, to conserve a limited pool of activated amino acids, amino acyl-*t*-RNA, GTP, ATP, etc., is albumin [10, 18]. This protein also appears to be more susceptible to ethanol than acute-phase reactants during inflammation (Table 1). It is interesting to note that ethanol potentiates the hepatotoxic effects of various compounds [6–8]. At the same time ethanol inhibits the acute-phase response which is a defensive adaptation to inflammatory stress.

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