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ENHANCEMENT OF ACUTE ETHANOL HEPATOTOXICITY UNDER CONDITIONS OF LOW OXYGEN SUPPLY AND ISCHEMIA/REPERFUSION

THE ROLE OF OXYGEN RADICALS

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Abstract—Using isolated hemoglobin-free perfused rat livers we studied the effect of low oxygen supply on ethanol hepatotoxicity in two models. In the first model resembling low blood supply, perfusion rate was lowered from 60 to 10 ml/min after a 30 min-equilibration phase and kept low for 60 min. As a consequence, oxygen consumption fell from 1.76 + 0.15 μmol/min/g to 0.51 ± 0.02 μmol/min/g. In the second model, total ischemia was accomplished by interruption of the perfusion for 30 min and was followed by reperfusion at a perfusion rate of 60 ml/min for a further 30 min. In this model, oxygen consumption returned immediately to normal values upon reperfusion. In both models, low oxygen supply had no toxic effects of its own on livers from fed rats. While ethanol (3 g/l) given under normoxic conditions led to a moderate hepatotoxicity, its application in both models of partial as well as total ischemia and reperfusion resulted in a marked liver damage as evidenced by a strong release of sorbitol dehydrogenase, glutamate–pyruvate-transaminase, lactate dehydrogenase and glutathione, as well as by an increase in hepatic calcium content. Inhibition of ethanol metabolism by 4-methylpyrazol prevented liver damage in both models indicating that metabolism of ethanol is a prerequisite for its toxicity to occur. Also, hepatotoxicity was inhibited partially by catalase and superoxide dismutase and nearly totally by deferrioxamine and allopurinol. Thus, reactive oxygen species which are produced during ethanol metabolism as well as under conditions of low oxygen supply are mediators of hepatic damage in both models employed.

Reactive oxygen species produced during the metabolism of acetaldehyde by aldehyde oxidase and/or xanthine oxidase seem to be mediators of acute ethanol hepatotoxicity [1], partly via the induction of lipid peroxidation [2–4]. On the other hand, an ethanol-induced increase in hepatic liver oxygen consumption led to the hypothesis that a relative hypoxia might be involved in the pathogenesis of alcoholic liver injury [5–7]. Using isolated hemoglobin-free perfused rat livers we have recently shown that ethanol toxicity was strongly potentiated under hypoxic conditions induced by gassing the perfusion medium with a mixture of 95% N_2 and 5% CO_2 instead of carbogen which is used under normoxic conditions [8]. Oxygen was activated under these conditions due to the presence of purine metabolites and acetaldehyde, which are oxidized in a xanthine-oxidase-catalysed reaction yielding superoxide and its interconversion products. These seemed responsible for direct toxic actions as well as for an inhibition of glycolysis, the main source of energy-rich phosphates under hypoxic conditions [8].

From a clinical point of view, low oxygen supply may result from an impairment of normal blood flow leading to ischemia. Narrowing of blood vessels will lead to a lower rate of organ perfusion resulting in a continuous lower supply of oxygen, i.e. partial ischemia. Such a situation may emerge for example during the course of cirrhotic transformation. In this case, blood is lowered in parts of the liver and may influence the hepatotoxic actions of ethanol if consumed under these circumstances. Another situation emerges, when a blood vessel is totally occluded for a short period; under such a condition, total organ ischemia will prevail as long as the occlusion is present and will be followed by full supply of blood (and oxygen) as soon as the occlusion is removed. In both cases, oxygen supply will be impaired. As we had observed that hypoxia enhances ethanol toxicity [8], we employed two models to study the effect of partial ischemia as well as total ischemia and reperfusion, which more closely mimic pathophysiological conditions, on the acute toxicity of ethanol towards the isolated perfused rat liver.

MATERIALS AND METHODS

Animals. Male Wistar rats (320–380 g; breeder: Winkelmann, Borchen) were used throughout. They had free access to feed (Altromin® pellets) and tap water until use.

Liver perfusion. Livers were removed under pentobarbital anesthesia, connected to a recirculating perfusion system and perfused with Krebs–Henseleit buffer according to published procedures [9]. The perfusion medium was continuously gassed

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with carbogen (95% O_2, 5% CO_2), oxygen partial pressure amounting to 600 mm Hg. The experiments were started after a 30-min equilibration period. Partial ischemia (low flow perfusion) was produced by lowering the flow rate from 60 to 10 ml/min 30 min after the start of the respective experiment and kept low for a further 60 min. Total ischemia was accomplished by interruption of perfusion for 30 min, followed by reperfusion at the original flow rate for a further 30 min. Oxygen concentrations of the influent and the effluent perfusate were measured using a Micro pH/Blood Gas Analyzer 413 (Instrumentation Laboratories) to allow for calculation of oxygen consumption. Bile was collected in portions every 30 min and weighed to estimate its flow rate.

Ethanol was added to the perfusion medium at a concentration of 3 g/l at the start of the respective experiment. 4-Methylpyrazol (0.4 mmol/l), superoxide dismutase (20 mg/l), catalase (20 mg/l), deferrioxamine (500 mg/l) or allopurinol (100 mg/l) were added at the start of the equilibration period in the appropriate experiment.

Biochemical determinations. The activities of lactate dehydrogenase (LDH), glutamate–pyruvate-transaminase (GPT) and sorbitol dehydrogenase (SDH) were determined in the perfusate using commercial kits of Boehringer-Mannheim. Ca^{2+}-concentrations in the liver (following acid extraction) and in the perfusate were measured colorimetrically also with reagents of Boehringer-Mannheim. Malondialdehyde (MDA) was measured in the perfusate by coupling to thiobarbituric-acid [10]. Total glutathione was determined according to Brehe and Burch [11]; oxidized glutathione (GSSG) was estimated by the same procedure after blocking reduced glutathione (GSH) with 2-vinylpyridine [12]. Ethanol concentrations in the perfusate were determined by means of gas-liquid chromatography [1].

Statistics. Means ± SE were calculated in the usual way. The difference between two means was checked with Dunnett's *t*-test for multiple comparisons [13] taking $P < 0.05$ as the limit of significance.

RESULTS

Two models were employed to study the effect of ischemia on ethanol toxicity towards the isolated perfused rat liver. In the first model resembling low blood supply, perfusion rate was lowered from 60 to 10 ml/min after 30 min and kept low for a further 60 min (partial ischemia; low flow perfusion). In the second model resembling temporary blood vessel occlusion, total ischemia was accomplished by interruption of the perfusion from min 30 to min 60 of the 90-min experiment (total ischemia/reperfusion). Ethanol was present at a concentration of 3 g/l from the start of the respective experiment.

Oxygen consumption and ethanol elimination

In control experiments at normal perfusate flow rate (60 ml/min), oxygen consumption lay around 2 μmol/g/min over the whole perfusion period (Fig. 1a and b). Lowering of the flow rate to 10 ml/min led to a drop in oxygen consumption to 0.5 μmol/g/min until the end of the experiment (Fig. 1a). In experiments with 30 min of total ischemia, oxygen consumption rate returned immediately to normal values after reperfusion (Fig. 1b). Ethanol addition

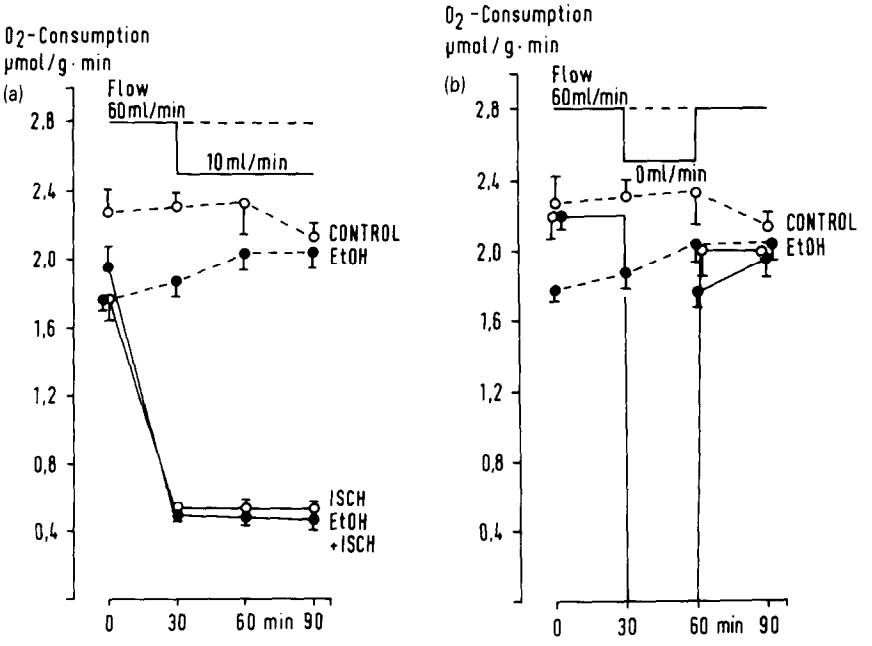

Fig. 1. Oxygen consumption by the isolated perfused rat liver: (a) under conditions of partial ischemia (low flow perfusion); (b) under conditions of total ischemia/reperfusion. Values are means ± SE, N = 5–6. (– – –) normal flow rate; (——) ischemic conditions (low flow perfusion or total ischemia/reperfusion); (○) no ethanol; (●) + 3 g/l ethanol.

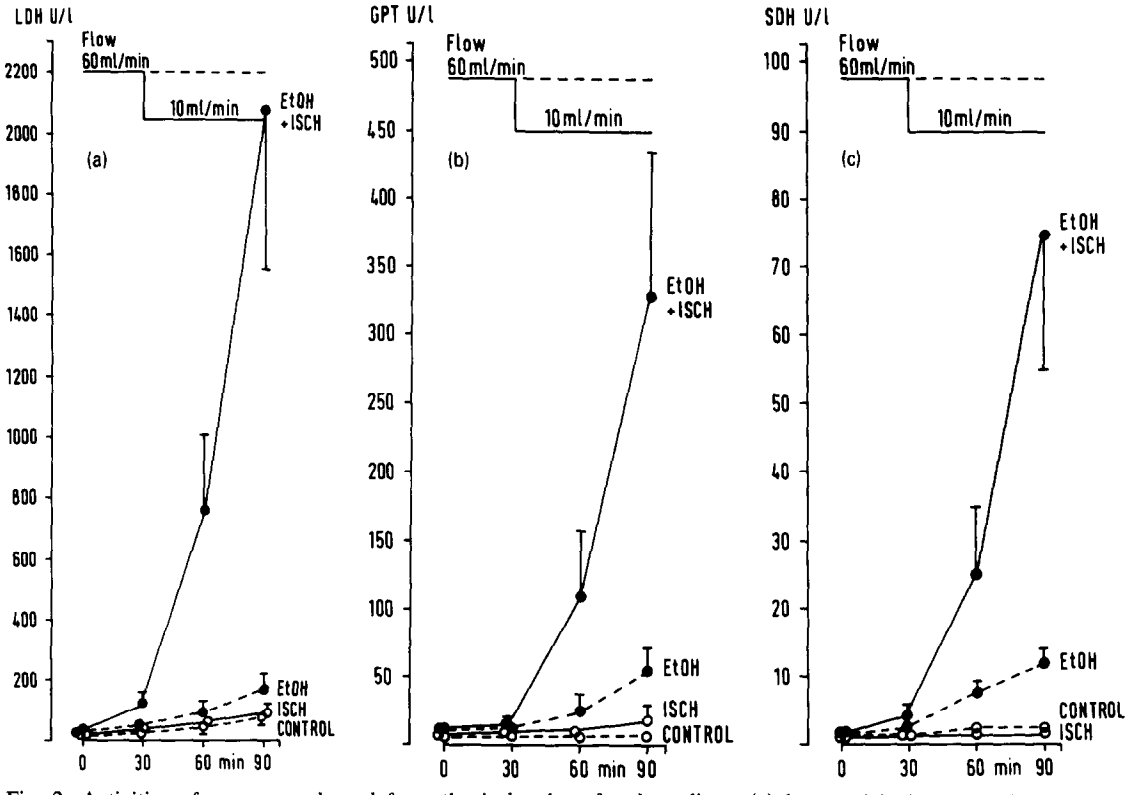

Fig. 2. Activities of enzymes released from the isolated perfused rat liver: (a) lactate dehydrogenase (LDH); (b) glutamate-pyruvate-transaminase (GPT); (c) sorbitol dehydrogenase (SDH). Values are means ± SE, N = 5–6. (- - -) normal flow rate; (——) partial ischemia (low flow perfusion); (○) no ethanol; (●) + 3 g/l ethanol.

had no further marked effect on oxygen consumption (Fig. 1a and b). Ethanol elimination amounted to 0.51 g/l/hr under normal flow conditions and was slightly lower (0.38 g/l/hr) in both models of ischemia (data not shown).

Hepatotoxic effects

While low flow perfusion had no toxic effect of its own on the livers of fed animals, ethanol treatment alone led to a moderate toxicity as evidenced by a slight increase in the release of LDH (Fig. 2a), GPT (Fig. 2b) and SDH (Fig. 2c). The toxicity of ethanol was strongly potentiated, however, under conditions of partial ischemia (Fig. 2a–c).

As a general measure of viability, bile flow was observed. In control experiments, bile flow decreased moderately in a time-dependent manner (Fig. 3). Ethanol alone had no effect on the rate of bile flow decrease, while low flow perfusion led to a slightly stronger drop in bile flow (Fig. 3). The combination of ethanol and low flow perfusion, however, resulted in a marked drop in bile flow to 8% of the original value after 30 min of the onset of partial ischemia (Fig. 3).

In control experiments, glutathione was released into the perfusate yielding a final concentration of 41.2 nmol/ml, 91% of which were in the oxidized state (Table 1). Addition of ethanol led to an

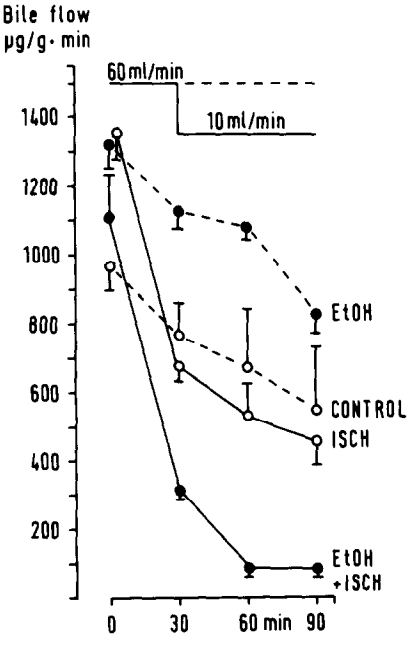

Fig. 3. Bile flow rate of the isolated perfused rat liver. Values are means ± SE, N = 5–6. (- - -) normal flow rate; (——) partial ischemia (low flow perfusion); (○) no ethanol; (●) + 3 g/l ethanol.

Table 1. Concentrations of malondialdehyde (MDA) and glutathione in the perfusates at the end of the experiments

Group	Treatment(s)			MDA (nmol/ml)	Total glutathione (GSH + 2GSSG) (nmol/ml)	GSSG (GSH-equivalents) (nmol/ml)
	Partial ischemia[a]	Ethanol[b]	Inhibitor			
1	−	−	−	0.10 ± 0.01	41.2 ± 7.5	37.4 ± 8.9
2	−	+	−	0.09 ± 0.02	77.4 ± 16.2*	57.4 ± 21.5
3	+	−	−	0.02 ± 0.01*	16.5 ± 3.4*	13.4 ± 3.7*
4	+	+	−	0.12 ± 0.07	76.6 ± 16.2*	73.1 ± 17.8*
5	+	+	4-MP[c]	0.08 ± 0.01	36.7 ± 6.7†	30.0 ± 8.0†
6	+	+	CAT[d]	0.10 ± 0.03	77.3 ± 18.7	59.4 ± 18.8
7	+	+	DFO[e]	0.07 ± 0.01	26.8 ± 7.3†	16.4 ± 3.1†
8	+	+	AP[f]	0.05 ± 0.01†	28.5 ± 3.1†	11.2 ± 1.4†

Values are means ± SE, N = 5–6.

[a] flow 60 ml/min during the first 30 min, followed by reduction to 10 ml/min for 60 min; [b]3g/l; [c]4-methylpyrazol (0.4 mmol/l); [d]catalase (20 mg/l); [e]deferrioxamine (500 mg/l); [f]allopurinol (100 mg/l).

* Statistically significant difference ($P < 0.05$) to group 1.

† Statistically significant difference ($P < 0.05$) to group 4, Dunnett's t-test.

enhanced release of glutathione (Table 1). Following low flow perfusion, GSH-release amounted to only 40% of the control value, most probably a direct effect of the lower flow rate; addition of ethanol under these conditions enhanced glutathione release to the same extent as seen with ethanol under normal flow conditions (Table 1). Release of malondialdehyde into the perfusate was similar in all groups except for the group subjected to partial ischemia only; here, again, the low rate of perfusion resulted in a substantially lower rate of MDA-release (Table 1).

While neither ethanol nor partial ischemia led to an accumulation of calcium in liver tissue, the combined treatment (ethanol + low flow perfusion) resulted in a 1.34-fold increase in hepatic calcium content (Table 2). The hepatic content of total glutathione was not changed as compared to controls upon the application of ethanol under normal flow conditions, and was slightly but not significantly decreased under conditions of partial ischemia in the absence of ethanol (Table 2). Treatment with ethanol in combination with low flow perfusion, however, resulted in a significant depletion of hepatic glutathione by 40% as compared to controls (Table 2).

Total ischemia and reperfusion alone had no marked effect on the release of enzymes by the liver (Fig. 4a–c), but the combination of ethanol and ischemia/reperfusion led to a marked hepatotoxicity as evidenced by a strong increase in the perfusate activities of LDH (Fig. 4a), GPT (Fig. 4b) and SDH (Fig. 4c). Upon the onset of total ischemia, bile flow rapidly decreased to about 10% of the original value in the absence and in the presence of ethanol (Fig. 5). In the absence of ethanol, bile flow recovered after reperfusion reaching control values at the end of the experiment, while in the presence of ethanol it remained low indicating sustained toxicity (Fig. 5). The potentiation of ethanol toxicity under conditions of total ischemia and reperfusion was furthermore evident by an enhanced release of glutathione into the perfusate (Table 3) as well as by a marked accumulation of Ca^{2+} in hepatic tissue (Table 4).

Table 2. Concentrations of Ca^{2+} and glutathione in the isolated perfused livers at the end of the experiments

Group	Treatment(s)			Ca^{2+} (μmol/g)	Total glutathione (GSH + 2GSSG) (μmol/g)	GSSG (GSH-equivalents) (μmol/g)
	Partial ischemia[a]	Ethanol[b]	Inhibitor			
1	−	−	−	1.39 ± 0.10	6.32 ± 0.31	0.06 ± 0.01
2	−	+	−	1.38 ± 0.09	6.71 ± 0.65	0.08 ± 0.01
3	+	−	−	1.22 ± 0.06	5.03 ± 0.55	0.15 ± 0.09
4	+	+	+	1.86 ± 0.18*	3.80 ± 0.63*	0.08 ± 0.01
5	+	+	4-MP[c]	1.38 ± 0.16†	5.61 ± 0.60†	0.06 ± 0.01
6	+	+	CAT[d]	1.36 ± 0.14†	4.41 ± 0.39	0.14 ± 0.06
7	+	+	DFO[e]	0.92 ± 0.11†	7.50 ± 0.93†	0.23 ± 0.03†
8	+	+	AP[f]	0.82 ± 0.05†	7.64 ± 0.54†	0.15 ± 0.10

Values are means ± SE, N = 5–6.

[a] flow 60 ml/min during the first 30 min, followed by reduction to 10 ml/min for 60 min; [b]3g/l; [c]4-methylpyrazol (0.4 mmol/l); [d]catalase (20 mg/l); [e]deferrioxamine (500 mg/l); [f]allopurinol (100 mg/l).

* Statistically significant difference ($P < 0.05$) to group 1.

† Statistically significant difference ($P < 0.05$) to group 4, Dunnett's t-test.

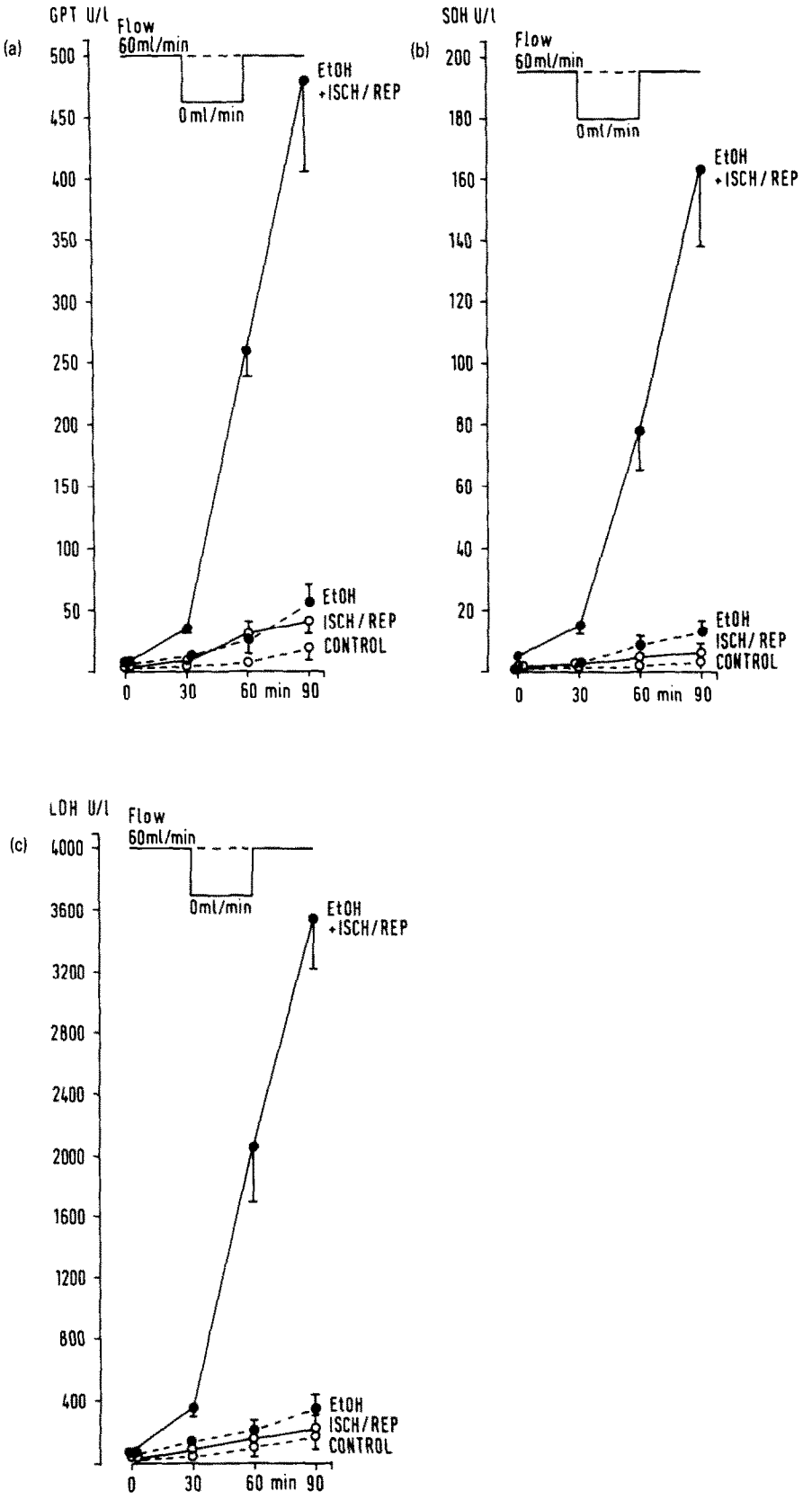

Fig. 4. Activities of the enzymes released from the isolated perfused rat liver: (a) lactate dehydrogenase (LDH); (b) glutamate–pyruvate-transaminase (GPT); (c) sorbitol dehydrogenase (SDH). Values are means ± SE, N = 5–6. (– – –) normal flow rate; (——) total ischemia + reperfusion; (○) no ethanol; (●) + 3 g/l ethanol.

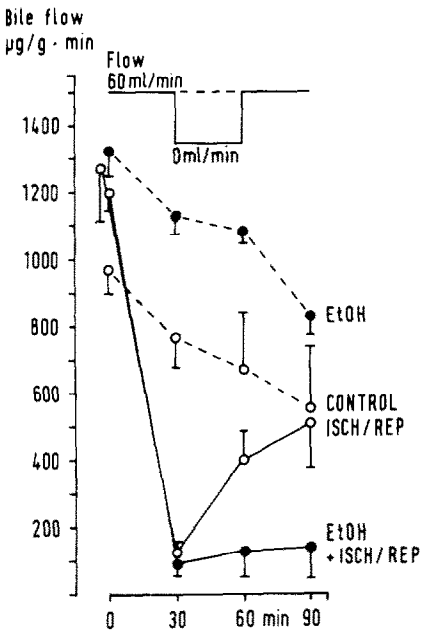

Fig. 5. Bile flow rate of the isolated perfused rat liver. Values are means ± SE, N = 5–6. (– – –) normal flow rate; (——) total ischemia + reperfusion; (○) no ethanol; (●) + 3 g/lg ethanol.

MDA-concentrations in the perfusate were elevated three-fold, indicating the occurrence of lipid peroxidation (Table 3). Furthermore, hepatic glutathione was depleted by 23% as compared to control values (Table 4).

Effects of enzyme inhibitors and scavengers of reactive oxygen species

The release of enzymes following ethanol and low-flow perfusion was inhibited by the alcohol dehydrogenase inhibitor 4-methylpyrazol, by the H_2O_2-scavenger catalase, by the iron-chelator deferrioxamine and by the xanthine oxidase inhibitor and radical scavenger allopurinol (Table 5). Differences in the potential of inhibitors (e.g. catalase) to suppress the release of individual enzymes is due to differences in cellular content of these enzymes and in the case of their release: LDH leaks more readily out of the cell; this may explain why catalase was not capable of inhibiting its release under conditions of partial ischemia, but prevented the leakage of GPT and SDH. Except for catalase, all agents also inhibited the release of glutathione into the perfusate (Table 1) as well as the GSH-depletion of the liver and the accumulation of calcium (Table 2).

In experiments with ethanol and total ischemia/reperfusion, all agents as well as superoxide dismutase inhibited the release of enzymes (Table 6) and of glutathione (Table 3) into the perfusate as well as the accumulation of Ca^{2+} in hepatic tissue (Table 4), the formation and secretion of MDA (Table 3) as well as the loss of hepatic glutathione (Table 4).

DISCUSSION

Partial ischemia (low flow perfusion) as well as total ischemia and reperfusion, while having no toxicity of their own in livers from fed animals, strongly potentiated the hepatotoxicity of ethanol. This enhancement of ethanol toxicity was evident despite an even lower rate of its metabolic degradation. These findings are in accordance with previous results from our laboratory which showed a potentiation of ethanol hepatotoxicity under hypoxic conditions [8]. The observation that livers from fed animals were resistant to damage due to ischemia indicates that, due to the presence of glycogen, anaerobic energy conservation reactions can maintain the basic physiological functions of liver cells under ischemic conditions. In unpublished experiments from our laboratory, livers depleted of glycogen by fasting the donor rats for 16 hr prior to

Table 3. Concentrations of malondialdehyde (MDA) and glutathione in the perfusates at the end of the experiments

Group	Treatment(s) Ischemia[a] + reperfusion	Ethanol[b]	Inhibitor	MDA (nmol/l)	Total glutathione (GSH + 2GSSG) (nmol/l)	GSSG (GSH-equivalents) (nmol/l)
1	–	–	–	0.10 ± 0.01	41.2 ± 7.5	37.4 ± 8.9
2	–	+	–	0.09 ± 0.02	77.4 ± 16.2	57.4 ± 21.5
3	+	–	–	0.06 ± 0.01*	28.4 ± 8.6	22.4 ± 7.5
4	+	+	–	0.35 ± 0.15*	154.0 ± 17.6*	145.0 ± 20.4*
5	+	+	4-MP[c]	0.13 ± 0.03†	55.4 ± 11.1†	40.4 ± 13.8†
6	+	+	SOD[d]	0.11 ± 0.02†	61.0 ± 10.6†	ND
7	+	+	CAT[e]	0.19 ± 0.09	92.7 ± 25.3	18.7 ± 15.7†
8	+	+	DFO[f]	0.10 ± 0.01†	39.6 ± 2.9†	ND
9	+	+	AP[g]	0.11 ± 0.02†	45.4 ± 4.8†	6.8 ± 4.1†

Values are means ± SE, N = 5–6.

[a] flow 0 ml/min from min 30 to min 60; [b] 3 g/l; [c] 4-methylpyrazol (0.4 mmol/l); [d] superoxide dismutase (20 mg/l); [e] catalase (20 mg/l); [f] deferrioxamine (500 mg/l); [g] allopurinol (100 mg/l).

* Statistically significant difference ($P < 0.05$) to group 1.

† Statistically significant difference ($P < 0.05$) to group 4, Dunnett's *t*-test.

ND: not determinable (below detection limit).

Table 4. Concentrations of Ca^{2+} and glutathione in the isolated perfused livers at the end of the experiments

Group	Treatment(s)			Ca^{2+} (μmol/g)	Total glutathione (GSH + 2GSSG) (μmol/g)	GSSG (GSH-equivalents) (μmol/g)
	Ischemia[a] + reperfusion	Ethanol[b]	Inhibitor			
1	−	−	−	1.39 ± 0.10	6.32 ± 0.31	0.06 ± 0.01
2	−	+	−	1.38 ± 0.09	6.71 ± 0.65	0.08 ± 0.01
3	+	−	−	1.50 ± 0.08	6.27 ± 0.49	0.08 ± 0.01
4	+	+	−	2.21 ± 0.23*	4.86 ± 0.81*	0.05 ± 0.01
5	+	+	4-MP[c]	1.76 ± 0.20	6.51 ± 0.81†	0.04 ± 0.01
6	+	+	SOD[d]	1.66 ± 0.20†	5.13 ± 0.25	0.01 ± 0.01
7	+	+	CAT[e]	1.57 ± 0.21†	6.15 ± 0.58	0.02 ± 0.01
8	+	+	DFO[f]	1.25 ± 0.06†	8.19 ± 0.53†	0.07 ± 0.06
9	+	+	AP[g]	1.25 ± 0.01†	7.49 ± 0.33†	ND

Values are means ± SE, N = 5–6.

[a] flow 0 ml/min from min 30 to min 60; [b] 3 g/l; [c] 4-methylpyrazol (0.4 mmol/l); [d] superoxide dismutase (20 mg/l); [e] catalase (20 mg/l); [f] deferrioxamine (500 mg/l); [g] allopurinol (100 mg/l).

* Statistically significant difference ($P < 0.05$) to group 1.

† Statistically significant difference ($P < 0.05$) to group 4, Dunnett's *t*-test.

ND: not determinable (below detection limit).

Table 5. Effect of inhibitors on enzyme release by isolated perfused rat livers following ethanol-treatment[a] and low-flow perfusion (10 ml/min for 60 min)

None	327 ± 108	75.0 ± 19.9	1086 ± 536
4-MP[c]	20 ± 8*	4.6 ± 1.8*	196 ± 79*
CAT[f]	137 ± 43*	37.2 ± 10.7*	1611 ± 423
DFO[g]	47 ± 34*	10.6 ± 8.7*	364 ± 218*
AP[h]	16 ± 7*	2.3 ± 1.0*	145 ± 41*

Values are means ± SE, N = 5–6.

[a] 3 g/l; [b] glutamate–pyruvate-transaminase; [c] sorbitol dehydrogenase; [d] lactate dehydrogenase; [e] 4-methylpyrazol (0.4 mmol/l); [f] catalase (20 mg/l); [g] deferrioxamine (500 mg/l); [h] allopurinol (100 mg/l).

* Statistically significant difference ($P < 0.05$) to the treatment group in the absence of inhibitors, Dunnett's *t*-test.

Table 6. Effect of inhibitors on enzyme release by isolated perfused rat livers following ethanol-treatment[a] and total ischemia/reperfusion (flow 0 ml/min from min 30 to min 60)

Inhibitor	GPT[b] (U/l × 90 min)	SDH[c] (U/l × 90 min)	LDH[d] (U/l × 90 min)
None	483 ± 64	163.0 ± 35.6	3548 ± 544
4-MP[e]	72 ± 17*	33.4 ± 11.9*	916 ± 356*
SOD[f]	161 ± 56*	54.0 ± 13.4*	1565 ± 577*
CAT[g]	221 ± 103*	58.1 ± 23.1*	1714 ± 698*
DFO[h]	16 ± 2*	1.9 ± 0.9*	214 ± 21*
AP[i]	20 ± 5*	4.8 ± 1.3*	188 ± 43*

Values are means ± SE, N = 5–6.

[a] 3 g/l; [b] glutamate–pyruvate-transaminase; [c] sorbitol dehydrogenase; [d] lactate dehydrogenase; [e] 4-methylpyrazol (0.4 mmol/l); [f] superoxide dismutase (20 mg/l); [g] catalase (20 mg/l); [h] deferrioxamine (500 mg/l); [i] allopurinol (100 mg/l).

* Statistically significant difference ($P < 0.05$) to the treatment group in the absence of inhibitors, Dunnett's *t*-test.

surgery exhibited a marked hepatotoxicity under conditions of partial ischemia or total ischemia and reperfusion. The same is true with livers subjected to hypoxia (95% N_2/5% CO_2 instead of carbogen) [14]. In the presence of ethanol, however, the liver loses its ability to cover its energy demand via anaerobic pathways as ethanol exerts an inhibitory effect on the glycolytic pathway [8, 15, 16].

The inhibition of ethanol hepatotoxicity by the alcohol dehydrogenase inhibitor 4-methylpyrazol indicates that, as in the case of "normal" oxygen supply [1], ethanol metabolism is a prerequisite for its toxicity to occur. The inhibitory action of superoxide dismutase and catalase indicates the involvement of superoxide and/or H_2O_2 as mediators of ethanol hepatotoxicity. The fact that both superoxide dismutase and catalase resulted in an inhibition of toxicity may seem discrepant, as superoxide dismutase will lead to an enhanced rate of H_2O_2-production. Our findings, however, suggest that the presence of both $^{\cdot}O_2^-$ *and* H_2O_2 is needed for hepatotoxicity to take place. Both will interact in a Haber–Weiss-type reaction to yield $^{\cdot}OH$ or $^{\cdot}OH$-like radicals. If one is missing (e.g. $^{\cdot}O_2^-$ in the presence of superoxide dismutase), this conversion will not take place and cytotoxicity is no more evident. The prevention of liver damage by deferrioxamine points at an involvement of iron ions either as catalysts of such interconversion reactions of reactive oxygen species or as direct initiators of oxidative damage. Deferrioxamine binds iron tightly yielding catalytically inactive complexes [17, 18]. In its presence, no more ionized iron is available for an interaction with primary reactive oxygen species ($^{\cdot}O_2^-$, H_2O_2) to yield more potent pro-oxidants. The suppression of hepatic damage by allopurinol, which is both an inhibitor of xanthine oxidase and a hydroxyl radical scavenger [19] strongly suggests the involvement of hydroxyl radicals as mediators of ethanol toxicity under ischemic conditions as well as xanthine oxidase being the most probable source of reactive oxygen species produced under these conditions and mediating oxidative stress and hepatotoxicity.

Further evidence for a role of oxidative stress in mediating the enhanced ethanol hepatotoxicity under ischemic conditions, apart from inhibitor studies, is the fact that, in both models, the glutathione released into the perfusate is mainly present in the oxidized form. Under conditions of oxidative stress intracellular glutathione is readily oxidized [20]. Oxidized glutathione has been detected under conditions of hypoxia and reoxygenation [21]. Thus, when released under conditions of enhanced membrane permeability due to toxic damage, GSSG is predominantly secreted. Also, the increased formation and secretion of malondialdehyde after ethanol treatment in the model of total ischemia/reperfusion and the inhibition of its formation in both models by scavengers of reactive oxygen species and metabolic inhibitors, support the idea of oxidative mechanisms being involved. Although malondialdehyde is a poor indicator of lipid peroxidation, its presence, together with our further findings, supports the general idea of an involvement of oxidative stress in our models.

In fact, the presence of purine catabolites produced during ischemia and acetaldehyde formed from ethanol as substrates of xanthine oxidase [22], which is present in its O_2-dependent form under ischemic conditions [23], provides two powerful sources of reactive oxygen species. As was the case under hypoxic conditions [1] the massive production of reactive oxygen seems to overwhelm the cellular antioxidative protection mechanisms. As a consequence, these reactive species mediate both a direct toxicity towards cellular macromolecules and a severe energy deficit due to the inhibition of glycolysis [1, 15, 16], the main source of anaerobic energy.

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