Endogenous hydroperoxide formation, cell volume and cellular K⁺ balance in perfused rat liver§

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Addition of benzylamine (0.5 mM) to isolated perfused rat liver led to a net release of K⁺ of $10.5 \pm 0.3 \mu\text{mol/g}$, which was accompanied by a decrease in liver mass by $9.3 \pm 0.4\%$ and a decrease of the intracellular water space by $13.7 \pm 0.6\%$, suggestive of hepatocellular shrinkage. Benzylamine had no effect on the perfusion pressure, and there was a close relationship between benzylamine-induced net K⁺ release and the accompanying decrease in liver mass. Benzylamine-induced net K⁺ release was sensitive to inhibition of monoamine oxidase by pargyline and increased with benzylamine flux through monoamine oxidase, suggesting its dependence on intracellular H₂O₂ formation. In line with this, infusion of H₂O₂ (but not of benzaldehyde, the other product of benzylamine metabolism) stimulated net K⁺ release from the liver. However, at a given H₂O₂ load K⁺ release was about 2–3-fold higher when H₂O₂ was generated intracellularly during the oxidation of benzylamine, as compared with exogenously delivered H₂O₂. Inhibition of catalase by 3-amino-

1,2,4-triazole (0.2 mM) significantly increased the benzylamine-induced net K⁺ release as well as the benzylamine-induced release of GSSG into bile, but had no effect on benzylamine oxidation at monoamine oxidase. In the presence of Ba²⁺ (1 mM) or in Ca²⁺-free perfusions, the benzylamine-induced net K⁺ efflux was diminished by 60–70% or about 30%, respectively. This was not explained by the 20–30% decrease in flux through monoamine oxidase observed under these conditions. The results suggest that metabolic generation of H₂O₂ inside the liver leads to a net K⁺ efflux and subsequent hepatocellular shrinkage. Net K⁺ efflux under these conditions is enhanced when catalase is inhibited, suggesting that the rate of both intracellular H₂O₂ generation and degradation can modulate cellular K⁺ balance and cellular volume. The data support the idea that oxidative stress may affect hepatocellular functions also by lowering the hepatocellular hydration state.

INTRODUCTION

Recent studies have shown that the hepatocellular hydration state is one important determinant of hepatocellular function (for review see Häussinger and Lang, 1991). Cellular hydration changes within minutes in perfused rat liver in response to hormones (Hallbrucker et al., 1991; vom Dahl et al., 1991a,b), amino acids, bile acids (Wettstein et al., 1990; Häussinger et al., 1992) and t-butyl hydroperoxide (Hallbrucker et al., 1993), a model compound frequently used to study oxidative stress (Sies, 1985). With respect to protein turnover, an increase in the hepatocellular hydration state apparently acts as an anabolic signal by inhibiting proteolysis and stimulating protein synthesis (Häussinger et al., 1991; Stoll et al., 1992). Conversely, cellular shrinkage acts like a protein-catabolic signal. The relationship between cellular hydration and protein turnover may be of clinical relevance, in view of the finding that a decrease in cellular hydration in skeletal muscle in patients with different severe diseases closely correlates with the extent of negativity of whole-body nitrogen balance (Häussinger et al., 1993). Accordingly, it was hypothesized that cellular shrinkage in skeletal muscle and liver in response to a variety of mediators of inflammation, including oxidative stress, might be one important trigger for the protein-catabolic state observed in critically ill patients (Häussinger et al., 1993). Indeed, experimental oxidative stress induced by infusion of t-butyl hydroperoxide in liver produces hepatocellular shrinkage due to opening of K⁺ channels in the plasma membrane (Sies et al., 1974; Hallbrucker et al., 1993). However, hydroperoxide infusion may be different from oxidative-stress

conditions *in vivo*, because here reactive oxygen species are formed intracellularly rather than being delivered via the portal vein. Thus, when t-butyl hydroperoxide is infused into perfused rat liver, the plasma membrane will be exposed first to the hydroperoxide before intracellular defence systems [for reviews see Sies (1985) and Chance et al. (1979)] such as glutathione peroxidase can come into play, whereas these defence systems could theoretically prevent K⁺-channel opening when the hydroperoxides are generated inside the cells. We therefore studied the effects of intracellularly generated H₂O₂ on hepatocellular K⁺ balance. The results show that endogenous formation of H₂O₂ can trigger cell shrinkage due to net K⁺ release from the liver and that this response depends on defence systems against oxidative stress, such as catalase activity.

MATERIALS AND METHODS

Liver perfusion

Livers from male Wistar rats (200–250 g body mass), fed *ad libitum* on stock diet (Altromin), were perfused as described previously (Sies, 1978) in a non-recirculating manner with bicarbonate-buffered Krebs–Henseleit saline plus L-lactate (2.1 mM), pyruvate (0.3 mM) and glucose (5 mM). In some experiments Ca²⁺ was omitted from the perfusion fluid ('Ca²⁺-free medium'). In experiments with BaCl₂ (1 mM), sulphate was omitted from the perfusion fluid. The perfusate was gassed with O₂/CO₂ (19:1); the temperature was 37 °C. Benzylamine and H₂O₂ were added to the influent perfusate by using precision micropumps.

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§ Dedicated to Professor Dr. G. Paumgartner on the occasion of his 60th birthday.

Determinations

Bile was sampled in 1 min intervals. Bile flow was determined from the secreted bile mass, assuming a specific mass of 1 g/ml. GSSG in bile and perfusate was determined as described by Akerboom and Sies (1981). Ammonia, urea, glutamate and glutamine in effluent perfusate were determined as described in Bergmeyer (1974).

The effluent K^+ concentration was continuously monitored with a K^+ -sensitive electrode (Radiometer, Munich, Germany). Net K^+ uptake or release was determined by planimetry of areas under curves and calibration was done by infusion of known amounts of KCl. When present, a baseline drift was taken into account. Such drifts were linear during the measurement period, amounted less than 0.5 mV/h and were observed in less than 20% of the experiments. The reason for these slight baseline drifts is unclear, but probably involves slow ion leakage from the reference electrode and warming phenomena of the electrodes exposed to the 37 °C perfusate. Corrections for such drifts were made by extrapolation of the slopes of the tracings found before and after benzylamine addition.

Liver cell volume was determined as intracellular water space by using a [^{14}C]urea/[3H]inulin wash-out technique described in detail by vom Dahl et al. (1991c), and liver mass was continuously monitored on a specially constructed balance pan as described by vom Dahl et al. (1991a,c).

The rate of proteolysis was measured as [3H]leucine release from isolated perfused livers of rats, which had been pretreated 16 h before the perfusion experiment by intraperitoneal injection of L-[4,5- 3H]leucine (200 μ Ci). In these experiments the influent perfusate contained in addition 100 μ M unlabelled leucine. For further details of proteolysis measurements see vom Dahl et al. (1991a).

Statistics

Data from different perfusion experiments are given as means $\pm$ S.E.M. (number of experiments).

Materials

Labelled inulin, leucine and urea were from Amersham (Frankfurt, Germany). L-Lactic acid was from Roth (Karlsruhe, Germany). H_2O_2 , benzylamine, pargyline (*N*-methyl-*N*-2-propynylbenzylamine), 3-amino-1,2,4-triazole, glutaminase (grade V) and t-butyl hydroperoxide were from Sigma (Munich, Germany). Fetal-calf serum was from Sebak (Aidenbach, Germany). Other enzymes and coenzymes were from Boehringer (Mannheim, Germany). All other chemicals were from Merck (Darmstadt, Germany).

RESULTS

Effect of extracellular H_2O_2 on cellular K^+ balance

As shown in Figure 1, addition of H_2O_2 (0.2 mM) to isolated perfused rat liver led to a net release of K^+ of $5.2 \pm 0.1 \mu\text{mol/g}$ ($n = 3$), which was completed within $10.4 \pm 0.3 \text{ min}$ and a net K^+ re-uptake of $3.6 \pm 0.2 \mu\text{mol/g}$ ($n = 3$) after removal of H_2O_2 from the influent perfusate. The amount of K^+ released under the influence of H_2O_2 was dependent on the portal H_2O_2 addition (Figure 2). The time course of H_2O_2 -induced net K^+ release from the liver resembled that observed upon infusion of another

hydroperoxide, i.e. t-butyl hydroperoxide, as described recently [compare Figure 1 with Figure 1 of Hallbrucker et al. (1993); see also Figure 9 in Sies et al. (1974)]. However, t-butyl hydroperoxide was more potent than H_2O_2 with respect to net K^+ release from the liver: when infused at a concentration of 0.2 mM, the t-butyl hydroperoxide-induced net K^+ release was $7.2 \pm 0.2 \mu\text{mol/g}$ ($n = 18$), whereas the H_2O_2 -induced net K^+ release was $5.2 \pm 0.1 \mu\text{mol/g}$ ($n = 3$). This difference could be due to a more effective clearance of H_2O_2 in the liver, because H_2O_2 is a substrate for catalase and glutathione peroxidase, whereas t-butyl hydroperoxide is only metabolized by the latter enzyme, but not by catalase (Sies and Summer, 1975; Sies, 1985). Like t-butyl hydroperoxide (0.2 mM) (Hallbrucker et al., 1993), H_2O_2 (0.2 mM) also led to a slight increase in the perfusion pressure, which was $2.0 \pm 0.1 \text{ cm H}_2\text{O}$ after a 10 min period of H_2O_2 infusion. As shown recently (Hallbrucker et al., 1993), the haemodynamic response to t-butyl hydroperoxide infusion, but not its effect on cellular K^+ balance, is explained by a stimulation of eicosanoid formation inside the liver.

Figure 1 Effect of H_2O_2 (0.2 mM) on K^+ balance in perfused rat liver

A representative tracing from a series of 3 similar experiments is shown.

Figure 2 Relationship between the hydroperoxide-induced net K^+ release from the liver and portal H_2O_2 addition

A H_2O_2 addition of 1 $\mu\text{mol/min per g}$ corresponds to an influent concentration of about 0.25 mM. When error bars are given, they refer to means $\pm$ S.E.M. ($n = 4$ different experiments).

Figure 3 Effect of benzylamine (0.5 mM) on K^+ balance in perfused rat liver in the absence (—) and presence (-----) of pargyline (0.1 mM)

Representative tracings from a series of 7 similar experiments are shown.

Effects of benzylamine on cellular K^+ balance and cell volume

In order to assess whether intracellularly generated H_2O_2 is also capable of stimulating net K^+ release from the liver, experiments with benzylamine were performed. Benzylamine is metabolized in liver at monoamine oxidase, yielding H_2O_2 , ammonia and benzaldehyde as reaction products (Oshino and Chance, 1977; Akerboom et al., 1981, 1982). As shown in Figure 3, infusion of benzylamine (0.5 mM) led to a net K^+ release ($10.5 \pm 0.3 \mu\text{mol/g}$; $n = 7$), followed by slow re-uptake of K^+ ($8.2 \pm 0.2 \mu\text{mol/g}$; $n = 4$) after cessation of the benzylamine infusion. However, when pargyline (0.2 mM), an inhibitor of monoamine oxidase, was present, the benzylamine-induced net K^+ release from the liver was inhibited by more than 95% (Figure 3, Table 1). Pargyline (0.1 mM) itself was without effect on cellular K^+ balance and did not affect H_2O_2 -induced net K^+ release: in the presence or the absence of pargyline (0.1 mM) the net K^+ release induced by infused H_2O_2 (0.2 mM) was 5.7 ± 0.2 or $5.2 \pm 0.1 \mu\text{mol/g}$ ($n = 3$) respectively. Thus, benzylamine oxidation by monoamine oxidase is apparently required for its effect on cellular K^+ balance. Because infusion of benzaldehyde (0.2 mM) or of NH_4Cl (at concentrations below 0.5 mM) had no significant effects on hepatocellular K^+ balance, whereas H_2O_2

induced marked K^+ release (Figures 1 and 2), the net K^+ release from the liver under the influence of benzylamine (0.5 mM) is probably due to intracellular H_2O_2 formation. In contrast with infused H_2O_2 or t-butyl hydroperoxide, benzylamine (0.5 mM) had no effect on the perfusion pressure (results not shown). Benzylamine (0.5 mM) only slightly affected cell viability; lactate dehydrogenase activity increased from 0.7 ± 0.1 to 3.5 ± 0.4 units/l (both $n = 3$) after a 20 min period of benzylamine infusion. In the presence of pargyline, lactate dehydrogenase activity in the effluent increased from 0.9 ± 0.2 to 1.7 ± 0.5 units/l (both $n = 3$) after a 20 min period of benzylamine infusion (0.5 mM).

Benzylamine-induced net K^+ release from the liver was accompanied by hepatocellular shrinkage. After a 30 min period of benzylamine (0.5 mM) infusion, i.e. after completion of K^+ efflux (Figure 3) the intracellular water space decreased by $81 \pm 5 \mu\text{l/g}$ of liver ($n = 4$), equivalent to a cell-volume decrease of $13.7 \pm 0.6\%$. In addition, benzylamine led to a decrease of liver mass, which paralleled the extent of benzylamine-induced net K^+ release (Figure 4).

Benzylamine-induced oxidative stress and net K^+ efflux from perfused rat liver

Because monoamine oxidase generates H_2O_2 and ammonia from benzylamine in equimolar amounts, changes of the nitrogen balance across the liver under the influence of benzylamine can be used to estimate the rate of H_2O_2 production. Here, the ammonia formed from benzylamine may either be washed out as such or be converted into urea, glutamate or glutamine. Thus the increase in ammonia plus urea-, glutamate- and glutamine-nitrogen release ('extra nitrogen release') from perfused rat liver upon addition of benzylamine was taken as a parameter to monitor benzylamine flux through monoamine oxidase and consequently to estimate the rate of benzylamine-induced H_2O_2 formation inside the liver, an approach previously used by others (Akerboom et al., 1982). This approach to estimating benzylamine flux through monoamine oxidase, however, relies on the assumption that benzylamine does not significantly affect the basal rate of proteolysis. Hepatic proteolysis was previously shown to be controlled by cell volume (Häussinger et al., 1990b,

Table 1 Effects of pargyline (0.1 mM), 3-amino-1,2,4-triazole (0.2 mM), Ba^{2+} (1 mM) and Ca^{2+} -free perfusion on benzylamine-induced net K^+ release and flux through monoamine oxidase

Benzylamine was infused for 20 min and the resulting net K^+ release was determined as the area under the curve as described in the Materials and methods section. Pargyline (0.1 mM) and 3-amino-1,2,4-triazole (0.2 mM) infusion was started 15 min before benzylamine addition. Both compounds had no effect on cellular K^+ balance. Benzylamine flux through monoamine oxidase was determined as benzylamine-induced increase in urea- plus ammonia- plus glutamate- plus glutamine-nitrogen release from the liver (for details see the text). Pargyline, 3-amino-1,2,4-triazole, Ba^{2+} or Ca^{2+} -free perfusions had no major effects on nitrogen release (urea- plus ammonia- plus glutamate- plus glutamine-nitrogen) from the liver in the absence of benzylamine; these rates were 0.55 ± 0.04 ($n = 3$), 0.58 ± 0.03 ($n = 14$), 0.53 ± 0.02 ($n = 5$) and 0.46 ± 0.02 ($n = 4$) $\mu\text{mol/min per g}$ respectively, and compare with a nitrogen release of 0.53 ± 0.02 ($n = 16$) $\mu\text{mol/min per g}$ in control perfusions (i.e. in the absence of pargyline, 3-amino-1,2,4-triazole, Ba^{2+} and with Ca^{2+} present at 1.25 mM). GSSG release into bile in the absence of benzylamine was 1.1 ± 0.1 nmol/min per g ($n = 13$); this value was 1.1 ± 0.1 and 1.9 ± 0.1 nmol/min per g (both $n = 4$) in the presence of aminotriazole (0.2 mM) and pargyline (0.1 mM) respectively. Data were obtained during metabolic steady states, which were achieved with respect to nitrogen release into the perfusate and GSSG release into bile within 10 min of benzylamine addition. Data are given as means $\pm$ S.E.M. (n): n.d., not determined.

Condition	Net K^+ release ($\mu\text{mol/g}$)	Benzylamine-induced monoamine oxidase flux ($\mu\text{mol/min per g}$)	Increase in GSSG release into bile (nmol/min per g)
Benzylamine (0.5 mM)	10.5 ± 0.3 (7)	0.48 ± 0.02 (4)	1.9 ± 0.1 (5)
+ pargyline	0.3 ± 0.1 (4)	-0.01 ± 0.00 (3)	0.1 ± 0.0 (4)
+ 3-amino-1,2,4-triazole	15.7 ± 0.5 (6)	0.50 ± 0.02 (4)	2.9 ± 0.1 (4)
+ $BaCl_2$	3.8 ± 0.1 (7)	0.34 ± 0.01 (5)	n.d.
Ca^{2+} -free	7.0 ± 0.2 (6)	0.37 ± 0.03 (4)	n.d.
Benzylamine (0.25 mM)	9.0 ± 1.2 (5)	0.29 ± 0.02 (5)	n.d.

Figure 4 Relationship between benzylamine-induced net K⁺ release and liver mass decrease

The benzylamine concentration in influent was varied from 20 μM to 1 mM in the absence (●) or presence (○) of aminotriazole (0.2 mM), and the corresponding net K⁺ release was compared with the simultaneously occurring decrease in liver mass. Aminotriazole enhances the benzylamine-induced net K⁺ release (see Figure 6), but the relationship between K⁺ release and mass decrease is maintained. (□) refers to the benzylamine (0.5 mM) effect in Ca²⁺-free perfusions. When error bars are shown, data are given as means ± S.E.M. ($n = 3-6$).

Figure 5 Effect of benzylamine addition on benzylamine-induced increase of nitrogen release in the absence (●) or presence (○) of 3-amino-1,2,4-triazole

Extra nitrogen release refers to the increase of urea- plus glutamine- plus glutamate-nitrogen plus ammonia release from the liver under the influence of benzylamine ('extra nitrogen release'). Extra nitrogen release under the influence of benzylamine reached a steady state within 10 min of benzylamine infusion and was determined between 15–20 min of benzylamine addition. A benzylamine addition of 1 μmol/min per g corresponds to an influent concentration of about 0.25 mM. When error bars are given the data refer to means ± S.E.M. ($n = 3-7$).

1991; vom Dahl et al., 1991a): hyperosmotic cell shrinkage stimulates proteolysis, but only when the pathway is preinhibited (vom Dahl et al., 1991a). In amino acid-free liver perfusions, proteolysis is known to be maximally active (Mortimore and Pösö, 1987) and cannot be further enhanced by hyperosmotic cell shrinkage when assessed as [³H]leucine release from perfused

Figure 6 Effect of benzylamine addition on benzylamine-induced net K⁺ release in the absence (●) or presence (○) of 3-amino-1,2,4-triazole

A benzylamine addition of 1 μmol/min per g corresponds to an influent concentration of about 0.25 mM. When error bars are given, the data refer to means ± S.E.M. ($n = 3-7$).

livers which had been prelabelled *in vivo* 16 h before the perfusion experiment (vom Dahl et al., 1991a). From this, the benzylamine-induced cell shrinkage is not expected to increase the proteolytic rate in our experiments and the benzylamine-induced alterations of nitrogen balance across the liver should reflect benzylamine metabolism only. This was indeed confirmed in a separate series of experiments, in which the influence of benzylamine (0.5 mM) was measured on proteolysis, the latter being determined as described recently (vom Dahl et al., 1991a) as [³H]leucine release from perfused livers which were prelabelled *in vivo* 16 h before the perfusion experiment by intraperitoneal injection of [³H]leucine. Within 80 min of perfusion, ³H label release into the effluent reached a steady state, which was maintained over the next 90 min (vom Dahl et al., 1991a). This rate of [³H]leucine release was set to 100%. Further addition of benzylamine (0.5 mM) for 20 min decreased ³H release by 9 ± 1% ($n = 3$). The reason for this slight inhibition of proteolysis by benzylamine, despite accompanying cell shrinkage, is unclear, but may relate to the formation of ammonia from benzylamine, which itself is known to be a potent inhibitor of proteolysis (Seglen, 1977). However, these data augment the view that the alterations of nitrogen balance across the perfused livers under the influence of benzylamine will largely reflect the rate of monoamine oxidase flux and are not significantly affected by effects on proteolysis.

As expected, inhibition of monoamine oxidase by pargyline (0.1 mM) fully abolished the extra nitrogen release after infusion of benzylamine (0.5 mM) (Table 1). As shown in Figure 5, extra nitrogen release from the liver under the influence of benzylamine was a saturable process, which paralleled the extent of benzylamine-induced net K⁺ efflux (Figure 6). There was a close correlation between H₂O₂ formation from benzylamine (assessed as extra nitrogen release) and the accompanying net K⁺ release (Figure 7).

H₂O₂ generated intracellularly during oxidation of benzylamine is metabolized by both catalase and glutathione peroxidase (Sies et al., 1973; Oshino and Chance, 1977; Chance et al., 1979; Akerboom et al., 1982). Involvement of the latter enzyme during metabolism of benzylamine in perfused liver was suggested by the increase in GSSG release into bile, from 1.0 ± 0.1 to 2.9 ± 0.1 nmol/min per g (both $n = 5$) upon addition of benzylamine (0.5 mM), as previously described by others (Oshino

Figure 7 Relationship between benzylamine flux through monoamine oxidase and net K^+ release from the liver in absence (●) and presence (○) of 3-amino-1,2,4-triazole (0.2 mM)

Flux of benzylamine through monoamine oxidase was assessed as extra nitrogen release from the liver after infusion of benzylamine (see legend to Figure 5). 3-Amino-1,2,4-triazole (0.2 mM) infusion was started 15 min before the addition of benzylamine; 3-amino-1,2,4-triazole itself had no effect on cellular K^+ balance and urea, glutamine, glutamate or ammonia release from the liver (results not shown). Monoamine oxidase flux was varied by infusing different concentrations of benzylamine (range 0.05–1.0 mM). When error bars are given, the data refer to means $\pm$ S.E.M. ($n = 3$ –7).

and Chance, 1977; Akerboom et al., 1982). When catalase was inhibited by aminotriazole (0.2 mM) (Margoliash et al., 1960), the benzylamine-induced net K^+ efflux was enhanced significantly (Figure 6, Table 1). Aminotriazole (0.2 mM) itself had no effect on hepatocellular K^+ balance or perfusion pressure, nor did 3-amino-1,2,4-triazole affect GSSG release into bile, which was 1.12 ± 0.03 and 1.07 ± 0.06 nmol/min per g (both $n = 4$) in the absence and presence of 3-amino-1,2,4-triazole (0.2 mM) respectively. Further, extra nitrogen release after addition of benzylamine was not affected by 3-amino-1,2,4-triazole (Figure 5, Table 1), indicating that this compound did not interfere with benzylamine oxidation by monoamine oxidase. Regardless of whether aminotriazole was present or absent, the correlation between benzylamine-induced net K^+ release and the decrease in liver mass was maintained (Figure 4). However, in the presence of aminotriazole, GSSG release into bile under the influence of benzylamine (0.5 mM) was significantly higher than in its absence (Figure 8), which might indicate that inhibition of catalase by this compound increased H_2O_2 metabolism at glutathione peroxidase. On the other hand, pargyline (0.1 mM) itself increased GSSG release into bile from 1.03 ± 0.07 to 1.94 ± 0.04 nmol/min per g (both $n = 4$); however, addition of benzylamine (0.5 mM) during continuous infusion of pargyline did not further increase GSSG release into bile [i.e. GSSG release increased upon addition of benzylamine from 1.94 ± 0.04 to only 2.05 ± 0.05 nmol/min per g (both $n = 4$)], in line with its inhibitory effect on monoamine oxidase (Table 1). Under all conditions tested, benzylamine did not induce a detectable GSSG release into the perfusate (results not shown).

Figure 8 Effects of pargyline (0.1 mM) (□) and 3-amino-1,2,4-triazole (0.2 mM) (○) on the benzylamine (0.5 mM)-induced GSSG release into bile

Control (●) refers to benzylamine-induced GSSG release into bile in the absence of pargyline or 3-amino-1,2,4-triazole. In all experiments, GSSG release into the perfusate was not detectable. For pargyline (□) or 3-amino-1,2,4-triazole (○), infusion of these compounds was started 15 min before the infusion of benzylamine (0.5 mM). Data are given as means $\pm$ S.E.M. ($n = 3$ for each condition).

Characterization of benzylamine-induced net K^+ efflux

As shown in Table 1, benzylamine flux through monoamine oxidase decreased by about 25% in the presence of $BaCl_2$ (1 mM) or in Ca^{2+} -free perfusions, whereas simultaneously the benzylamine-induced net K^+ efflux was inhibited by 60–70% and by about 30%, respectively. This inhibition of benzylamine-induced net K^+ release from the liver by Ba^{2+} or in Ca^{2+} -free perfusions was not explained by the 25% inhibition of H_2O_2 generation by monoamine oxidase under these conditions, because in control perfusions (Ba^{2+} absent, $[Ca^{2+}] = 1.25$ mM) variation of benzylamine flux through monoamine oxidase between 0.35 and $0.5 \mu\text{mol/min per g}$ had little effect on the benzylamine-induced net K^+ release (Figure 7). In fact, lowering of the influent benzylamine concentration from 0.5 to 0.25 mM decreased monoamine oxidase flux from 0.48 ± 0.02 ($n = 4$) to $0.29 \pm 0.02 \mu\text{mol/min per g}$ ($n = 5$), but lowered net K^+ release only from 10.5 ± 0.3 ($n = 7$) to $9.0 \pm 1.2 \mu\text{mol/g}$ ($n = 5$) (Figure 7; see also Table 1). Thus benzylamine-induced net K^+ release is sensitive to inhibition by Ba^{2+} and to a smaller extent is dependent on extracellular Ca^{2+} . The sensitivity of benzylamine-induced net K^+ efflux to Ba^{2+} and Ca^{2+} -free perfusions is similar to that recently described for *t*-butyl hydroperoxide-induced net K^+ efflux from perfused rat liver (Hallbrucker et al., 1993), but differs markedly from that observed in response to hypo-osmotic liver exposure (Häussinger et al., 1990a).

In the presence of Ba^{2+} , benzylamine led to a slight increase of portal pressure, by $3.2 \pm 0.8 \text{ cmH}_2\text{O}$ ($n = 5$), whereas no pressure effects were exerted by the monoamine under all other conditions employed. Vasoconstriction by benzylamine in the presence of Ba^{2+} may explain why benzylamine (0.5 mM) led to a decrease in liver mass of $7.1 \pm 0.3\%$ ($n = 3$) under these conditions, although the relationship depicted in Figure 4 would predict only a 3% mass decrease in response to a benzylamine (0.5 mM)-induced net K^+ efflux of $3.8 \mu\text{mol/g}$, as occurs in the presence of Ba^{2+} .

DISCUSSION

Benzylamine-Induced K^+ release

The data in this paper show that benzylamine leads to a marked net K^+ efflux from the liver, which apparently requires flux of benzylamine through monoamine oxidase, because inhibition of the enzyme by pargyline blocked the benzylamine effect on cellular K^+ balance (Figure 3, Table 1). Intracellular H_2O_2 production from benzylamine seems causally related to the net K^+ efflux, because (i) qualitatively similar effects were achieved by infusion of H_2O_2 (Figures 1 and 2), (ii) benzaldehyde and ammonia (at concentrations below 0.5 mM) had no effect on cellular K^+ balance, and (iii) inhibition of H_2O_2 degradation by catalase by 3-amino-1,2,4-triazole markedly enhanced the K^+ release in response to infused benzylamine (Figures 6 and 7, Table 1). These findings extend previous notions that oxidative stress exerts influence on ion-transport systems in the plasma membrane (Sies et al., 1974; Saha et al., 1992; Elliott and Schilling, 1992; Ruppertsberg et al., 1991; Hallbrucker et al., 1993) by demonstrating that cellular ion balance is affected also when H_2O_2 is generated metabolically inside the liver cells. It was recently shown that hydroperoxides such as t-butyl hydroperoxide induce net K^+ release from the liver by opening K^+ channels in the plasma membrane (Hallbrucker et al., 1993). Thus, regarding the mechanism of benzylamine-induced net K^+ efflux, it is well conceivable that K^+ -channel opening under the influence of H_2O_2 generated from benzylamine is at least involved in triggering the net K^+ release. This view is also augmented by the similar sensitivity of t-butyl hydroperoxide-induced and benzylamine-induced net K^+ release to Ba^{2+} and Ca^{2+} -free perfusions (Table 1; compare with data given by Hallbrucker et al., 1993). Oxidative stress was repeatedly shown to induce an intracellular Ca^{2+} signal (Bellomo et al., 1982, 1987; Sies et al., 1984). The sensitivity of benzylamine-induced net K^+ efflux from the liver to Ba^{2+} and Ca^{2+} -free perfusions (Table 1) may suggest that intracellular Ca^{2+} is involved in mediating the K^+ efflux (probably by K^+ -channel opening) in response to H_2O_2 generated intracellularly during benzylamine oxidation. In this respect, it is noteworthy that Ca^{2+} -activated K^+ channels have been described in rat hepatocytes (Baer and Petersen, 1987). Alternatively, modification of critical thiol groups of the K^+ -channel protein could produce the K^+ -channel activation, as suggested by recent patch-clamp studies (Ruppertsberg et al., 1991) and the demonstration that net K^+ efflux from the liver under the influence of t-butyl hydroperoxide parallels GSSG release from the liver (Hallbrucker et al., 1993). However, as shown in Table 1, a benzylamine-induced net K^+ efflux of about $10 \mu\text{mol/g}$ is accompanied by only a 2–3-fold increase in GSSG release from the liver, whereas an about 40-fold increase in GSSG release was found when a comparable net K^+ release occurred under the influence of exogenous t-butyl hydroperoxide (Hallbrucker et al., 1993).

It was recently shown that hyperosmotic cell shrinkage increases basal and t-butyl hydroperoxide-stimulated GSSG release into bile, probably in part due to a shrinkage-induced inhibition of NADPH generation via the pentose phosphate shunt (Saha et al., 1992). Thus, both benzylamine-induced H_2O_2 formation and cell shrinkage may contribute to the increased GSSG release into bile.

Possible physiological significance

The present study suggests that regulation of cellular K^+ balance and probably K^+ -channel activity by intracellular hydroperoxides

may have relevance *in vivo*. It even appears that H_2O_2 generated by monoamine oxidase is more effective in releasing K^+ than are exogenous hydroperoxides. A benzylamine (0.5 mM)-induced net K^+ efflux of about $10 \mu\text{mol/g}$ (Table 1) occurs when the rate of intracellular H_2O_2 generation is $0.4\text{--}0.5 \mu\text{mol/min per g}$ (as assessed by the change in nitrogen balance), but, in order to achieve a comparable net K^+ efflux by infusion of H_2O_2 , a portal H_2O_2 load about twice as high is required (compare Figures 2 and 7). One explanation for this could be that more hepatocytes in the liver acinus are exposed to H_2O_2 when it is generated inside the hepatocytes, whereas during H_2O_2 infusion only upstream, but not downstream, hepatocytes are exposed, due to efficient hydroperoxide clearance already by upstream cells. Many H_2O_2 -generating reactions in liver are known (for review, see Chance et al., 1979), and it is likely that H_2O_2 generated at different subcellular sites may differ in its potency to induce net K^+ release, because differences may exist in the accessibility to either catalase or glutathione peroxidase. Clearly, the activity of defence systems against oxidative stress modulates the K^+ depletion under the influence of intracellularly generated H_2O_2 , as suggested by the experiments with the catalase inhibitor 3-amino-1,2,4-triazole (Table 1, Figures 6 and 7). Both pargyline and 3-amino-1,2,4-triazole itself had no effect on cellular K^+ balance, indicating that endogenous H_2O_2 formation in perfused liver in the absence of added benzylamine occurs at a rate which does not measurably affect plasma-membrane K^+ -channel activity.

The present study suggests that the intracellular balance between hydroperoxide formation and consumption may be a critical parameter which determines cellular K^+ balance and the hepatocellular hydration state (Figure 4). Oxidative stress, as it occurs during inflammation, could therefore induce cellular shrinkage and trigger some metabolic responses of the cell. This could, for example, contribute to the protein catabolic state observed during inflammation. In line with this would be the recent observation that a loss of skeletal-muscle cell hydration occurs in humans with different severe diseases, the extent of which correlates closely with the negativity of whole-body nitrogen balance in these patients (Häussinger et al., 1993). This suggestion may appear at first glance not to be compatible with the finding in the present paper that benzylamine-induced cell shrinkage slightly inhibited, but did not enhance, liver proteolysis. However, one has to keep in mind that the experiments reported here were carried out in the absence of known inhibitors of proteolysis (e.g. amino acids, insulin), which renders proteolysis insensitive to cell shrinkage (vom Dahl et al., 1991a) and that oxidative stress due to benzylamine metabolism is also accompanied by the formation of ammonia, which itself is known to be a potent inhibitor of proteolysis (Seglen, 1977).

N.S. is the recipient of a Fellowship from the Deutsche Akademische Austauschdienst. Expert technical assistance was provided by Mrs. C. Möcklin. This study was supported by the Deutsche Forschungsgemeinschaft through Sonderforschungsbereich 154 and the Gottfried-Wilhelm-Leibniz-Prize, the Fonds der Chemischen Industrie and the Schilling Stiftung.

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