Pergamon

Biochemical Pharmacology, Vol. 48, No. 9, pp. 1683–1692, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00341-6

ROLE OF THIOL HOMEOSTASIS AND ADENINE NUCLEOTIDE METABOLISM IN THE PROTECTIVE EFFECTS OF FRUCTOSE IN QUINONE-INDUCED CYTOTOXICITY IN RAT HEPATOCYTES

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(*Received* 21 *April* 1994; *accepted* 26 *July* 1994)

Abstract—Freshly-isolated rat hepatocytes were exposed in glucose (15 mM) or fructose (5 mM) medium to menadione (2-methyl-1,4-naphthoquinone) (85 μM) or 1,4-naphthoquinone (NQ) (50 μM). Menadione and NQ are closely related quinones and have an approximately equal potential to induce redox cycling. However, NQ has a higher potential to arylate and is more toxic than menadione. During 2 hr of incubation, cell viability, thiol status, adenine nucleotide level and lactate production were determined. LDH-leakage was used as a measure of cell viability. In glucose medium, exposure of hepatocytes to menadione or NQ resulted in a faster excretion rate of oxidized glutathione as compared to those cells in fructose medium. As a result, quinone-exposed hepatocytes in fructose medium retained higher amounts of oxidized glutathione. Menadione-exposed hepatocytes in fructose medium exhibited a diminished rate of transthiolation of protein thiols with oxidized glutathione as compared to those cells in glucose medium. The adenine nucleotide level of hepatocytes in glucose medium was markedly higher than in fructose medium. This was caused by an ATP decrease in hepatocytes in fructose medium resulting in a low energy charge (E.C.) (0.6) as compared to hepatocytes in glucose medium (0.9). Only menadione caused a decrease in the E.C. in glucose medium while NQ caused a decrease of all three adenine nucleotides. In fructose medium, quinone-exposed hepatocytes showed no change in their adenine nucleotides as compared to control cells. Despite the higher oxidized glutathione content and the lower ATP level of NQ-exposed hepatocytes in fructose medium, they had a better viability than those cells in glucose medium. From our results we conclude that a high ATP content is not always beneficial for cell survival.

Key words: menadione; cytotoxicity; rat hepatocytes; fructose; protection

Fructose has been shown to protect hepatocytes against toxic insults (e.g. paracetamol [1], nitrofurantoin [2], menadione, cyanide, *t*-butyl hydroperoxide and cystamine [3]) as well as against other sources of damage (e.g. anoxia [4] and hypoxia [5, 6]). However, fructose does not protect hepatocytes against $HgCl_2$ which binds covalently to thiol groups [3]. GAPDH‡ (EC 1.2.1.12), a key enzyme in glycolysis, can partially be inhibited by thiol oxidizing agents (e.g. H_2O_2, menadione) [7, 8]. On the other hand, iodoacetic acid, a compound known to alkylate thiol groups, is able to completely abolish GAPDH activity [9]. From these results it can be concluded that glycolysis is more sensitive to alkylating agents than thiol oxidizing agents. In hepatocytes, the phosphorylation rate is higher for fructose than for glucose. Therefore, it is thought that fructose protects hepatocytes against cell death under stressful conditions by providing glycolysis-derived ATP [1, 3, 5, 6]. However, this is in contradiction with the use of fructose to deplete ATP in hepatocytes [10, 11].

Menadione (2-methyl-1,4-naphthoquinone), a well-studied quinone, is capable of both oxidizing and arylating cellular thiols and is thought to cause its cytotoxicity by disturbing energy metabolism [8, 12, 13]. Redegeld *et al.* [12] showed that menadione causes a decrease in the energy charge (E.C.) $[(ATP + \frac{1}{2}ADP)/(AMP + ADP + ATP)]$ [14] prior to the onset of the loss of cell viability and that intermediates of energy metabolism are able to delay the onset of cell death [12]. Quinones also have the potency to transport electrons and therefore might interfere with oxidative phosphorylation as well. De Haan and Charles [15] have shown that the reduced form of menadione, menadiol, is able to donate electrons to complex II, thereby uncoupling oxidative phosphorylation. In previous experiments we compared the cytotoxic effects of NQ and menadione in freshly-isolated rat hepatocytes. These two quinones are closely related but NQ has a higher

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‡ Abbreviations: GSSG, oxidized glutathione; GSH, reduced glutathione; NQ, 1,4-naphthoquinone; LDH, lactate dehydrogenase; E.C., energy charge; mBBr, monobromobimane; DTT, dithiothreitol; TBA, tetrabutylammonium hydroxide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

capacity to arylate and causes a faster onset of cell death in shaking cultures of rat hepatocytes than menadione [16]. This higher cytotoxicity was shown to be accompanied by a larger decrease in the E.C. in NQ-exposed cells as compared to menadione-exposed hepatocytes [17]. Because of its higher arylation capacity, NQ might be a more potent inhibitor of glycolysis than menadione and fructose might be unable to protect hepatocytes against NQ.

Silva *et al.* [2] provided evidence for the hypothesis that fructose protects hepatocytes against nitrofurantoin toxicity by changing glutathione metabolism. They found that nitrofurantoin-exposed hepatocytes in fructose medium retained higher amounts of GSH as compared to those cells in glucose medium. They suggested that fructose protects by lowering GSSG excretion, thereby permitting the hepatocytes to reduce GSSG and counteract oxidative stress. Moreover, fructose might favour NADPH production since it lowers the ATP/ADP ratio [10] which activates the two dehydrogenases in the pentose phosphate pathway [18, 19]. In addition, the shift in thiol-disulphide equilibrium towards disulphides during quinone metabolism favours the hexose monophosphate shunt [20, 21]. As a result, the cell is provided with NADPH to counteract oxidative stress.

In order to study the role of cellular energy metabolism and the relative importance of arylation and redox cycling in quinone toxicity, we exposed freshly-isolated rat hepatocytes in fructose medium or glucose medium to menadione or NQ. We compared the changes in cell viability, thiol status and adenine nucleotide levels. Our results show that fructose protected the hepatocytes against cell death induced by NQ and that this protection was accompanied by a lower ATP content of hepatocytes in fructose medium. Furthermore, there was a greater amount of GSSG of quinone-exposed cells in fructose medium than in glucose medium.

MATERIALS AND METHODS

Materials

NQ, menadione and tetrabutylammonium-hydroxide were purchased from Janssen Chimica (Beerse, Belgium). Dithionitrobenzene was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). HEPES, BSA, Triton X-100, EDTA, DTT, glutathione and NADH were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Collagenase B was from Boehringer (Mannheim, F.R.G.). All other chemicals were of analytical grade.

Animals

Male Hannover Wistar rats (Han: WIST, 190–300 g) were purchased from Harlan CPB (Zeist, The Netherlands). They were fed *ad libitum* a TNO-Institute grain-based open-formula diet (TNO-Toxicology and Nutrition Institute, Zeist, The Netherlands) and had free access to drinking water. Rat hepatocytes were isolated using the two-step collagenase perfusion technique described by Seglen [22] as modified by Paine *et al.* [23].

Incubations

The experiments were performed in shaking 25 mL erlenmeyer flasks (9 mL cell suspension per flask, 10^6 cells/mL) under an atmosphere of 95% O_2/5% CO_2. Incubations were performed in a Krebs–Henseleit buffer supplemented with 20 mM HEPES, 0.2% BSA, 15 mM glucose or 5 mM fructose, pH 7.4 at 37°. After 15 min of preincubating the hepatocytes, substrates were added in DMSO (0.4% of final volume).

Biochemical assays

The percentage leakage of lactate dehydrogenase (LDH, EC 1.1.1.27) in the medium was used as an indication of cell viability. LDH activity was measured according to Bergmeyer *et al.* [24].

Protein thiol groups were determined using Ellman's reagent [25] according to Sedlak & Lindsay [26] as modified by Albano *et al.* [27]. In short: for the determinations, 3 mL of the cell suspension were centrifuged (3 min, 250 *g*), the supernatant removed and 400 μL of 3.5% (w/v) $HClO_4$ added to the cell pellet. After centrifugation (5 min, 1500 *g*) the supernatant was removed and used for the determination of the adenine nucleotides. The cell pellet was washed twice with 1 mL 5% TCA, and re-suspended in PBS/EDTA(5 mM)/SDS(0.5% w/v). In order to clarify the suspension, 1 or 2 drops of 1 N NaOH were added. After this procedure, samples were treated according to Albano *et al.* [27].

Protein was determined according to Lowry *et al.* [28] as modified by Rutten *et al.* [29].

GSH, GSSG and mixed disulphides were determined by HPLC after derivatization with monobromobimane (mBBr) essentially as described by Cotgreave & Moldéus [30], Fahey *et al.* [31] and Newton *et al.* [32], with some modifications. In short:

Glutathione. Samples of 100 μL cell suspension were taken and centrifuged (3 min, 250 *g*), supernatant was removed and the pellet resuspended in 100 μL Krebs–Henseleit medium (—BSA). Glutathione was derivatized by adding 100 μL 2 mM mBBr dissolved in 50 mM *N*-ethylmorfoline and incubating this mixture for 5 min in the dark. After addition of 25 μL 40% TCA, protein was removed by centrifugation (5 min, 1500 *g*). The supernatant was stored at −20° until use.

Total intracellular glutathione. Samples were treated the same as for glutathione except for the reducing step: after resuspension in 100 μL Krebs–Henseleit medium (—BSA), 5 μL 25 mM DTT was added and this mixture was left shaking for 30 min at room temperature. For derivatization, 5 mM mBBr was used.

Total extracellular glutathione. Samples of 100 μL cell suspension were taken and centrifuged (3 min, 250 *g*) and the supernatant was reduced with 5 μL 25 mM DTT for 30 min, shaking at room temperature. After reduction, samples were treated the same as for glutathione. For derivatization, 5 mM mBBr was used.

Formation of mixed disulphides. For the determination of mixed disulphides, 200 μL cell suspension was centrifuged (3 min, 250 *g*) and the pellet was resuspended in 100 μL Krebs–Henseleit buffer

(—BSA). 25 μL 40% TCA was added, mixed and centrifuged (5 min, 1500 *g*). The pellet was washed twice with 1 mL 5% TCA and resuspended in 100 μL PBS + 1% SDS. This mixture was neutralized by adding 5 μL saturated $NaHCO_3$ and reduced with 5 μL 25 mM DTT for 60 min at room temperature by shaking vigorously. Then the mixture was derivatized with 5 mM mBBr and treated in the same way as glutathione.

Glutathione derivatives were determined on the HPLC using a Chromsep C18 RP column (200 × 3 mm, 1 × i.d., Chrompack, Middelburg, The Netherlands) at 35° and a flow rate of 1 mL/min. Buffer A: 128 mL methanol and 2.5 mL acetic acid diluted to 1 L with glass distilled water, adjusted to pH 3.9 with 4 N NaOH; buffer B: 900 mL methanol and 2.5 mL acetic acid, diluted to 1 L with glass distilled water. Glutathione-monobromobimane derivatives were eluted using a linear gradient from 0% B at 0 to 7 min, to 50% B from 8 to 17 min. Samples of 20 μL were injected and column effluents were detected at $\lambda_{ex} = 385$ nm, $\lambda_{em} = 480$ nm. Typical retention time for the conjugate was 4 min.

Adenine nucleotides

Intracellular ATP, ADP and AMP levels were determined using HPLC. From the supernatant used (see protein thiol groups), 350 μL were neutralized with 650 μL K_2HPO_4(0.8 M). Throughout the procedure, samples were kept on ice. After 15 min, the insoluble potassium perchlorate was removed by centrifugation (5 min, 1500 *g*). Samples were stored at −20° until use.

A Chromsep C18 RP column (200 × 3 mm, 1 × i.d., Chrompack, Middelburg, The Netherlands) was used for separations: buffer A: 50 mM $(NH_4)H_2PO_4$, 25 mM TBA; buffer B: 200 mM $(NH_4)H_2PO_4$, 25 mM TBA. Buffer A and Buffer B were both adjusted to pH 3.9 with H_3PO_4 and filtered through a 0.45 μm Millipore filter. The nucleotides were eluted using a linear gradient from 0% B at 1 min to 100% B at 16 min, followed by 100% B from 16 to 35 min at a flow rate of 1 mL/min. Samples of 20 μL were injected and column effluents were monitored at 260 nm. Typical retention times (min): AMP, 4.5; ADP, 14.5; ATP, 27.5.

Lactate

For lactate determination, 250 μL cell suspension were taken and mixed with 250 μL PBS/EDTA(0.05 mM)/Triton(0.05% w/v). The samples were shaken vigorously for 5 min and deproteinized with 7.5 μL 35% $HClO_4$. After mixing and centrifuging (5 min, 1500 *g*), 450 μL of the supernatant were neutralized with 9 μL 5 M NaOH. Neutralized samples were centrifuged for 5 min at 1500 *g* and lactate was determined in the supernatant as described by Gawehn and Bergmeyer [33].

RESULTS

Viability

During incubation, the viability of control cells remained high in both glucose and fructose medium (Fig. 1). Cells exposed to menadione started to lose viability (22% LDH leakage at $t = 2$ hr) when exposed in glucose medium but not in fructose medium. NQ in glucose medium caused a slow, progressive loss of cell viability resulting in 44% LDH leakage after 2 hr. In fructose medium this leakage was only 28%.

Thiol status

Protein thiols were depleted by NQ up to 58% within 5 min, whereas there was a more gradual decrease in time, from 14% after 5 min up to 40% at 2 hr, after incubation of the cells with menadione (Fig. 2). This decrease in protein thiols induced by both quinones was not influenced by the carbohydrate source in the medium.

The total glutathione content—the sum of GSH and GSSG—of hepatocytes exposed to menadione or NQ decreased progressively for 2 hr (Fig. 3a). Cells exposed to menadione in glucose medium as compared to fructose medium showed a faster loss of glutathione content. This effect was not seen for NQ.

Depletion of GSH was almost complete after 5 min irrespective of quinone or medium used (Fig. 3b).

The initial formation of mixed disulphides (Fig. 3c) was higher for menadione than NQ. Furthermore, the menadione-induced formation was higher in glucose medium than fructose medium. NQ-induced formation of mixed disulphides was the same in glucose and fructose media. In Fig. 4, the different forms of glutathione after 5 min (a) and 120 min (b) are represented as stacked bar diagrams. The amount of GSSG was calculated by subtracting the amount of mixed disulphides and GSH from the total amount of glutathione. Hepatocytes exposed to menadione in fructose medium contained more oxidized glutathione as compared to menadione-exposed hepatocytes in glucose medium. Fructose had no effect on NQ-induced accumulation of oxidized glutathione and formation of mixed disulphides. The accumulation of oxidized glutathione was equal to that caused by menadione in fructose medium. Much of the oxidized glutathione was excreted by the hepatocytes as seen in Fig. 4. Hepatocytes exposed to quinones excreted more glutathione than control cells. Furthermore, the excretion rate of GSSG of cells was lower in fructose medium than in glucose medium. The amount of excreted glutathione was similar for menadione and NQ. There was no loss in the total amount (intra- and extracellular) of glutathione during incubation.

Energy status

The E.C. of the hepatocytes was dependent on the medium used: cells in fructose medium exhibited a lower E.C. (0.6) than those in glucose medium (0.9) (Fig. 5). This lower cellular E.C. in fructose medium was due to the lower ATP content of these cells as seen in Fig. 6. There was a large decrease in the E.C. in menadione-exposed cells in glucose medium at 90 and 120 min, the result of a decrease in ATP and an increase in AMP. The E.C. of quinone-exposed cells in fructose medium decreased very slowly during incubation. Lactate production (Fig. 7) was very low in hepatocytes in glucose

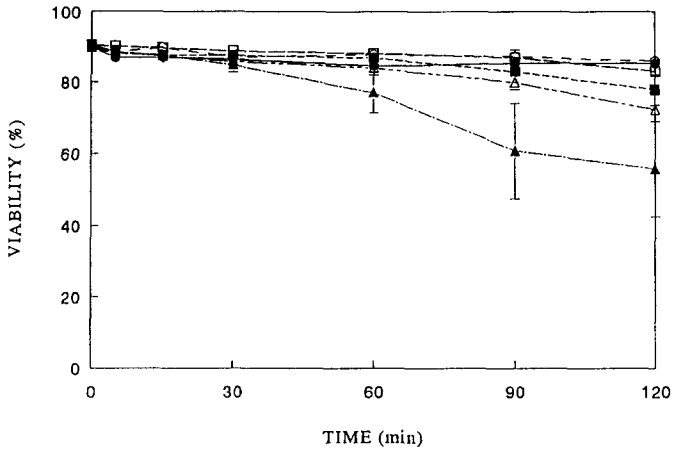

Fig. 1. Effects of exposure of freshly-isolated rat hepatocytes to NQ or menadione on cell viability (% measured as the amount of LDH retained by the cells). Hepatocytes were exposed for 2 hr in glucose medium (filled symbols) or in fructose medium (open symbols): control (0.4% DMSO) (○), NQ (50 μM) (△), menadione (85 μM) (□). The results are presented as the means ± SEM of four experiments, with hepatocytes derived from four different animals.

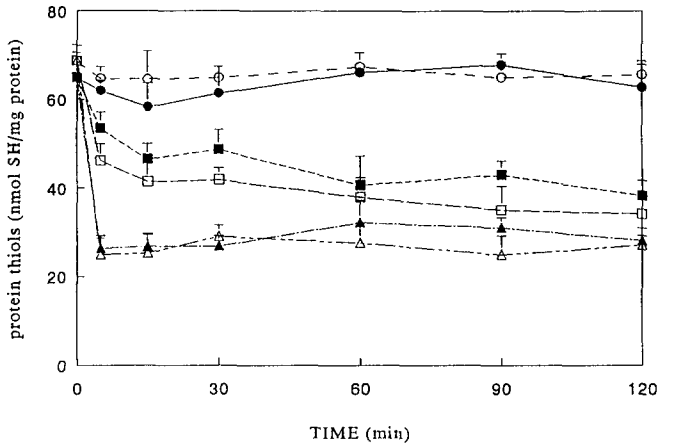

Fig. 2. Effects of exposure of freshly-isolated rat hepatocytes to NQ or menadione on protein thiol groups (nmol SH/mg protein). Hepatocytes were exposed for 2 hr in glucose medium (filled symbols) or in fructose medium (open symbols): control (0.4% DMSO) (○), NQ (50 μM) (△), menadione (85 μM) (□). The results are presented as the means ± SEM of four experiments, with hepatocytes derived from four different animals.

medium and was unchanged when the hepatocytes were exposed to menadione or NQ. Hepatocytes in fructose medium produced high amounts of lactate. The initial rates of production were equal for control and quinone-exposed cells. However, after 1 hr the production of lactate in control cells reached a maximum whereas the amount of lactate in quinone-exposed cells increased until the end of the experiment, with menadione-exposed hepatocytes producing the greatest amount.

DISCUSSION

In the experiments presented here, we used moderate toxic concentrations of menadione and NQ. Since NQ is more toxic than menadione, hepatocytes were exposed to a lower concentration of NQ than menadione. At these concentrations, the quinones clearly affected the thiol status but did not cause massive cell death of exposed hepatocytes.

Glycolysis

In our experiments we did not find evidence for an impairment of glycolysis by 50 μM NQ or by 85 μM menadione, since quinone-exposed hepatocytes in fructose medium produced high amounts of lactate. The initial rate of lactate production in fructose medium was equal for control and quinone-exposed

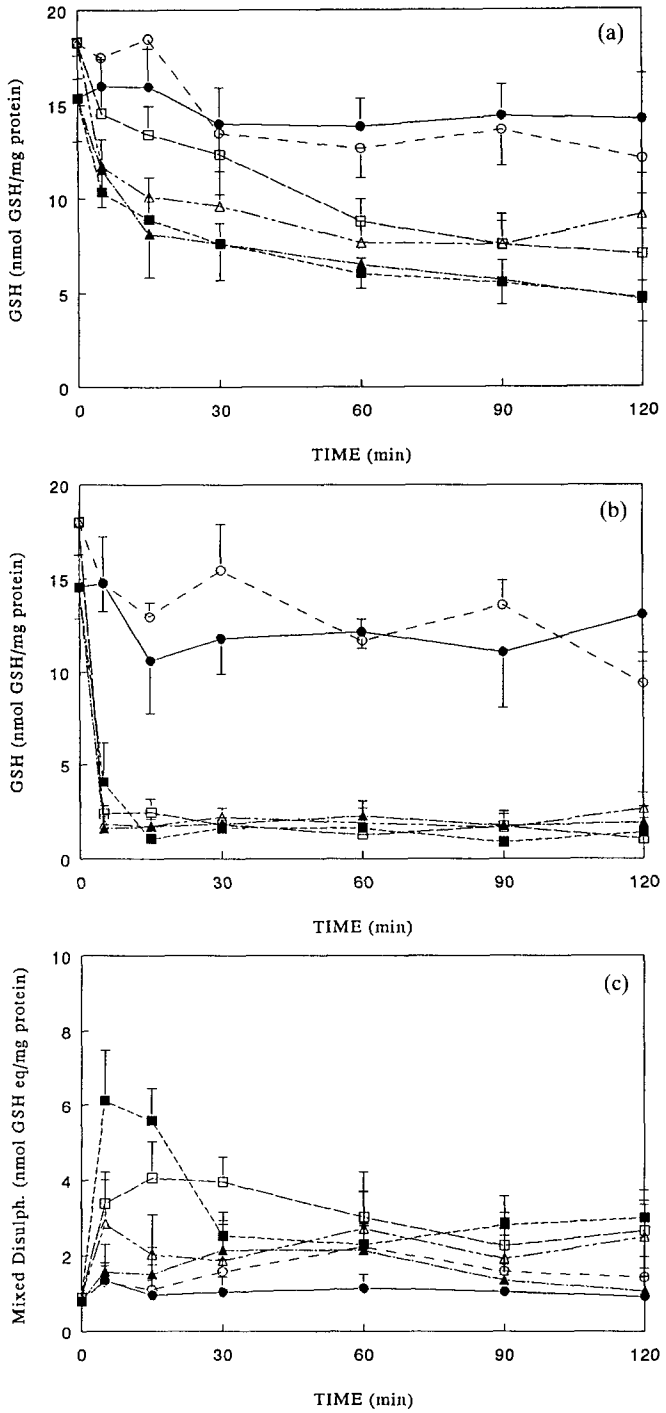

Fig. 3. Effects of exposure of freshly-isolated rat hepatocytes to NQ or menadione on glutathione status: total glutathione (a), GSH (b) and mixed disulphides (c) (nmol GSH/mg protein). Hepatocytes were exposed for 2 hr in glucose medium (filled symbols) or in fructose medium (open symbols): control (0.4% DMSO) (○), NQ (50 μM) (△), menadione (85 μM) (□). The results are presented as the means ± SEM of four experiments, with hepatocytes derived from four different animals.

cells. However, lactate production in control cells levelled off after 1 hr, indicating that an equilibrium was reached in production and utilization of lactate. In contrast, quinone-exposed cells continued to produce lactate. This indicates that there is a continuous demand for the products of glycolysis, e.g. ATP and/or intermediates for the citric acid cycle. Hepatocytes in glucose medium did not

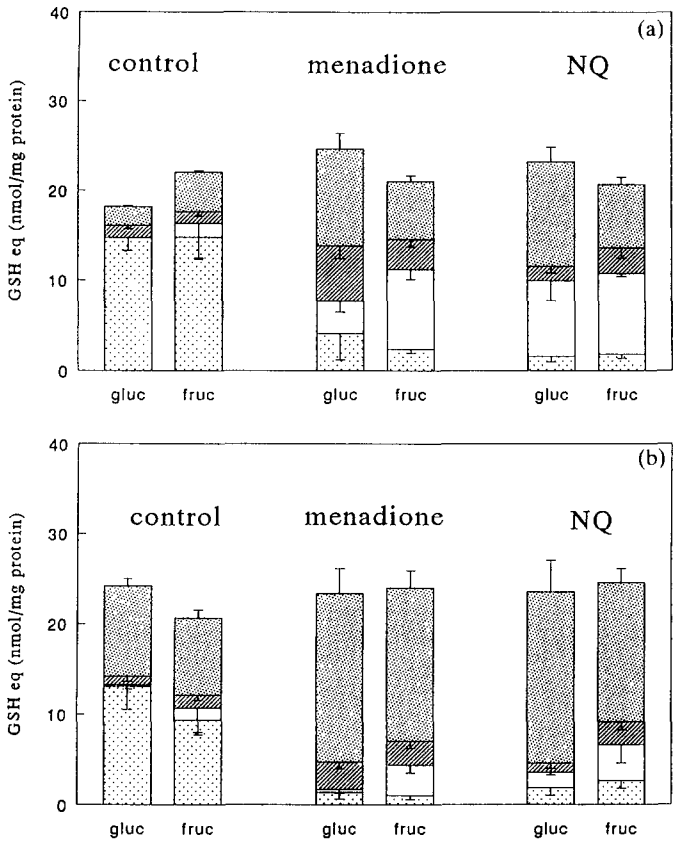

Fig. 4. The different forms of glutathione after 5 min (a) and 120 min (b) exposure to menadione or NQ in freshly-isolated rat hepatocytes (nmol GSH/mg protein): GSH (▨), GSSG (□), mixed disulphides (▨) and excreted glutathione (▨). The results are presented as the means ± SEM of four experiments, with hepatocytes derived from four different animals.

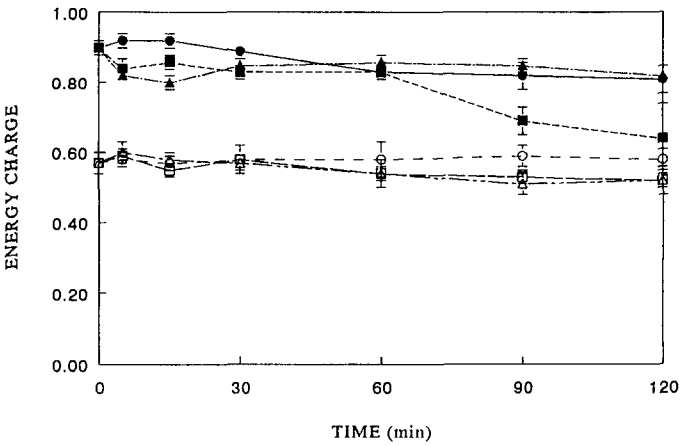

Fig. 5. Effects of exposure of freshly-isolated rat hepatocytes to NQ or menadione in glucose medium (filled symbols) or in fructose medium (open symbols) on the energy charge: control (0.4% DMSO) (○), NQ (50 μM) (△), menadione (85 μM) (□). The results are presented as the means ± SEM of four experiments, with hepatocytes derived from four different animals.

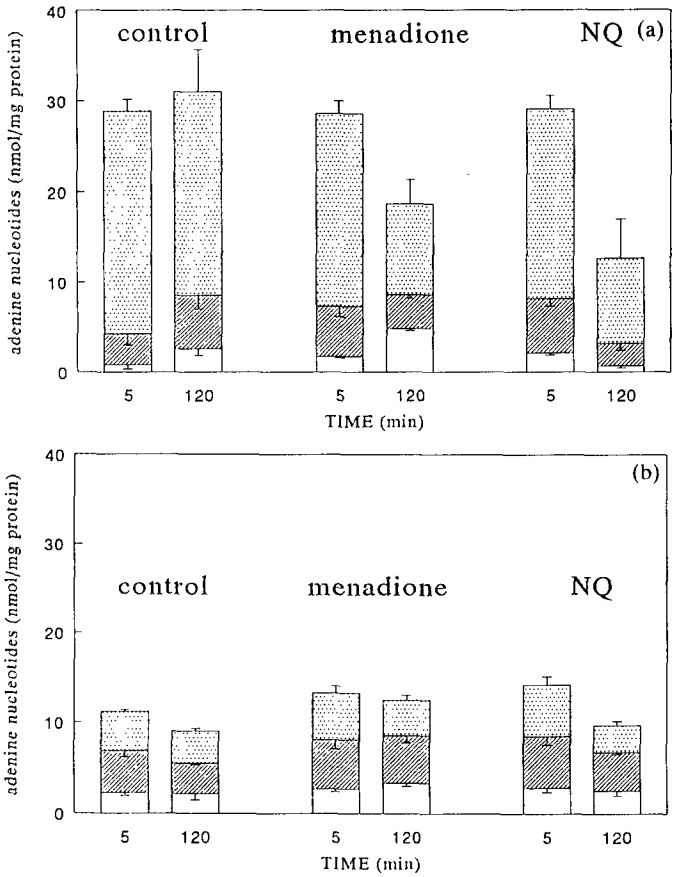

Fig. 6. The adenine nucleotides in glucose medium (a) and fructose medium (b) (nmol/mg protein): AMP (□), ADP (▨) and ATP (▩). Hepatocytes were exposed for 2 hr to menadione or NQ. The results are presented as the means ± SEM of four experiments, with hepatocytes derived from four different animals.

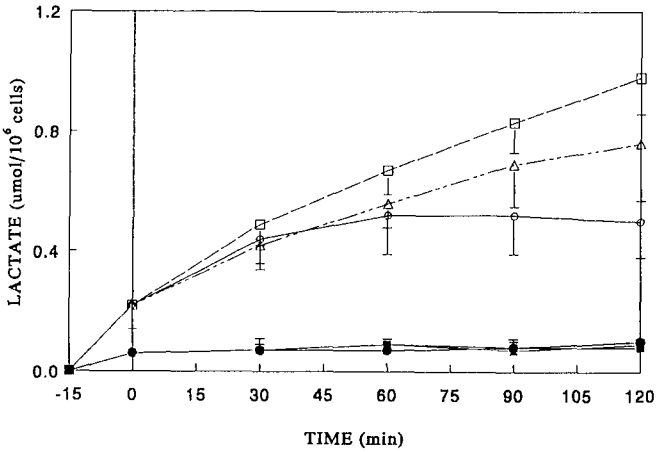

Fig. 7. Lactate production of freshly-isolated rat hepatocytes in glucose medium (filled symbols) or in fructose medium (open symbols) (μmol/10^6 cells). Hepatocytes were exposed for 2 hr: control (0.4% DMSO) (○), NQ (50 μM) (△), menadione (85 μM) (□). The results are presented as the means ± SEM of four experiments, with hepatocytes derived from four different animals.

produce lactate. As a result of the different lactate concentrations in fructose and glucose medium, hepatocytes in fructose medium will have a lower cytosolic pH than cells in glucose medium [3, 34]. Nieminen *et al.* [3] have shown that acidification protects rat hepatocytes against various toxic compounds, including $HgCl_2$. However, this acidification did not contribute to the protection provided by fructose against menadione-induced toxicity. On the other hand, the possibility that acidification does contribute to fructose protection against NQ, which is thought to exert its toxicity by arylation, can not be excluded. This possibility is presently under investigation. The high rate of lactate production in control cells in fructose medium suggests that those hepatocytes are no longer subject to the Pasteur effect (inhibition of glycolysis under aerobic conditions) [35]. This loss of control is the result of bypassing hexokinase and phosphofructokinase, the first two regulatory sites of glycolysis [34]. Pyruvate kinase, which is suppressed by a high ATP content, is relieved by the low ATP content of hepatocytes in fructose medium (ca. 4 nmol/mg protein). This low ATP content, ca. six times lower than in glucose medium (ca. 24 nmol/mg protein), is caused by the high rate of fructose phosphorylation. In order to maintain a high rate of glycolysis, hepatocytes need fast regeneration of NAD^+ which can be obtained by reducing pyruvate to lactate. This may be an explanation for the high lactate production in fructose medium.

Thiol homeostasis

One consequence of the decrease in ATP concentration, as found in fructose medium, can be a decrease in the velocity of ATP-dependent processes, e.g. GSSG excretion [36]. Indeed, our studies confirmed the results of Silva *et al.* [2] that hepatocytes in fructose medium, as compared to glucose medium, excrete lower amounts of GSSG when subjected to an oxidative challenge. However, our results deviate from data of Silva *et al.* [2] by showing higher amounts of intracellular GSSG rather than GSH, which may be the result of differences between the extent of oxidative challenge caused by quinones or nitrofurantoin. No indications were found for glutathione arylation by either quinone. This can be the result of a fast GSH oxidation and subsequent excretion of GSSG. Furthermore, GSH loss might be compensated by *de novo* GSH synthesis. However, arylation is likely to be involved in protein thiol depletion. This is indicated by both the higher loss of protein thiols (Fig. 2) and the lower formation of mixed disulphides (Fig. 3c) in NQ-exposed hepatocytes.

We found that fructose had no effect on the extent of protein thiol depletion. Moreover, protein thiols were depleted to the same extent as with higher and much more cytotoxic concentrations (200 μM) of quinones previously used [17]. This indicates that processes following protein thiol depletion are not the only factors determining the onset of loss of cell viability. A remarkable effect of fructose on the metabolism of GSSG was seen in hepatocytes after 5 min of menadione exposure. In glucose medium, most of the intracellular GSSG consisted of mixed disulphides. In fructose medium, fewer mixed disulphides were formed and GSSG predominated. This indicates that the linear relationship between the cellular GSSG/GSH ratio and the formation of mixed disulphides [37], catalysed by thiol transferases, is altered in fructose medium. This interesting phenomenon needs further exploration.

Adenine nucleotides

Menadione caused a decrease in the E.C. after 2 hr in glucose medium, suggesting an impairment of cellular ATP generation and an activation of adenylate kinase. NQ-induced cell death in glucose medium was not preceded by a decrease in the E.C. whereas in previous experiments, 200 μM NQ caused a fall in the E.C. within 5 min, followed by massive cell death within 1 hr. This indicates that different disturbances of cellular metabolism are caused by NQ at 50 μM as compared with 200 μM. The decrease in the adenine nucleotide content of NQ-exposed hepatocytes in glucose medium coincided with LDH leakage. Therefore, this decrease probably results from increasing plasma membrane permeability.

Many metabolic processes are regulated by either the E.C. or the ATP level and in many cases there is a direct utilization of ATP. Silva *et al.* [2] and our group (this paper) found that fructose causes a decrease in GSSG excretion. Mäenpää *et al.* [38] have shown *in vivo* that fructose metabolism results in a decrease in DL-[1-^{14}C]leucine incorporation into rat liver protein. Therefore, ATP depletion caused by fructose metabolism might result in a decrease in processes that are disadvantageous under certain circumstances (i.e. GSSG excretion). Fructose might also decrease energy consuming processes not essential for short term survival (synthesis of some proteins).

Recently, some groups have emphasized the importance of mitochondrial membrane potential in retaining cell integrity [39–43]. They have shown that the collapse of this potential, resulting from a permeability transition, was closely associated with the onset of loss of cell viability. Cyclosporin inhibits this permeability transition [44] and was shown to protect against oxidant-mediated cell death [41, 42]. The permeability transition of mitochondria occurs after Ca^{2+} accumulation in the mitochondria and can be induced by P_i and other compounds (for an extensive review see Ref. 45). Zoeteweij *et al.* [46] have shown that calcium-induced cell death of cultured rat hepatocytes is delayed by omitting P_i from the incubation media. Furthermore, it has been shown that fructose is able to deplete intracellular P_i [4, 19, 38, 47]. Here, we found indirect evidence of P_i depletion by fructose since there was a decrease in adenine nucleotides in fructose medium, indicating a stimulated deamination of AMP. P_i inhibits this process [7]. Thus, P_i depletion caused by fructose might be a very important event in protecting mitochondrial integrity. This possibility is presently under investigation.

In conclusion, the different effects of NQ and menadione on the E.C. of hepatocytes in glucose medium indicate that at these concentrations the quinones affect different processes in the cell. Fructose does not seem to reduce the extent of

quinone-induced oxidative stress as indicated by the high amount of GSSG in quinone-exposed cells. The results presented here indicate that hepatocytes in fructose medium may show a number of changes, including a lower ATP availability, cytosolic acidification and a decreased P_i content. It is probably the combination of these factors that contributes to improved cell survival. Although hepatocytes in glucose medium contained more ATP, these cells were more vulnerable to the toxicity of the quinones than cells in fructose medium. Therefore, under certain circumstances, a high ATP content is not beneficial.

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