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Expression of Cytochrome P-450s and Glutathione *S*-Transferases in the Rat Liver during Water Deprivation: Effects of Glucose Supplementation

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Pharmacokinetic profiles of therapeutic agents change in dehydrated animals. The present study was designed to determine the expression of xenobiotic-metabolizing enzymes in the rat liver and the effect of glucose supplementation during water deprivation.

Deprivation of water intake, which reduced food intake, resulted in no significant change in the cytochrome P-450 1A2, 2B1/2, 2C11 and 3A1/2 expression. Cytochrome P-450 2E1, however, was three-fold induced with an increase in the mRNA. Rehydration of 48-h water-deprived rats for the next 24 h with free access to foods restored the P-450 2E1 level to that of the control, although rehydration with 20% food supply failed to normalize the P-450 2E1 expression. Water deprivation caused a reduction in the plasma insulin level, which was prevented by rehydration with a sufficient food supply. The plasma insulin level was inversely related to the P-450 2E1 expression. Glucose feeding instead of foods during dehydration prevented P-450 2E1 induction in the absence of recovering the plasma insulin level. Western blot analysis revealed that the hepatic rGSTA2 level was 30% decreased in dehydrated rats, whereas the rGSTA3, M1 and M2 expression was not affected. Suppression of rGSTA2 accompanied a reduction in the mRNA. Glucose feeding further reduced rGSTA2 expression.

The data indicated that expression of major P-450s and glutathione *S*-transferases, except P-450 2E1, was not greatly affected by water deprivation and that the P-450 2E1 induction and a decrease in plasma insulin resulted from the reduction in food intake but not from dehydration *per se*. Glucose supplementation restored P-450 2E1 expression but further suppressed rGSTA2 expression during water deprivation.

INTRODUCTION

Water deprivation may occur in isolated situations by a natural disaster and less harsh dehydration may be associated with certain clinical situations such as polyuria, diarrhorea and hyperthermia. Hormonal and pathophysiological changes occur during dehydration.[1–3] In addition, water deprivation causes a reduction in food intake[4] to prevent elevations in extracellular fluid osmolarity and sodium concentration. The potential rate of adverse reactions of drugs as well as their metabolism and disposition would be greatly affected by water deprivation as well as by its accompanying reduction in food intake.

The metabolic basis and pharmacokinetic profiles in the altered physiological state should be established for patients taking medication therapy. The dosing regimen should be modified appropriately to minimize the adverse effects of therapeutic agents as well as to improve therapeutic effectiveness. Studies have shown that water deprivation affects drug disposition kinetics.[5,6] For example, water deprivation modulates total body clearance and the distribution of gentamicin.[7] Changes in the expression of hepatic detoxifying enzymes would also affect the pharmacodynamic and pharmacokinetic profiles of therapeutic agents.

The content of total cytochrome P-450 and the activity of aminopyrine *N*-demethylase have been shown to be decreased in water-deprived animals, whereas aniline hydroxylase activity was increased.[4] In particular, cytochromes P-450 2E1 and P-450 3A1/2 are inducible by starvation.[8] In the present study, we were interested in studying the effect of acute water deprivation on the expression of major cytochrome P-450 and glutathione *S*-transferase (GST) isoforms, and in establishing the respective role of dehydration and reduction in food intake. Given the clinical application of intravenous infusion of 5% glucose in water during dehydration with hypernatraemia, the effect of glucose feeding on the altered expression of the enzymes was determined further during water deprivation. The results of this study will provide information on the detoxifying enzyme expression, which will assist in establishing the medication regimen.

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EXPERIMENTAL

Materials

[α-^{32}P]dCTP (3000 mCi mmol^{-1}) was purchased from New England Nuclear (Arlington Heights, IL, USA). Biotinylated donkey anti-goat IgG and biotinylated goat anti-rabbit IgG were supplied from Gibco BRL (Gaithersburg, MD, USA) and Life Technologies (Gaithersburg, MD, USA), respectively. Random prime-labelling kit was purchased from Promega (Madison, WI, USA). Monoclonal mouse anti-rat P-450 1A1/2, 2B1/2, 2C11 and 3A1/2 antibodies were obtained from Oxford Biomedical Research (Oxford, MI, USA). Commercial assaying kits for the plasma insulin and glucose were purchased from Diagnostic Products Co. (Los Angeles, CA, USA) and Boehringer Mannheim (Boehringer Mannheim, Germany), respectively. Most of the reagents in the molecular studies were supplied from Sigma Chemical Co. (St Louis, MO, USA).

Animal treatment

Animal studies were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. Male Sprague-Dawley rats (ca. 250 g) were purchased from Korea Food and Drug Administration (Seoul, South Korea) and maintained in a clean room at the Animal Care Centre for Pharmaceutical Research, College of Pharmacy, Seoul National University, at a temperature of 20–23°C with 12 h light/dark cycles and a relative humidity of 50%. Animals were caged under the supply of filtered pathogen-free air and water *ad libitum*. For the time-course study, rats were deprived of drinking water for 24, 48 or 72 h. A group of rats were allowed free access to water and foods for 24 h after 48 h of water deprivation (i.e. rehydration), whereas another group of rats were subjected to water deprivation with the restricted food supply that was equal to that consumed by pair-fed dehydrated rats (i.e. 7 g per animal per day). Food intake and body weight were recorded every day during the experiments. A group of animals were allowed access to glucose at the daily amount of ca. 15 g per animal as a substitute for foods. Data points consisted of at least three independent experiments.

Isolation of microsomal and cytosolic proteins

Hepatic microsomal and cytosolic fractions prepared by differential centrifugation were washed in pyrophosphate buffer and stored in 50 mM TRIS acetate buffer (pH 7.4) containing 1 mM EDTA and 20% glycerol. The subcellular preparations were stored at –70°C until use. Protein content was determined by the method of Lowry *et al*.[9]

Immunoblot analysis

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to previously published procedures.[10,11] Microsomal and cytosolic proteins were separated by 7.5% and 12% gels, respectively, and electrophoretically transferred to nitrocellulose paper. The nitrocellulose paper was incubated with anti-rat cytochrome P-450 antibody or anti-rat GST antibody, followed by incubation with biotinylated secondary antibody. For P-450 immunoblottings, filters were allowed to react with monoclonal mouse anti-rat P-450 1A1/2, 2B1/2, 2C11 and 3A1/2 antibodies. The filters were incubated with goat anti-mouse IgG as the secondary antibody and developed using 5-bromo-4-chloro-3-indolylphosphate–4-nitroblue tetrazolium chloride.

Isolation of total RNA

Total RNA was isolated using the improved single-step method of thiocyanate–phenol–chloroform RNA extraction according to the method of Puissant and Houdebine.[12]

Preparation of cDNA probes

Specific cDNA probes for P-450 2E1 and rGSTA2 genes were amplified by reverse transcriptase polymerase chain reaction using the selective primers[10,11] and cloned in the pGEM+T vector (Promega, Madison, WI, USA).

Northern blot hybridization

Northern blot analysis was carried out as described previously.[11] Briefly, total RNA isolated from rat livers was resolved by electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde and transferred to nitrocellulose paper. The blot was incubated with hybridization buffer containing 50% deionized formamide, 5 $\times$ Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidine and 0.1% bovine serum albumin; Pentex Fraction V), 0.1% sodium dodecylsulfate (SDS), 200 μg ml^{-1} of sonicated salmon sperm DNA and 5 $\times$ SSPE (1 $\times$ SSPE: 0.15 M NaCl, 10 mM NaH_2PO_4, and 1 mM disodium ethylenediamine tetraacetate, pH 7.4) at 42°C for 1 h without a probe. Hybridization was performed at 42°C for 18 h with a heat-denatured cDNA probe, which was random prime-labelled with [α-^{32}P]dCTP. Filters were washed in 2 $\times$ SSC (standard saline citrate) and 0.1% SDS for 10 min at room temperature twice and in 0.1 $\times$ SSC and 0.1% SDS for 10 min at room temperature twice. Filters were finally washed in the solution containing 0.1 $\times$ SSC and 0.1% SDS for 60 min at 60°C. The membranes were stripped and rehybridized with a labelled probe complementary to 18S rRNA to quantify the amount of RNA loaded onto the membranes.

Scanning densitometry

Scanning densitometry was performed with a Microcomputer Imaging Device, Model M1 (Imaging Research, St Catharines, Ontario, Canada).

Plasma insulin and glucose contents

The plasma insulin content was determined by radioimmunoassay using Coat-A-Count insulin (Diagnostic Products Co, Los Angeles, CA, USA) according to the

manufacturer's protocol. The radioactivity of [^{125}I]insulin was counted using a gamma-counter (Cobra 5010 Quantum, Packard Instrument Co, Meriden, CT, USA). The glucose level was assayed by an automatic blood chemistry analyser (Hitachi 747, Tokyo, Japan).

Data analysis

One-way analysis of variance (ANOVA) procedures were used to assess significant differences among treatment groups. For each significant effect of treatment, the Newman–Keuls test was used for comparisons of multiple group means.

RESULTS

Effects of water deprivation on cytochrome P-450s

Effects of water deprivation on the expression of xenobiotic-metabolizing enzymes were assessed in the rat liver. Restriction of water intake of rats resulted in no significant change in the levels of cytochrome P-450 1A2, 2B1/2, 2C11 and 3A1/2 (Fig. 1A). P450 2E1 was induced in a time-dependent manner with a 3-fold increase being noted at 48–72 h of water deprivation (Fig. 1A). Northern blot analysis also showed that the level of P-450 2E1 mRNA was increased during water deprivation (Fig. 1B). This result was consistent with that of Western blot analysis.

Rehydration and glucose feeding on P-450 2E1 expression

The basis for the altered P-450 2E1 expression during water deprivation was assessed further. Rehydration of 48-h water-deprived rats for 24 h with sufficient food supply significantly prevented the induction of P-450 2E1 (Fig. 2A). Because water deprivation reduced food consumption by 80% (ca. 7 g per day per water-deprived animal vs >35 g per day per control animal), we designed an experiment to assess whether restricted food consumption accompanied by water deprivation affected the enzyme expression. Rehydration with limited feeding (i.e. 7 g per day) after 48 h of water deprivation failed to prevent the P-450 2E1 induction, raising the possibility that induction of P-450 2E1 by water deprivation might result from the change in food consumption, but not from dehydration (Fig. 2A). Given the reduction in food consumption as a result of water restriction, further studies were conducted to determine the effect of glucose feeding on the inducible P-450 2E1. Rats fed glucose (i.e. 15 g per day) instead of foods during 3 days of water deprivation showed no induction of hepatic P-450 2E1. Rehydrated rats fed glucose also showed no P-450 2E1 induction (Fig. 2A).

The P-450 2E1 mRNA level was measured in rehydrated rats with limited food supply (i.e. 20% of the amount consumed by control rats). Whereas rehydration with sufficient food supply showed no increase in the P-450 mRNA, rehydration with 20% of the control food supply caused a comparable increase in the mRNA to that in dehydrated animals (Fig. 2B). Glucose

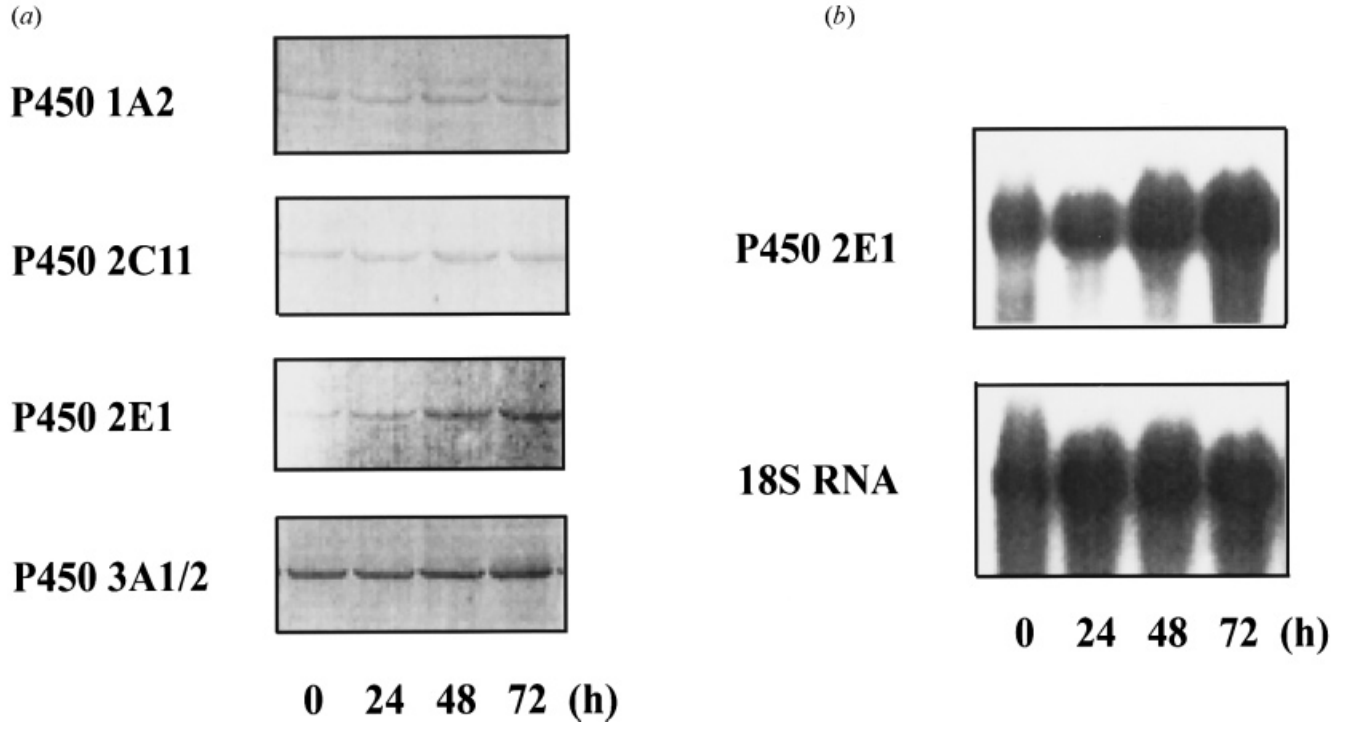

Figure 1. (A) Hepatic P-450 1A2, 2C11, 2E1 and 3A1/2 expression after water deprivation. Immunoblot analyses were carried out with hepatic microsomal proteins isolated from rats at 0, 24, 48 or 72 h of water deprivation. Each lane was loaded with 10 μg of microsomal proteins. (B) Time-course of hepatic P-450 2E1 mRNA level. Northern blot analysis showed P-450 mRNA levels in total RNA fractions (20 μg of each) produced from rats at the above time points. Amounts of mRNA loading were assessed by rehybridization of the blot with a labelled probe for 18S rRNA.

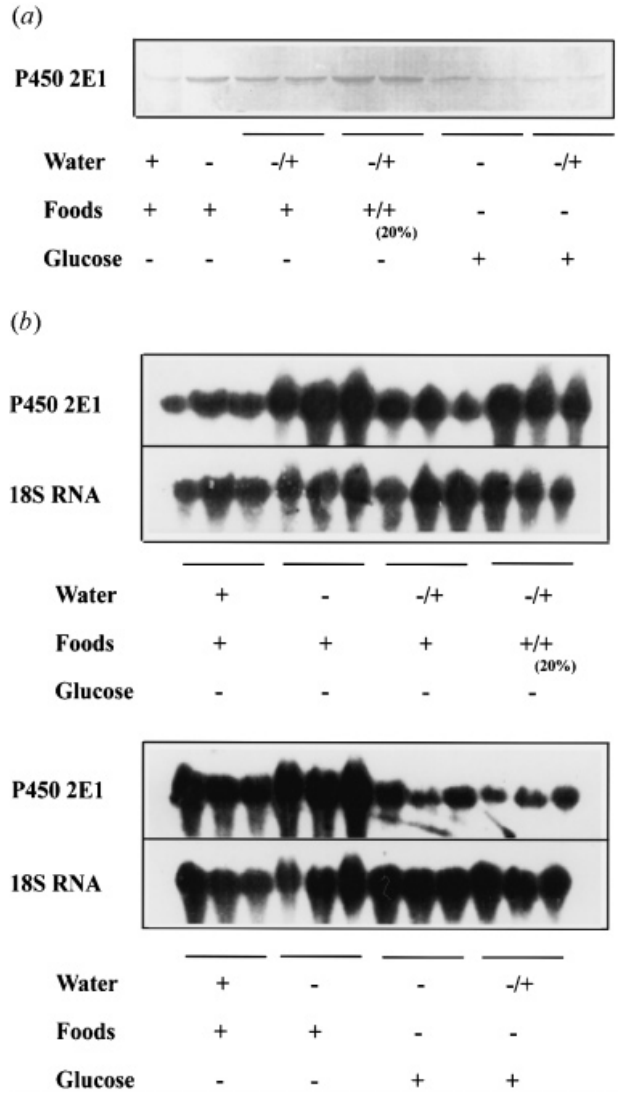

Figure 2. (A) Hepatic P-450 2E1 expression in dehydrated (–) or rehydrated (–/+) rats. Rats were rehydrated for 24 h with sufficient (+) or a limited supply of foods (20% of control) after 48 h of water deprivation. Glucose was substituted for foods with or without rehydration. (B) Representative Northern blot analysis for hepatic cytochrome P-450 2E1 level in dehydrated or rehydrated rats.

feeding instead of foods caused no increase in the mRNA in dehydrated or rehydrated rats; rather, the mRNA level was decreased in rats fed glucose (Table 1). This was in parallel with the change in P-450 2E1 protein expression.

Plasma glucose and insulin levels

The plasma glucose and insulin levels were determined in dehydrated and rehydrated animals. The plasma insulin content was 50% decreased in water-deprived rats (Table 2). Rehydration with sufficient foods, however, prevented the reduction in the plasma insulin level. Hence, the P-450 2E1 induction by water deprivation was inversely related to that of the plasma insulin level. This relationship was confirmed by the recovery of P-450 2E1 expression in rats rehydrated with free access to foods, in which the plasma insulin returned to the control (Table 2). The plasma insulin level in dehydrated or rehydrated rats fed glucose instead of foods was comparable to that in rehydrated rats with limited food supply (i.e. 20%). Although P-450 2E1 induction by water restriction was completely prevented by glucose feeding, the suppressed insulin level in either dehydrated or rehydrated rats failed to be restored by glucose administration; rather, the plasma insulin level seemed to be decreased in glucose-fed rats. Hence, the plasma insulin appeared to affect the expression of P-450 2E1.

The plasma glucose level was assayed in dehydrated and rehydrated rats. Whereas water deprivation caused no significant change in the plasma glucose content, rehydration with sufficient food supply significantly increased the blood glucose level. The blood glucose level in rats rehydrated for 24 h with limited food supply was comparable to that in control rats (Table 2). The plasma glucose level in rats fed glucose instead of foods was not different from that in control animals. These results showed the possibility that induction of P-450 2E1 by water deprivation might result from a change in glucose utilization in conjunction with suppression in the insulin level, but not with a change in the extracellular water volume.

Expression of GST

Expression of GST proteins also was determined during water deprivation. Western blot analysis showed that the level of rGSTA1/2 protein was 30% decreased after 72 h of water deprivation, whereas the expression of rGSTA3, rGSTM1 and rGSTM2 was not changed significantly (Fig. 3).

A change in rGSTA2 mRNA level after water deprivation was consistent with that of the protein (Fig. 4). Northern blot analysis revealed that the rGSTA2 mRNA level was decreased significantly in water-deprived rats, whereas the mRNA level was increased

Table 1. Relative P-450 2E1 mRNA level in the livers of dehydrated or rehydrated rats

Water	+	–	–/+	–/+	–	–/+
Foods	+	+	+	+/(20%)	–	–
Glucose	–	–	–	–	+	+
P-450 2E1 mRNA	1	2.6 ± 0.3*	1.0 ± 0.2**	2.2 ± 0.2	0.4 ± 0.1**	0.4 ± 0.1**

Hepatic P-450 2E1 mRNA levels were determined in the livers of dehydrated (–) or rehydrated (–/+) rats with sufficient (+) or limited food supply (20% of control) by Northern blot analysis, followed by scanning densitometry. Data represent the mean ± SD from three separate experiments. One-way analysis of variance was used for comparisons of multiple group means, followed by Newman–Keuls test: *$P < 0.05$ compared to control rats; **$P < 0.01$ compared to rats at 72 h of water deprivation. (Relative mRNA level in control rats = 1).

Table 2. Plasma insulin and glucose levels in dehydrated or rehydrated rats

Water	+	–	–/+	–/+	–	–/+
Foods	+	+	+	+/(20%)	–	–
Glucose	–	–	–	–	+	+
Insulin (μIU ml^{-1})	16 ± 4	8 ± 2**	15 ± 7	5 ± 3**	5 ± 2**	5 ± 2**
Glucose (mg dl^{-1})	153 ± 14	129 ± 27	237 ± 62*	153 ± 51	153 ± 11	153 ± 26

Rats were subjected to dehydration (–) or rehydration (–/+) with sufficient (+) or limited food supply (20% of control). Glucose was substituted for foods to dehydrated or rehydrated rats. The plasma insulin and glucose levels were determined as described in Methods. Data represent the mean ± SD from five separate experiments. One-way analysis of variance was used for comparisons of multiple group means followed by Newman–Keuls test: $*P < 0.05$ and $**P < 0.01$ compared to control rats.

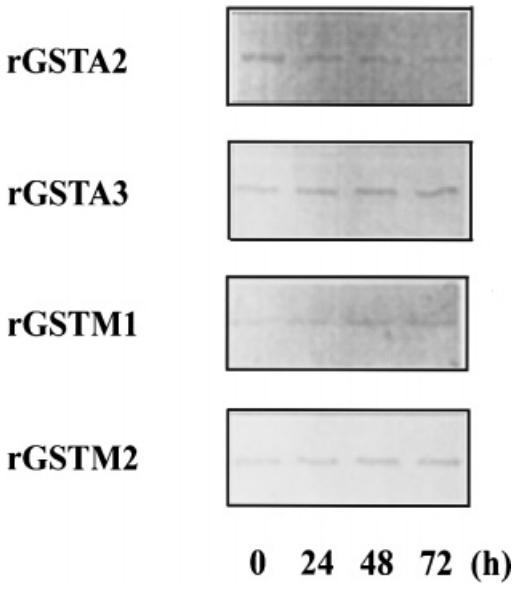

Figure 3. Major GST levels in the liver. Western immunoblot analyses were carried out with rat hepatic cytosol (3 μg each) produced from rats at 0, 24, 48 or 72 h of water deprivation. Results were confirmed by repeated experiments.

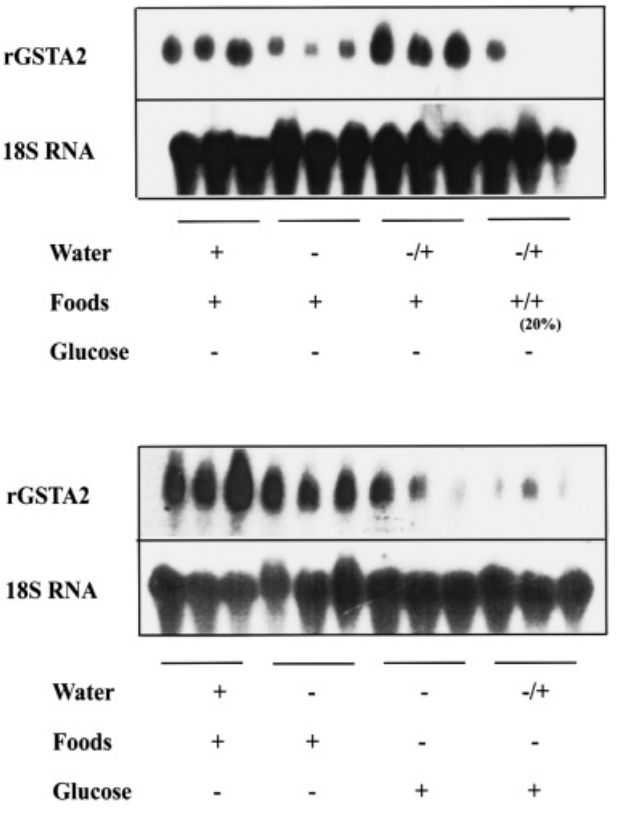

Figure 4. Northern blot analysis for rGSTA2 mRNA. Northern blot analysis was performed with total RNA fractions (20 μg each) isolated from control, dehydrated or rehydrated rats with sufficient or limited food supply. Amounts of RNA loading were assessed by rehybridization of the blots with a probe for 18S rRNA. Results were confirmed by repeated experiments.

in the state of rehydration. Rehydration with 20% of the control food supply caused a reduction in rGSTA2 mRNA relative to the control. The rGSTA2 mRNA level was, however, recovered by rehydration with sufficient food supply, whereas rehydration of rats fed a limited amount of foods (i.e. 7 g per day) showed no recovery in the mRNA. The level of hepatic rGSTA2 mRNA was diminished in water-deprived or rehydrated rats fed glucose instead of foods (Fig. 4). Hence, changes in rGSTA2 expression by water deprivation and/or rehydration appeared to be associated closely with glucose supply as well as with food intake.

Discussion

Studies have shown that the pharmacokinetics of therapeutic agents change in the dehydration state.[5–7] Alterations of renal clearance and extracellular fluid volume would significantly limit excretion of drugs. Changes in detoxifying enzyme expression and in the volume of drug distribution would account for the altered pharmacokinetics and pharmacodynamics in the state of dehydration.

Studies showed that the enzyme responsible for hydroxylation of the aromatic ring was stimulated by water deprivation[6] and that microsomal aniline hydroxylase activity was increased by water deprivation.[4] The present study demonstrated that expression of major cytochrome P-450s was not affected by water deprivation, whereas cytochrome P-450 2E1 was induced with a parallel elevation of the mRNA level. Cytochrome P-450 2E1 was induced during water deprivation with a reduction in food intake. Both increases in the P-450 2E1 protein and mRNA levels by water deprivation were similar to those obtained by starvation experiment.[13] Hence, induction of P-450 2E1 by water deprivation would be due to the reduction of food intake and presumably due to a subsequent decrease in glucose utilization. Either dehydration or rehydration alone, however, failed to affect the expression of P-450 2E1, showing that the induction of P-450 2E1 by water deprivation was not associated

with a change in the hydration state. Despite the common induction of P-450 2E1 by both water deprivation and starvation, cytochrome P-450 3A1/2 was not induced in water-restricted rats, which clearly differed from that by starvation.[14] The difference may result from changes in extracellular water volume and the level of mineralocorticoid.

It has been suggested that P-450 2E1 expression in hepatocytes and HepG2 cells was affected by insulin and thyroid hormones as a result of altered mRNA stability.[15,16] The altered enzyme expression returned to that of the control by glucose administration, but not by rehydration alone. Either dehydration or rehydration with a limited food supply reduced the plasma insulin level in rats, which was inversely related to the expression of hepatic P-450 2E1. Interestingly, however, glucose feeding instead of foods prevented P-450 2E1 induction during water deprivation in spite of its suppression of the plasma insulin content. The results of this study also showed that the plasma insulin level may not be an exclusive determinant for P-450 2E1 expression. Presumably, other factors such as glucose and growth hormone(s) would also be involved in the regulation of P-450 2E1 expression. Expression of P-450 2E1 was increased by the elevation of fatty acid oxidation and blood concentrations of ketone bodies.[17–20] This may be associated with hormonal change(s) and glucose utilization.

Cytochrome P-450 2E1 is active in the metabolism of a variety of small organic molecules.[21] The reactive intermediates produced from xenobiotics catalysed by P-450 2E1 are frequently capable of binding covalently to tissue macromolecules, such as proteins, and causing tissue damage.[22,23] Cytochrome P-450 2E1 plays a critical role in the metabolism of nitrosamines—potent carcinogens that require metabolic activation to exert their carcinogenic effects.[11,24,25] Conversely, suppression of P-450 2E1 may contribute to inhibition of colon and liver carcinogenesis by chemical carcinogens. The present study showed that glucose feeding prevented the induction of P-450 2E1 by water deprivation. Thus, the clinical use of 5% glucose in water for dehydrated patients with hypernatraemia would be appropriate in reducing the P-450 2E1-mediated production of toxic reactive intermediates from a wide variety of xenobiotics.

Hepatic GST expression was monitored in water-deprived rats in this study. We showed that major GST enzymes were not altered during water deprivation. The expression of rGSTA2 was slightly reduced. This change may also result from the reduction in food consumption. This is supported by a decrease in rGSTA2 mRNA in response to glucose supplementation. Hence, the metabolic activity of the phase II conjugating enzyme may be decreased slightly during dehydration.

Dehydration causes tissue injury, presumably due to reduction in the organ perfusion rate. Decreases in the apparent volume of distribution and the total clearance would increase drug concentrations.[26] Tissue accumulation of certain drugs significantly increased the toxicity of the drugs, with increased drug concentrations at tissues.[7] Dehydration causes an increase in the haematocrit with decreased food intake,[4,27] which would affect the blood haemodynamics as well as energy metabolism. In addition to the haemodynamic factor, the changes in drug-metabolizing enzyme expression would be associated with the altered pharmacological and toxicological responses.

In summary, the present study demonstrated that expression of major P-450s and glutathione *S*-transferases, except P-450 2E1, was not greatly affected by water deprivation and that the P-450 2E1 induction resulted from the reduction in food intake but not from dehydration *per se*. Glucose supplementation restored P-450 2E1 expression during water deprivation but further suppressed rGSTA2 expression.

Acknowledgements

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