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Intraischemic metabolic effects of different disaccharides on protected canine kidneys*

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Summary. The addition of the dissaccharides maltose (10, 20, 30 mM) and sucrose (30, 60 mM) to Bretschneider's organ protective HTK solution was evaluated to improve renal protection by an enhanced glycolytic energy supply. Canine kidneys were perfused for 8 min with either HTK solution or HTK solution containing additional disaccharides. After nephrectomy the kidneys were incubated at 25 °C and metabolic parameters were determined at regular intervals. Maltose and sucrose are slowly cleaved during renal ischemia but maltose distinctly faster than sucrose. Maltose increases intraischemic ATP supply. However, 30 mM maltose was no better than 10 mM. 60 mM sucrose was about as effective for glycolysis as 10 mM maltose. However, possibly due to fructose release there was an accelerated decrease of adenine nucleotides with sucrose. Although fructose enters glycolysis it seems to have negative side-effects. Hence, probably neither sucrose nor fructose are appropriate for renal substrate supply during ischemia.

Key words: HTK-solution - Ischemia - Kidney - Metabolism - Saccharides

Introduction

The transition from aerobic to anaerobic conditions at the onset of ischemia leads not only to a decrease of the total metabolic rate but also to a shift in the individual metabolic pathways. For example, glycolysis becomes the only relevant pathway for energy supply during ischemia.

Since the kidney normally contains little glucose and glycogen, renal glycolysis is limited by the substrate reserves. However, it can be increased by an exogenous glucose supply in order to improve intraischemic energy provision [13]. In contrast to anoxia, substrate provision must occur in the preischemic phase either by infusion prior to the protective perfusion or as an additive to the protective solution. Renal loading with glycolytic substrates by glucose infusions is limited by the tolerance limit to plasma glucose levels and by the washout effect of the subsequent protective perfusion. Glucose addition to a buffered protective solution allows a higher substrate supply. An ample provision of readily usable glucose, however, possibly leads to a disadvantageous metabolic stress by an excessive cellular substrate load [6, 15, 16].

Disaccharides must be cleaved before reabsorption which delays the cellular uptake of their components. As additives to the buffered HTK-solution they may allow a plentiful renal endowment with glycolytical substrates during ischemia without an immoderate increase in metabolic stress. Hence, we investigated the effects of maltose and sucrose on renal intraischemic energy metabolism when these saccharides were added to Bretschneider's HTK solution used for canine renal protection. In control groups HTK solution with a glucose or fructose additive or unmodified HTK solution were used.

Material and methods

Mongrel dogs weighing 31 kg on the average were allowed free access to water up to the induction of anaesthesia but feeding (Nagut Vipromix, Dr. Müller KG, Lage/Lippe, FRG) was stopped 24 h before. After premedication with 90 mg piritramide (Dipidolor®, Janssen GmbH, Düsseldorf, FRG) and 0.5 mg atropine (Atropinsulfat Braun, B. Braun Melsungen AG, Melsungen, FRG) anaesthesia was induced with 5-10 mg/kg thiopental sodium (Trapanal®, Byk Gulden, Konstanz, FRG) and maintained with halothane (Halothan Hoechst, Hoechst, Frankfurt, FRG) in combination with a 3:1 N_2O/oxygen ventilation and small doses of fentanyl as necessary (Fentanyl, Janssen GmbH, Düsseldorf, FRG).

After median laparotomy the kidneys were exposed. During the preparation about 500 ml/h Tutofusin® for volume maintenance (Pfrimmer & Co, Erlangen, FRG) and as a standard 500 ml 5% glucose (25 g) (Glucose 5, B. Braun Melsungen AG, Melsungen,

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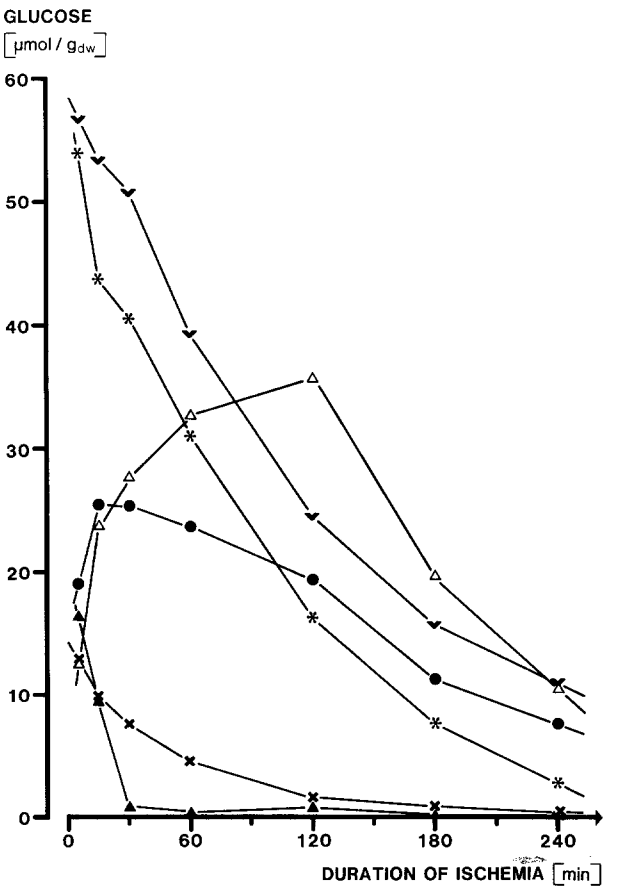

Fig. 1. Free glucose content ($\bar{x}$) in canine kidneys during 240 min of ischemia at 25°C. Groups according to the composition of the protective solution: (×) HTK-solution ($n=21$); (*) HTK-solution and increased (50–75 g) glucose premedication ($n=7$); (v) HTK-solution with 10 mM glucose ($n=5$); (●) HTK-solution with 10 mM maltose ($n=4$); (△) HTK-solution with 60 mM sucrose without mannitol ($n=2$); (▲) HTK-solution with 30 mM fructose without mannitol ($n=1$)

FRG) were infused [13]. In some experiments, however, the dogs received 1,000–1,500 ml (50–75 g) glucose. Then 1,250 I.U. heparin (Heparin-Natrium Braun, B. Braun Melsungen AG, Melsungen, FRG) and 0.1 mg/kg furosemide (Lasix®, Hoechst, Frankfurt, FRG) were injected. Fifteen minutes later the kidneys were perfused for eight minutes with a chilled (5–8°C) protective solution. For details of the perfusion technique see [14].

As protective solutions either the original HTK solution (Kardioplegische Lösung HTK nach Bretschneider, Köhler-Pharma, Alsbach-Hähnlein, FRG) or HTK solutions modified by sugar additon (vide infra) were used. The composition of Bretschneider's HTK solution is as follows: NaCl 15 mM, KCl 9 mM, MgCl 4 mM, histidine 180 mM, histidine-HCl 18 mM, K^+-α-ketoglutarate 1 mM, tryptophan 2 mM, mannitol 30 mM. The calculated osmolarity is 310 mOsm/liter.

The kidneys were removed after perfusion and incubated at 25°C in the respective protective solution. At regular intervals during ischemia specimens (cortex/medulla ratio 2:1) were taken for biochemical analyses. After homogeneisation and deproteinisation with 3.5% perchloric acid, lactate [9], glucose [3] and adenine nucleotides (Adenosine triphosphate (ATP): Testkombination ATP, Boehringer Mannheim GmbH, Mannheim; adenosine di/monophosphate (ADP/AMP): Testkombination ADP/AMP, Boehringer Mannheim GmbH, Mannheim, FRG) were determined in the neutralised supernatant. Alternatively, intrarenal pH in the outer medulla was measured via stick-in electrodes (LOT 403-M3, Dr. Ingold KG, Frankfurt) and a pH-meter (Digital pH/Millivolt-Meter 611, Orion Research, Cambridge).

For statistical analysis the Wilcoxon, Mann and Whitney U-test was used. Data are given as mean values and standard errors of the mean.

Experimental groups

According to the saccharides added to the HTK solution five groups were classified:

- HTK-group with standard glucose premedication: 21 kidneys were used for biochemical analyses, 10 kidneys for pH measurements.
- HTK with higher glucose premedication (50–75 g): 7 kidneys were used for biochemical analyses.
- HTK + maltose addition (standard glucose premedication): In case of 10 mM maltose in the HTK solution 4 kidneys were examined biochemically and 4 kidneys were used for pH measurement. With 20 mM maltose 2 kidneys served for metabolite determinations. When 30 mM were added, a HTK solution was prepared in which mannitol was omited to prevent too high an increase in osmolality. 2 kidneys were used for analyses, 2 for pH.
- HTK + sucrose addition (standard glucose premedication): Either 30 or 60 mM sucrose were added to a HTK solution in which mannitol was omited. With both concentrations 2 kidneys were examined biochemically. In addition, in 2 kidneys pH was measured after perfusion with the 60 mM sucrose solution.
- HTK + fructose addition (standard glucose premedication): 30 mM fructose was added to a HTK solution without mannitol with which one kidney was perfused and examined biochemically.

Results

Renal intraischemic glucose content

When disaccharides had been added to the protective solution the course of the renal glucose content differed in principle from the situation in which glucose was the main substrate. Whereas a renal substrate load with glucose resulted in an exponential glucose decrease from initially high values, with disaccharides glucose rose during ischemia up to a maximum until it declined again with longer ischemia times (Fig. 1). With 10 mM maltose the highest glucose value (26 µmol/g_{dw}) was already reached after 15 min, with 30 mM maltose a maximal concentration of 44 µmol/g_{dw} glucose was measured after 60 min of ischemia. With 30 mM sucrose there was an initial one hour plateau at about 14 µmol/g_{dw} after which glucose levels declined. With the addition of 60 mM sucrose to the protective solution a maximal renal glucose content of 36 µmol/g_{dw} was found after 120 min. Fructose addition, on the other hand, led to a rapid disappearance of glucose in the renal tissue (Fig. 1).

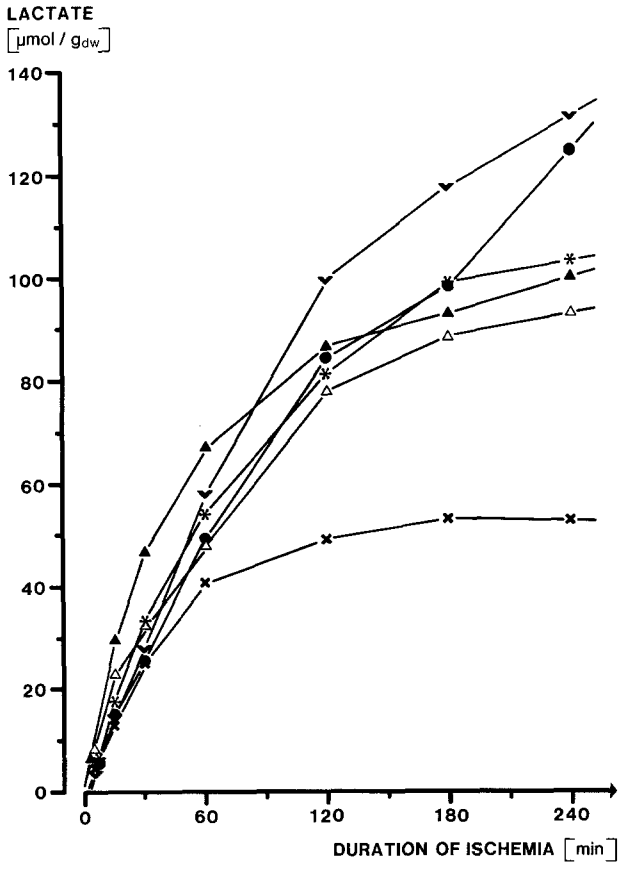

Fig. 2. Lactate content ($\bar{x}$) in canine kidneys during 240 min of ischemia at 25°C. Groups according to the composition of the protective solution: (×) HTK-solution ($=21$); (*) HTK-solution and increased (50–75 g) glucose premedication ($n=7$); (v) HTK-solution with 10 mM glucose ($n=5$); (●) HTK-solution with 10 mM maltose ($n=4$); (△) HTK-solution with 60 mM sucrose without mannitol ($n=2$); (▲) HTK-solution with 30 mM fructose without mannitol ($n=1$)

Lactate production during ischemia

The rise in renal substrate reserves resulted in an increased intraischemic lactate production. The addition of 10 mM glucose to the protective solution was distinctly more efficient with respect to lactate increase, especially between 60 and 240 min of ischemia, than a 10 mM maltose additive in spite of double the amount of glucose molecules. Only after 4 h of ischemia did the renal lactate content of both groups approach each other (Fig. 2). However, against the pure HTK solution 10 mM maltose had significantly ($P<0.005$) increased the renal lactate content at 120 min of ischemia. Only the higher sucrose concentration (60 mM) clearly increased lactate production against the rate in kidneys protected with the unmodified HTK solution. Fructose (30 mM) stimulated lactate formation in about the same measure as a higher (50–75 g) glucose premedication (Fig. 2).

Renal ATP and SAN

All saccharides retarded the intraischemic ATP decay but only with distinctly higher concentrations disaccharides were about as effective as a 10 mM glucose solution additive (Figs. 3–5). After 120 min of ischemia the renal ATP content was significantly ($P<0.005$) higher with 10 mM maltose than after perfusion with unmodified HTK solution. With rising maltose concentrations ATP became lower (Fig. 3). With fructose the ATP concentration at 30 min of ischemia (4.5 µmol/g_{dw}) was the highest of all groups. After 120 min, however, ATP was hardly elevated compared to kidneys protected without saccharide addition to the protective solution (Fig. 3). The renal ATP content was linearly dependent on the anaerobic lactate production (Fig. 4).

Disaccharides, contrary to glucose, did not show unequivocal improvements in the renal SAN (ATP + ADP + AMP) content. With 60 mM sucrose the adenine nucleotides were even somewhat lower than after the application of the pure HTK solution. The lowest SAN contents were found with fructose; at about 60 min of ischemia SAN was less than half the correspondent value after HTK protection (4.1 vs. 10.2 µmol/g_{dw}) (Fig. 5).

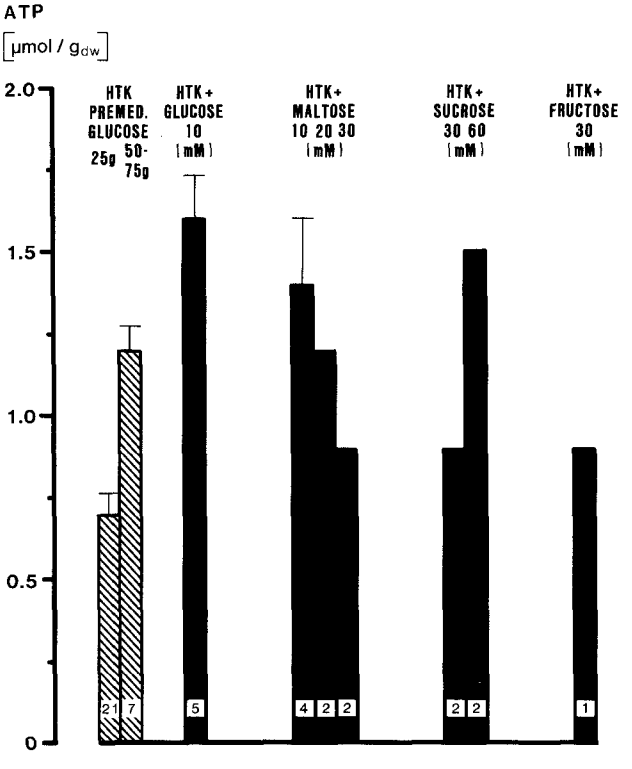

Fig. 3. Renal ATP content ($\bar{x} \pm$ SEM) after 120 min of ischemia at 25°C under different additives to the protective solution

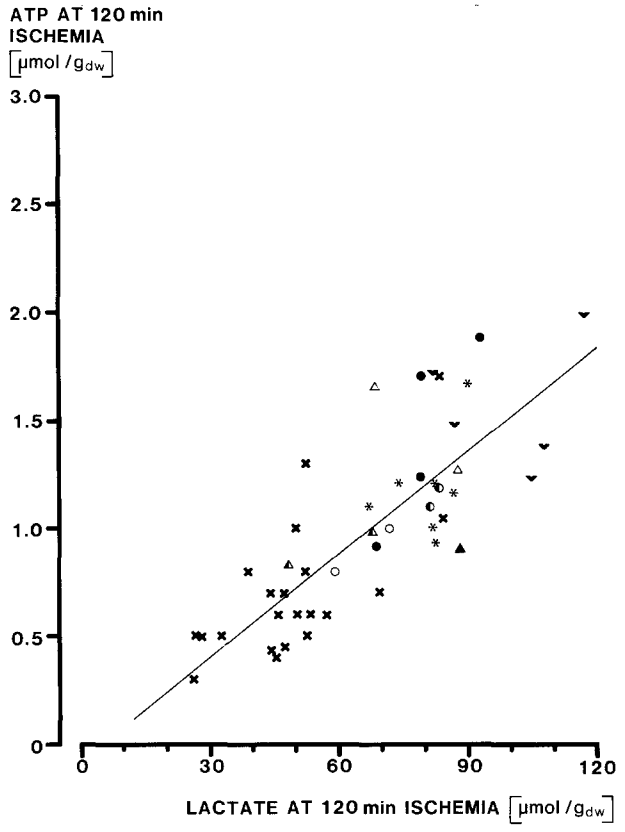

Fig. 4. Interrelation between renal ATP and lactate content after 120 min of ischemia at 25°C. Protection: (×) HTK-solution ($n=21$); (*) HTK-solution after increased (50–75 g) glucose premedication ($n=7$); (v) HTK-solution with 10 mM glucose ($n=5$); (●) HTK-solution with 10 mM maltose ($n=4$); (◐) HTK-solution with 20 mM maltose ($n=2$); (○) HTK-solution with 30 mM maltose ($n=2$); (▲) HTK-solution with 30 mM sucrose without mannitol ($n=2$); (△) HTK-solution with 60 mM sucrose without mannitol ($n=2$); (▲) HTK-solution with 30 mM fructose without mannitol ($n=1$). Correlation line for the HTK-protected kidneys with both standard and elevated glucose premedication ($n=28$): ×; *): $y=0.016+0.015\times$; $r=0.785$

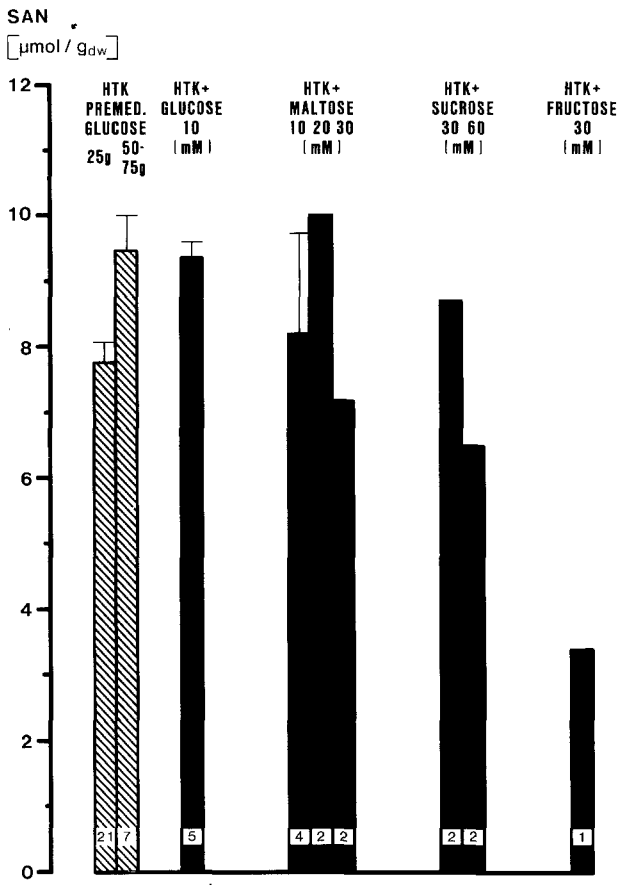

Fig. 5. Renal SAN content ($\bar{x}\pm$SEM) after 120 min of ischemia at 25°C under different additives to the protective solution

pH in the outer medulla

The renal pH in the outer medulla decreased during ischemia starting from values of about 7.3 in all groups. With glucose and perhaps to some extent endogenous glycogen as the main substrate a plateau was reached consistently in the course of ischemia although on different levels depending on the nature and amount of the prevailing substrate. Up to 120 min the pH with the addition of 10 mM glucose was comparable to the pH with 30 mM maltose. Whereas the pH with glucose hardly changed afterwards, it further decreased with 30 mM maltose. On the other hand the pH with 10 mM maltose fell slower than with 30 mM maltose or 10 mM glucose. Despite a higher lactate production, however, the pH after renal perfusion with HTK + 60 mM sucrose was comparable to corresponding values after HTK protection (Fig. 6).

Discussion

Recently it has been shown that a low content of glucose and glycogen in kidneys protected with a substrate free solution limits intraischemic glycolysis [15]. Although addition of glucose to the protective solution does increase glycolysis (Figs. 2, 4), a substrate such as glycogen, apart from its higher glycolytic energy yield, would be perhaps more favourable due to its slow cleavage avoiding a metabolic stress as a consequence of too high a load with easily usable substrate at the onset of ischemia [16]. Disaccharides must be cleaved before their components can reach the intracellular space which delays the intracellular substrate accumulation as seen in the course of the renal glucose concentrations during ischemia (Fig. 1). Since maltase is present in the kidney [19] the metabolic utilisation of an external disaccharide would be easier

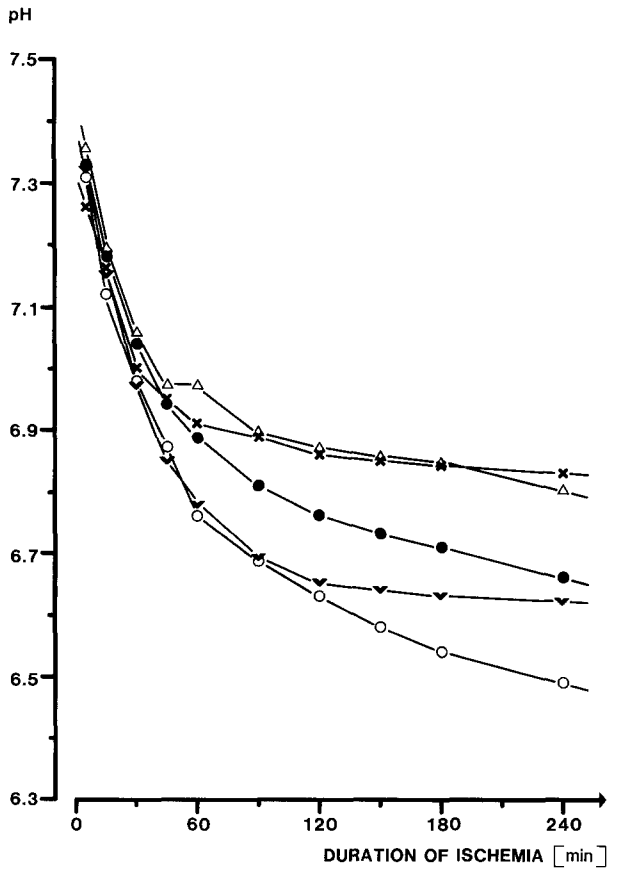

Fig. 6. Renal pH ($\bar{x}$) in the outer medulla of canine kidneys during 240 min of ischemia at 25°C under different additives to the protective solution: (×) HTK-solution ($n=10$); (v) HTK-solution with 10 mM glucose ($n=5$); (●) HTK-solution with 10 mM maltose ($n=4$); (○) HTK-solution with 30 mM maltose ($n=2$); (△) HTK-solution with 60 mM sucrose without mannitol ($n=2$)

for maltose than for sucrose. Because of its very slow cleavage in the kidney sucrose has been added to protective solutions as an osmotic agent [1, 8]. Nevertheless, in osmotically relevant concentrations sucrose obviously has also metabolic effects.

Sucrose in a 30 mM concentration increased both ATP and SAN content. However, an additional increase from 30 to 60 mM further enhanced only ATP but not SAN. SAN became even lower than in HTK protected kidneys. The reason for this phenomenon may lie in a phosphate trapping due to fructose metabolism.

Fructose given parenterally in high dosage to rats results in large accumulations of phosphorylated carbohydrate metabolites in the renal proximal tubule where fructokinase and fructose-1-P aldolase activities have been found [7]. The straight segment has the highest fructokinase but a lower fructose-1-P aldolase than the proximal convoluted part. Hence, with a fructose load fructose-1-P rises most in the straight part of the proximal tubule which also suffers the greatest changes. Nevertheless, a considerable amount of fructose-1-P accumulates in the proximal convoluted segments, as well [7]. So much phosphate is thereby sequestered as to cause a drastic fall in phosphate and, as a consequence of a phosphate dependence of oxidative metabolism [4] and glycolysis [21], a decrease of ATP [7]. As ATP inhibits 5′-nucleotidase, and as phosphate inhibits AMP deaminase, AMP arising in the tissue is liable to undergo enhanced dephosphorylation and deamination under the conditions occuring after fructose loading [24].

It is conceivable that similar reactions in the proximal tubule occured under the ischemic conditions of our experiments. Since the two enzymes peculiar to fructose metabolism, fructokinase, and fructose-1-P aldolase are confined to the proximal tubule [7], in more distal nephron parts fructose was probably degraded directly via glycolysis. Hence, in our overall tissue specimens the higher intraischemic ATP supply in distal nephron parts due to glycolytic degradation of the sucrose components fructose and glucose [15] may have superposed on the effects of fructose metabolism in the proximal tubule. With a fructose additive to the HTK solution the contribution of the glucose portion of sucrose on glycolytic ATP supply was absent. Hence, the unfavourable disinhibition of AMP degradation by fructose metabolism could take effect in a drastic SAN decrease at only a slight ATP elevation in the overall tissue specimens.

The fructose effects on glycogenolysis cannot be examined with sucrose due to the simultaneous glucose release. With only a fructose additive, however, a sharp decline of the renal glucose content was observed (Fig. 1). Since phosphorylation of the glycogen phosphorylase is necessary for glycogen degradation, an inhibition of glycogenolysis by phosphate trapping in the proximal tubule may have contributed to the rapid glucose disappearance with fructose. On the other hand, the glycogen content is lower in the proximal tubule than in more distal segments [2]. Since glucose disappeared completely, fructose probably also affected glucose and glycogen metabolism in the distal nephron parts. Fructose is reported to increase phosphorylase a in the liver [11, 17, 20, 22]. Thus, fructose possibly stimulates glycogenolysis in the ischemic distal nephron thereby depleting endogenous substrate reserves even during early ischemia. In addition, a direct stimulation of glycolysis is likely to occur, not only due to the substrate effect of fructose but also by virtue of fructose 2,6-bisphosphate accumulation, a potent stimulator of glycolysis [10]. This interpretation is supported by the steep lactate increase in the first

hour of ischemia (Fig. 2) and a temporarily even slower ATP decrease at the onset of ischemia with 30 mM fructose than with an 10 mM glucose solution additive. Later on, however, distinct ATP concentration differences existed in favour of the glucose group (Fig. 3).

In spite of higher lactate production the pH with 60 mM sucrose was not lower than pH values in HTK protected kidneys (Fig. 6). It is rather improbable that sucrose would not have increased glycolysis only in the outer medullar region where the pH measurements were done if it had enhanced glycolysis in other renal sites. Hence, sucrose had possibly reduced the H^+ extrusion out of the cells which is unfavourable in the presence of a buffer in the extracellular space [5, 12, 18].

Corresponding to a lower lactate production, maltose in a 10 mM concentration was slightly less effective on glycolytic ATP supply than 10 mM glucose (Figs. 2–4). The decrease in pH was retarded in relation to glucose (Fig. 6). The results with higher concentrations suggested that an augmentation of the maltose content in the HTK solution beyond 10 mM would not be advantageous. The findings do not allow a final decision whether glucose or maltose is superior. If, however, the cellular energy content must not fall below a crucial limit to maintain viability [15, 23], a moderate substrate addition in form of maltose or glucose to the protective solution could become a means to further improve renal ischemia tolerance provided that such an addition is not associated with intolerable negative side-effects, for instance on cellular membranes or volume regulation.

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