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The impacts of experimental necrotizing pancreatitis on hepatocellular ion homeostasis and energetics: An in vivo nuclear magnetic resonance study

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Background. *Liver dysfunction may be an early event or the end result of multiple organ dysfunction (MOD) in necrotizing pancreatitis. This study measured the early changes in hepatocellular ions and energetics associated with such conditions.*

Methods. *Twenty-five rats, prepared with a ^{23}Na and ^{31}P double-tuned nuclear magnetic resonance surface coil secured over the dome of the liver, were randomized into 5 groups: control, 10 and 20 minutes of total inflow ischemia, pancreatitis induced by deoxycholic acid (DCA), and sham-DCA (saline injection). Dysprosium-$TTHA^{3-}$ solution was used to separate the intracellular and extracellular sodium peaks.*

Results. *In rat liver, 20 minutes of total inflow occlusion caused irreversible depletion of high-energy phosphates. Changes at 2 hours after the onset of DCA-pancreatitis are compared with changes after 20 minutes of ischemia (mean ± SEM). Although the DCA-pancreatitis animals did not become hypotensive until 1 hour after the induction of pancreatitis, the changes in hepatic intracellular ions and energetics began soon after such an insult. At 2 hours after the onset of pancreatitis, hepatocellular pH_i and $[NA^+]_i$ were 6.99 ± 0.16 and 78.4 ± mmol/L, respectively ($P<.01$, compared with sham animals). A similar pattern of changes in hepatic bioenergetics also occurred. After the onset of pancreatis, the hepatic cytostolic phosphorylation potential decreased with time ($y=0.654$ —$0.004t$, where t is time in minutes and $r^2 = 0.967$ and the rate of hepatic hydrolysis of adenosine triphosphate increased progressively ($y = 0.702t + 91.363$, where t is time in minutes and $r^2 = 0.969$. These changes correlated well with the accumulated $[Na]_i$.*

Conclusions. *Unresuscitated necrotizing pancreatitis caused severe hepatocellular acidosis, profound sodium accumulation, and bioenergy depletion early in its course. These effects were as severe as those induced by total liver ischemia. Liver dysfunction may be an early, not terminal, event of MOD in necrotizing pancreatitis. (Surgery 1998;124:372-9.)*

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MULTIPLE ORGAN DYSFUNCTION (MOD) is a major complication of circulatory shock caused by hemorrhage, sepsis, or conditions that render the patient at risk for major fluid shift. It is the most important complication of necrotizing pancreatitis, accounting for the high mortality rate associated with this disease.[1,2] Severe acute pancreatitis is characteristically associated with a marked increase in the microvascular permeability. The subsequent large volume loss from the intravascular into the interstitial space leads to distant organ hypoperfusion. These processes result in alteration or disruption in the functions of all organ systems, leading to hemodynamic instability, respiratory insufficiency and failure, acute kidney failure, liver dysfunction, coagulopathy, and macrophage abnormality. With the advances in critical care, few patients with severe acute pancreatitis die from direct consequences of hypovolemia; most deaths occur later in the course of illness, often a result of sepsis or MOD.[2, 3]

Because of its central role in metabolic homeostasis and host defense, the liver has an important role in the prevention or development of sepsis or MOD during critical illness. Although liver failure is usually thought to be a terminal event of lung and kidney failure, liver dysfunction may play a much earlier role in the

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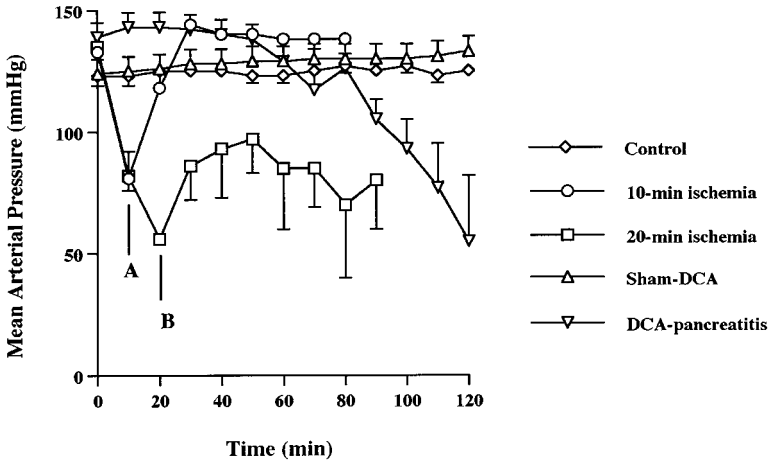

Fig. 1. Changes in MAP during ischemia/reperfusion and during necrotizing pancreatitis. **(A)** The point of reperfusion in the 10-minute ischemia group. **(B)** The 20-minute ischemia group. The hypotension was statistically significant in both groups during ischemia, but only in the 20-minute ischemia group during reperfusion ($P < .01$, ANOVA for repeated measurements). Animals with pancreatitis became significantly hypotensive only at 80 minutes after the onset of pancreatitis ($P < .05$, ANOVA for repeated measurements).

development of MOD. There is evidence that hepatic dysfunction occurs early after hemorrhage and persists despite adequate fluid resuscitation.[4-6] This persistent hepatic dysfunction after fluid resuscitation may be accounted for, in part, by a progressively compromised microvascular flow in the liver that eventually leads to tissue ischemia and cellular dysfunction.[7] Furthermore, experimental biochemical liver support may delay the transition to decompensated shock and the subsequent development of MOD.[8-10] These observations suggest that a progressive liver dysfunction may be the basis for the eventual failure of other organ systems in critically ill patients. Therefore, early detection and prevention of such liver dysfunction are logical and crucial in the care of patients at risk for development of MOD.

This study evaluated the use of nuclear magnetic resonance (NMR) spectroscopy in in vivo monitoring of early hepatic dysfunction. We measured the changes in hepatocellular pH and sodium concentration, cytosolic phosphorylation potential, and adenosine triphosphate (ATP) hydrolysis rate in necrotizing pancreatitis and compared these changes with those of total hepatic inflow ischemia. We tested the hypothesis that unresuscitated necrotizing pancreatitis adversely affects the liver early in its course in a magnitude equivalent to that of total hepatic inflow ischemia.

METHODS

Experimental model. Our animal protocol was approved by the Animal Use and Care Administrative Advisory Committee of the University of California at Davis School of Medicine. Adult male Sprague-Dawley rats (weighing 360 to 420 g each) were injected with an intraperitoneal dose of sodium pentobarbital (65 mg/kg). General anesthesia was maintained by additional intravenous injections of sodium pentobarbital (6.5 mg/kg) as needed, and body temperature was kept at 37° C using a water-circulating heating pad. The carotid artery and the jugular vein were isolated through a midline neck incision and cannulated with PE-50 polyethylene tubing (Becton Dickinson & Co, Sparks, MD). A celiotomy was performed and the xiphoid process was removed. In the ischemic protocol, the common hepatic artery and the portal vein were isolated, and a 4-mm adjustable vascular occluder (In Vivo Metric, Healdsburg, Calif.) was placed around both vessels. In the pancreatitis protocol, the common hepatic duct was ligated near the hilum of the liver; an extraduodenal incision on the common bile duct was made and the common pancreaticobiliary duct was cannulated with a PE-10 polyethylene tube (Becton Dickinson & Co). The double-tuned ^{31}P and ^{23}Na NMR surface coil was secured over the dome of the liver at the intersection of the three lobes. The liver was covered with plastic wrap, and the abdomen was closed watertight in two layers.

Experimental protocols. After being randomized into 5 groups ($n = 5$ each), the animals underwent the preceding procedures. They were placed in a custom-built NMR probe, in supine position with

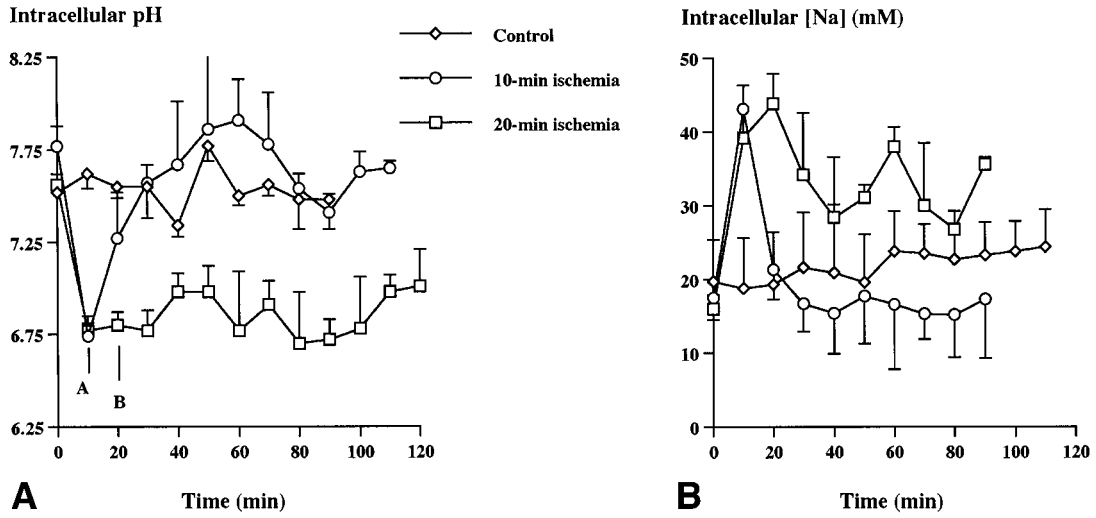

Fig. 2. Changes in hepatocellular ion homeostasis during ischemia and reperfusion. **(A)** Intracellular acidification persisted throughout reperfusion in the 20-minute ischemia group ($P < .01$, ANOVA for repeated measurements). **(B)** There was some restoration of sodium homeostasis in the 20-minute ischemia group, but $[Na]_i$ remained twofold higher than baseline at 1 hour after reperfusion ($P < .05$, ANOVA for repeated measurements).

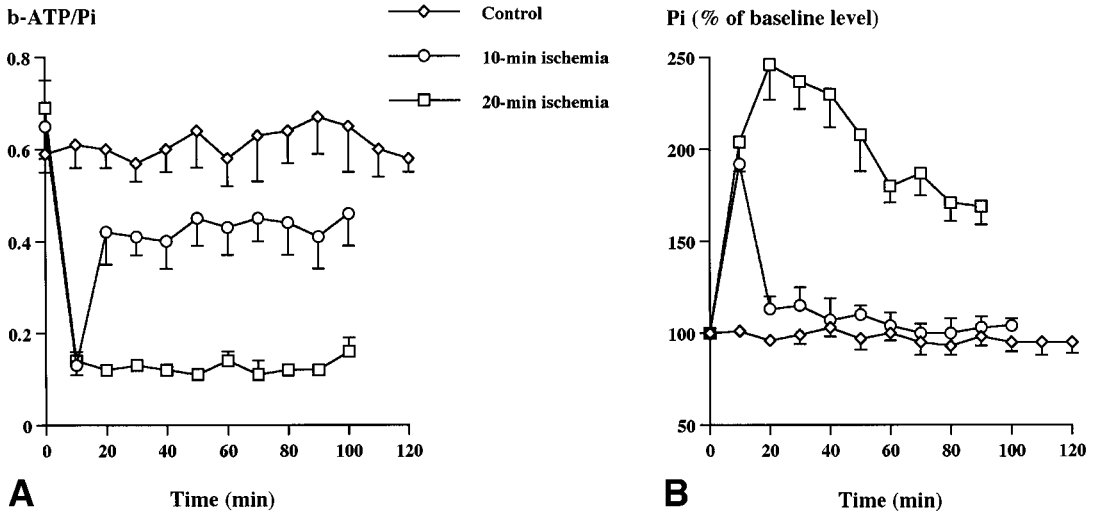

Fig. 3. (**A**) Impaired cytosolic phosphorylation potential persisted during reperfusion only in the 20-minute ischemia group ($P < .01$, ANOVA for repeated measurements). **(B)** The rate of hepatocellular ATP hydrolysis remained elevated during reperfusion only in the 20-minute ischemia group ($P < .01$, ANOVA for repeated measurements).

body temperature maintained at 37° C using a stream of warm air while in the magnet. After preparation for NMR spectroscopy, the animals underwent 1 hour of baseline data collection. Two protocols were studied: total hepatic inflow ischemia and necrotizing pancreatitis. In the ischemic protocol, control animals underwent the preparation without actual occlusion of hepatic inflow. The other two groups had total hepatic inflow occlusion for 10 and 20 minutes, followed by 1 hour of reperfusion. In the pancreatitis protocol, the animals received a retrograde injection of the common pancreaticobiliary duct with 100 μL of either a 20% (wt/vol) solution of deoxycholic acid (DCA-pancreatitis) (Sigma Chemical Co, St Louis, Mo) or normal saline (Sham-DCA) with constant pressure monitoring (< 20 cm H_2O).

NMR spectroscopy. All experiments were conducted using the GE Omega 300 spectrometer equipped with a 7-T horizontal-bore supercon-

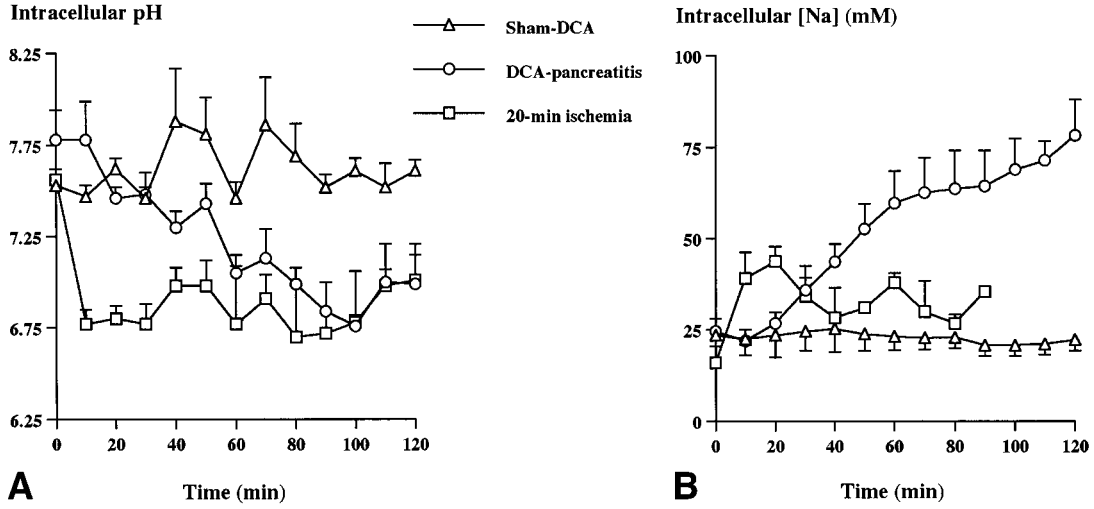

Fig. 4. (A) Changes in hepatocellular pH and **(B)** sodium concentration during pancreatitis. At 2 hours after the onset of pancreatitis, these changes were as severe as or worse than those associated with 20 minutes of inflow ischemia.

ducting magnet (GE NMR Instruments, Fremont, Calif). The NMR surface coil was a custom-built, two-turn circular coil probe constructed from enameled copper wire, with impedance matched and double-tuned to 79.4685 MHz for ^{23}Na and 121.6148 MHz for ^{31}P, respectively. Magnetic field homogeneity was obtained with the animal at the center of the field by shimming on the ^{23}Na free induction decay (FID) from the rat liver, with a typical linewidth of 60 to 80 Hz for the ^{23}Na resonance. $DyTTHA^{3-}$ solution (250 mmol/L), prepared from dysprosium oxide (Molycorp Inc., Louviers, Colo) and triethylenetetraminehexaacetic acid (TTHA, Sigma Chemical Co), as previously described,[11] was infused intravenously at the rate of 4.55 mmol/kg/hr. After 1 hour of intravenous infusion, a steady state was reached with a chemical shift difference of 2.5 to 3.0 ppm between the intracellular and extracellular Na resonances, and the infusion rate was reduced to and maintained at 2.9 mmol/kg/hr.

Interleaved ^{23}Na (60-μs excitation pulse, predelay time of 50 ms, acquisition time of 250 ms and 1904 scans) and ^{31}P (40-ms excitation pulse, predelay time of 1.5 sec, acquisition time of 1.5 sec and 272 scans) spectra were obtained using 2K and 4K data points, respectively, over a Å4000 Hz sweep width. The FID was Fourier-transformed after baseline correction and multiplication by an exponential function corresponding to 10-Hz line broadening for the ^{23}Na nucleus and 30-Hz line broadening for the ^{31}P nucleus.

The intracellular ^{23}Na concentration ($[Na^+]_i$) was calculated from the calibrated area under the unshifted peak of the ^{23}Na spectra after subtracting out the extracellular peak.[12] The intracellular pH was calculated from the chemical shift difference between the inorganic phosphate (P_i) peak and the α-ATP peak using the following equation[13] $pH = 6.75 + \log_{10}\{(x - 10.85)/(13.25 - x)\}$ where x is the chemical shift (ppm) of P_i peak relative to the α-ATP peak. Hepatic bioenergetics were determined from the resonance areas of the β-ATP and P_i peaks.

Data analysis. The results are reported as mean ± SEM. Analysis of variance (ANOVA) for repeated measurements was performed to examine changes within groups and between groups (Statview 4.5, Abacus Concepts, Inc., Berkeley, Calif). A *P* value less than .05 was considered significant. When results were significant for within-group changes, Dunnett's multiple range post hoc test was performed to identify the times at which the values differed from baseline. When significant differences among the groups existed, post hoc contrast tests were performed to identify the differences.

RESULTS

The mean arterial pressure (MAP) of all 5 groups of animals during the various protocols is shown in Fig. 1. MAP was stable in the control and sham-DCA groups. Animals with total hepatic inflow ischemia experienced immediate hypotension, with severity a function of the duration of ischemia. Ten minutes of total hepatic inflow occlusion induced a transient hypotension from 133 ± 2 mm Hg to 81 ± 11 mm Hg ($P < .05$), and within 20

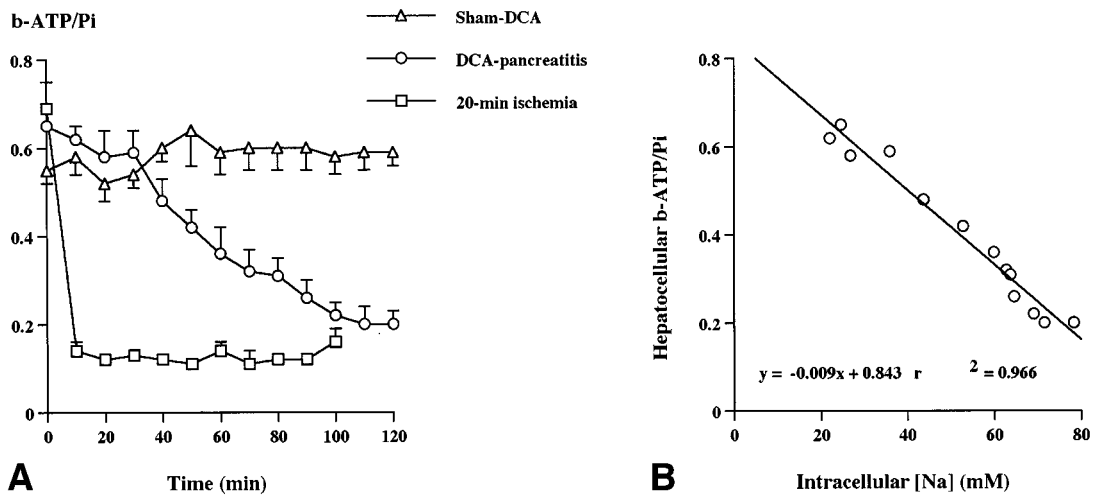

Fig. 5. Changes in hepatic cytosolic phosphorylation potential **(A)** during pancreatitis. Note that the increase in $[Na]_i$ preceded the depletion of hepatic high-energy phosphates and the relation between intracellular Na buildup and the high-energy phosphate content.

minutes after the start of reperfusion, MAP reverted to and remained at baseline level. In animals with 20 minutes of total hepatic inflow ischemia, MAP decreased to as low as 56 ± 2 mm Hg ($P < .05$) and, during reperfusion, it remained depressed. One hour after the start of reperfusion, MAP remained low, at 80 ± 20 mm Hg ($P < .05$), in the animals with 20 minutes of ischemia, compared with 138 ± 1 mm Hg in animals with only 10 minutes of ischemia. In the DCA-pancreatitis group, MAP remained normal for 1 hour after the onset of pancreatitis before it began to decrease. By 2 hours after the onset of necrotizing pancreatitis, MAP was 55 ± 27 mm Hg ($P < .05$), as low as that in animals after 20 minutes of total hepatic inflow ischemia.

Liver ischemia and reperfusion. Total hepatic inflow ischemia induced rapid accumulation of both intracellular proton and sodium (Fig. 2). Intracellular pH (pH_i) dropped quickly to 6.74 ± 0.07 and 6.77 ± 0.08 in the 10- and 20-minute ischemia groups, respectively ($P < .05$). With reperfusion, pH_i returned to baseline level of 7.54 ± 0.1 within 20 minutes in the 10-minute ischemia group. The livers with 20 minutes of total inflow ischemia remained acidotic at 6.72 ± 0.11 1 hour after reperfusion ($P < .05$). With ischemia, hepatic $[Na^+]_i$ increased from 17.5 ± 3 mmol/L to 43 ± 3.2 mmol/L in the 10-minute ischemia group and from 16 ± 1.9 mmol/L to 43.8 ± 4.1 mmol/L in the 20-minute ischemia group. One hour after reperfusion, $[Na^+]_i$ was 15.3 ± 3.4 mmol/L in the 10-minute ischemia group, but it remained elevated in the 20-minute ischemia group, at 26.8 ± 2.5 mmol/L ($P < .05$).

The ratio β-ATP/P_i, as an index of cytosolic phosphorylation potential, declined from a baseline level of 0.65 ± 0.07 and 0.69 ± 0.06 to 0.13 ± 0.02 and 0.14 ± 0.02, respectively, in the first 10 minutes of ischemia (Fig 3, *A*). If the ischemia was limited to only 10 minutes, the liver regained some of its cytosolic phosphorylation potential, with its β-ATP/P_i ratio being 0.42 ± 0.02 at 60 minutes after reperfusion. If the ischemia was 20 minutes, there was no evidence of recovery of the liver's cytosolic phosphorylation potential, with the β-ATP/P_i ratio remaining depressed at 0.12 ± 0.01 after 1 hour of reperfusion. The P_i content, an index of intracellular utilization or hydrolysis of ATP, rose quickly to 192% ± 9% of baseline level after 10 minutes of ischemia and 246% ± 19% after 20 minutes of ischemia (Fig. 3, *B*). With reperfusion, hepatocellular P_i returned to baseline level only in the 10-minute ischemia group and remained elevated in the 20-minute ischemia group at 171% ± 10% of baseline level 1 hour after the start of reperfusion.

DCA-pancreatitis and hepatocellular changes. Although the DCA-pancreatitis animals did not develop systemic hypotension for 1 hour after induction of pancreatitis (Fig. 1), the changes in hepatic intracellular ion homeostasis and energetics began soon after such an insult (Figs. 4 through 6). Hepatocellular acidosis developed linearly with time ($y = 7.701 - 0.008t$, where t is time in minutes and $r^2 = 0.831$). Similarly, the accumulation of hepatocellular sodium follows the equation $y = 0.481x + 23.103$, where t is time in minutes and $r^2 = 0.945$. At 2 hours after the onset of pancreatitis, hepatocellular pH_i and $[Na^+]_i$ were 6.99 ±

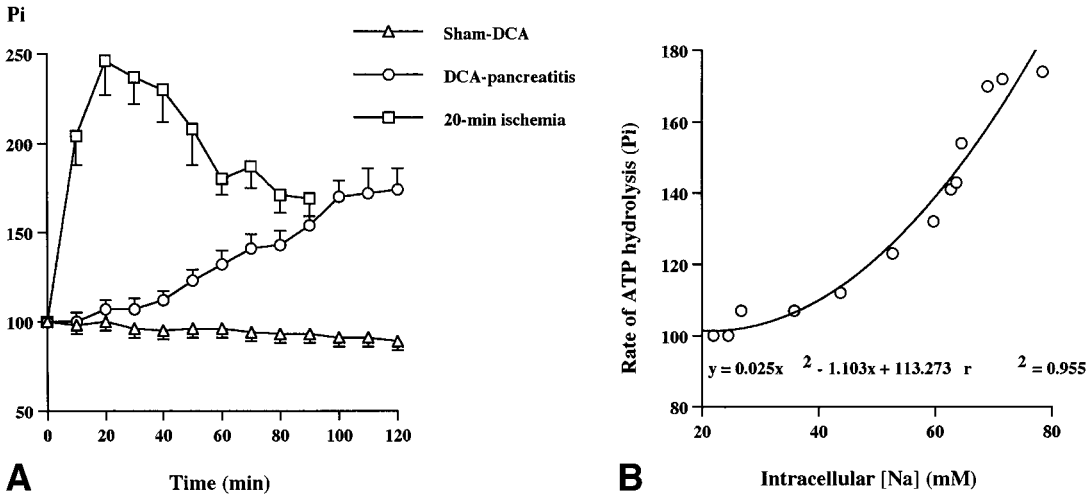

Fig. 6. The rate of hepatocellular ATP hydrolysis **(A)** increased in response to the accumulated $[Na]_i$ during pancreatitis.

0.16 and 78.4 ± 9.8 mmol/L, respectively. A similar pattern of changes in hepatic bioenergetics also occurred. After the onset of pancreatitis, the hepatic cytosolic phosphorylation potential decreased with time ($y = 0.654 - 0.004t$, where t is time in minutes and $r^2 = 0.967$) (Fig. 5), and the rate of hepatic hydrolysis of ATP increased progressively ($y = 0.702t + 91.363$, where t is time in minutes and $r^2 = 0.969$) (Fig. 6). These changes correlated well with the accumulated $[Na]_i$.

DISCUSSION

The liver is a critical organ for the body regulation of metabolism and substance clearance, and clinical evaluation of hepatic dysfunction remains a difficult task. Recognition and assessment of liver damage and recovery have been traditionally done with a liver enzyme profile based on circulatory level of synthesized products such as albumin, metabolic end-products such as total bilirubin, or enzymes released by hepatocyte damage. Unfortunately, these classic liver function tests only assess hepatocyte injury, and they have a very poor predictive value in the detection of liver dysfunction.[14] The large functional capacity of the liver and its ability to regenerate make such assessment of liver function difficult. In other words, these tests can only confirm the presence of hepatocyte injury, but they cannot quantitate the level of impaired liver function. There have been promising reports on novel in vivo liver function assessment based on the measurement of monoethylglycinexylidide, a metabolite of lidocaine.[15-17] The potential problem with this method is that the first pass, mixed function oxidative process, required to convert lidocaine into its metabolite requires blood flow passing through the splanchnic circulation, which may be impaired in the shock state.

NMR monitoring of hepatocellular disturbances associated with various systemic insults provides a continuous assessment of early hepatic dysfunction and detection of impending hepatic failure in critically ill patients. Our data on hepatic ischemia demonstrated that both hepatocellular cytosolic phosphorylation potential and its ability to correct intracellular acidosis were quickly impaired within 10 minutes after total inflow occlusion. These findings were consistent with previous reports by others.[12, 18] Furthermore, irreversible liver damage occurred if total inflow occlusion lasted longer than 20 minutes. We also found that intracellular sodium loading occurred along with intracellular acidification, independent of the duration of ischemia. This suggested that the accumulation of $[Na^+]_i$ may not simply be a result of hepatocellular ATP depletion but rather an attempt of the cells to correct the intracellular acidosis via the Na-H antiport. Such a pH-stimulated cell membrane transporter, the Na-H antiport, has been identified in rat hepatocytes.[19] In other words, an increase in sodium uptake, not a decrease in sodium extrusion, may account for the build-up in $[Na^+]_i$ during ischemia. With reperfusion, the acidification process resulting from anaerobic metabolism stopped, the sodium influx slowed down, and $[Na^+]_i$ returned to normal level owing to Na extrusion by Na-K-ATPase activity. The cell rebuilt its energy potential, and the rate of utilization returned to normal level in reversible ischemia. In

irreversible ischemic cases, intracellular acidosis persisted, hepatocellular cytosolic phosphorylation potential remained impaired, and the rate of ATP hydrolysis remained high, suggesting that energy consumption remained active to restore intracellular ion homeostasis, in this case, to offload the accumulating sodium. Depletion of ATP eventually occurred, Na-K-ATPase became deactivated, and $[Na^+]_i$ continued to rise, resulting in activation of other cascades leading to eventual cell death.

Our data also demonstrated that hepatocellular injury occurred early in the course of necrotizing pancreatitis. Although MAP was maintained near baseline level during the first hour of the insult, statistically significant hepatocellular changes began as early as 30 to 40 minutes after the onset of acute pancreatitis. This suggests that resuscitation based on cardiovascular parameters alone may not be adequate to prevent hepatic dysfunction in acute pancreatitis. Furthermore, the changes in hepatocellular ion homeostasis during acute pancreatitis precedes the depletion of high-energy phosphates, suggesting that deactivation of Na-K ATPase is neither the principal nor the initial event of the cascade leading to hepatocellular dysfunction. In fact, there is a correlation between the rate of hepatocellular utilization of ATP and the accumulation of intracellular Na after the onset of necrotizing pancreatitis. In other words, it may be the increased $[Na]_I$ that drove up hepatocellular consumption of bioenergetics. Therefore, prevention of cell swelling resulting from sodium loading may be more important than attempts to resupply the cells with high-energy phosphates. Preventing or limiting hepatocellular sodium accumulation during acute pancreatitis may help preserve the liver's cytosolic high-energy phosphate content and avoid activation of other sodium-stimulated cascades leading to cell death. The progression of liver dysfunction is also linear to time, and by 2 hours after the onset of acute pancreatitis, the degree of liver dysfunction is as severe as that caused by 20 minutes of total inflow ischemia. This experimental observation confirms the clinical impression of the need of early recognition of shock and aggressive fluid resuscitation in severe acute pancreatitis.[1-3] Necrotizing pancreatitis is known to release proinflammatory cytokines and ascites-associated factors that can induce distant organ dysfunction.[20-22] It remains unclear whether these toxic substances also have direct effects on the liver, and it is the resultant liver dysfunction that leads to disturbances in distant organ homeostasis and their eventual failure. Although our experiment does not conclusively confirm the later scenario, the data do support the contention that liver dysfunction occurs early in patients with necrotizing pancreatitis, or in patients who are at risk for development of distant organ dysfunction, as previously reported in animal studies of hemorrhagic or septic shock.[4-7,10]

We conclude that experimental necrotizing pancreatitis induces hepatocellular acidosis, intracellular sodium accumulation, and depletion of hepatic bioenergetics early in its course. These changes are severe and progressive, and they may be the result of increased activities in cell membrane transport mechanism leading to an increased utilization rate of high-energy phosphates to restore intracellular homeostasis. Liver dysfunction may develop early and progressively in necrotizing pancreatitis and it is not a terminal event of multiple organ dysfunction. Preservation of liver function may be as important as resuscitation of the cardiopulmonary system during the initial management of severe acute pancreatitis.

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DISCUSSION

Dr Alden H. Harken (Denver, Colo). I am surprised by the difference between the 10- and 20-minute ischemia and am startled that the 20-minute period seems so irreversible. What is your interpretation of the sodium data? With a surface coil, although NMR technology is very powerful, you really are getting an average sodium concentration both intracellularly and extracellularly. Also, if you were looking at phosphorus and sodium, you must have also had a strong hydrogen ion signal. If you superimpose that on your sodium data, can you then look at both intracellular and extracellular compartments and total fluid accumulation within the gland?

Dr Ho. We used a protocol similar to what you describe using the Langendorff isolated heart perfusion apparatus, which is a better model for this technique. There was no significant difference when we used a double-tuned sodium and phosphorus spectroscopy compared with a single 31 phosphorus spectroscopy as far as intracellular pH was concerned. With regard to intracellular and extracellular sodium concentrations, we use the dysprosium to separate the two.

Dr William G. Cheadle (Louisville, Ky). There is a rather spectacular drop in intracellular pH, regardless of whether it is pancreatitis or ischemia. What was the extracellular serum pH at this time? Perhaps intracellular changes predate extracellular pH changes that we see clinically.

Dr Ho. I don't think that we have adequately addressed the relationship between extracellular and intracellular pH yet. However, data based on the Langendorff heart perfusion model in our laboratory suggest that it is more important to maintain intracellular pH first.

Dr Max R. Langham (Gainesville, Fla). Is preservation of the liver as important as resuscitation of the cardiopulmonary system? Since the drop in intracellular pH and other numbers are all gradual, isn't one directly related to the other? If you had another group that had control, for example, of cardiopulmonary resuscitation, would the results in the liver be dramatically different?

Dr Ho. I think you're absolutely correct on that. I intended to perform this experiment in an unresuscitated model. The idea is to raise awareness that you not only need to resuscitate the blood pressure and the cardiac index but also to pay attention to hepatic function. Ultimately, we may confirm that Dr Ranson's criteria are quite good. The problem with his criteria is that he used an index of hepatocellular damage, not dysfunction. So by the time you pick it up, it's too late. We believe that we have a better technology to detect the liver dysfunction early on. Ultimately, we can use it as a way to ensure that we adequately resuscitate the patient with acute pancreatitis.