J Anesth (1995) 9:65–69

Journal of
Anesthesia

Effects of ulinastatin on rat renal energy metabolism and blood flow in hemorrhagic shock

MASAAKI UEKI, SATOSHI YOKONO, and KENJI OGLI

Department of Anesthesiology & Emergency Medicine, Kagawa Medical School, 1750–1 Ikenobe, Miki-cho, Kita-gun, Kagawa, 760–07 Japan

Abstract: The effect of ulinastatin on rat renal energy metabolism and blood flow in hemorrhagic shock was studied by ^{31}P nuclear magnetic resonance spectroscopy. Hemorrhagic shock was induced by withdrawing blood from the left carotid artery into a reservoir until mean femoral arterial blood pressure stabilized as 20 mmHg. Ulinastatin (50 000 units·kg^{-1}; UTI group, n = 10) or saline (0.9% NaCl; NS group, n = 10) was injected continuously during 30 min of hemorrhagic shock. Next, the total volume of blood shed in the reservoir was transfused into the right femoral vein over a period of 5 min. In the UTI group, 23.2 ± 15.1% of adenosine triphosphate (ATP) remained and intracellular pH (pHi) was 6.77 ± 0.07 at 30 min of hemorrhagic shock. However, ATP was not detected and pHi showed severe acidosis (pHi: 6.49 ± 0.04) in the NS group. After the transfusion of shed blood, the UTI group exhibited higher ATP levels and pHi values than the NS group. Rats treated with UTI maintained mean arterial blood pressure and renal blood flow at significantly higher values than those administered NS.

Ulinastatin improved the energy metabolism of the shocked kidney. We believe that ulinastatin maintains mitochondrial function against hemorrhagic shock by its membrane-stabilizing actions and might contribute beneficially in hemorrhagic shock.

Key words: Hemorrhagic shock, Ulinastatin, Energy metabolism, ^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMR), Renal blood flow

Introduction

It has been shown that during shock in animals, there is an increase in the activities of trypsin [1], plasmin [2], kininogenase [3] and so on in the blood, and a decrease in the activity of serum plasmin inhibitor. This suggests that protease activities might play an important role in the pathogenesis and development of shock. In this regard, it can be suggested that an enzyme inhibitor attenuates the development of shock through the inhibition of lysosomal enzyme. Ulinastatin (Miraclid, Mochida Pharmaceutical, Tokyo, Japan) is an acid glycoprotein with a molecular weight of 67 000. Ohnishi et al. reported that ulinastatin improves survival time and hemodynamic status in experimental shock. They suggested that ulinastatin might attenuate the activities of lysosomal enzyme released into the serum during shock [4]. However, anti-shock mechanisms of ulinastatin in hemorrhagic shock and its metabolic effects have not been fully studied at the cellular level.

The purpose of our study was to evaluate the cellular and metabolic effects of ulinastatin on the renal blood flow and energy metabolism monitored by ^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMR) in hemorrhagic shock.

Methods

Animals

This study was approved by the Kagawa Medical School Animal Investigation Committee. Twenty male Wistar rats (JCL-Wistar, Hamamatsu, Japan; body weight 375–400 g) were used in this study. The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg body wt, Abbot, North Chicago, IL, USA). After tracheotomy, anesthesia was maintained with isoflurane (0.7%, Abbot) under artificial ventilation with a volume-limited respirator (EVM-50A, Aika, Tokyo, Japan). Polyethylene catheters (PE-50, Clay Adams, Parsippany, NJ, USA) were inserted into the femoral artery to monitor blood pressure and into the femoral vein for injection. Mean arterial

Address correspondence to: M. Ueki
Received for publication on June 6, 1994; accepted on October 12, 1994

blood pressure was monitored with a polygraph (366, NEC San-ei Instruments, Tokyo, Japan). The left kidney was exposed by a dorsal incision for ^{31}P-NMR observation and renal blood flow measurement. After completion of all surgery, rats were given heparin (500 U·kg^{-1} i.v.).

^{31}P-NMR

^{31}P-NMR spectra were obtained on a GSK 270 WB spectrometer (6.3T, JEOL, Tokyo, Japan) operating at 109.25 MHz for phosphorous. The kidney was attached to a five-turn surface coil (10 mm in diameter). To eliminate the effect of surrounding tissues on the NMR signal, a thin copper sheet was inserted to provide physical separation between the surrounding muscles and the kidney. The rat was placed in the bore of the magnet in a vertical head-up position. The body temperature was maintained at 36° ± 1°C throughout the experiment using temperature controller of our own design. The pulse width was 10 μsec and 400 scans were accumulated at 1.0-s intervals.

Since the β-peak of adenosine triphosphate (β-ATP) is the only peak that was unique to ATP on the spectrum, tissue ATP levels were assessed by comparing changes in the area of β-ATP. Intracellular pH (pHi) was calculated from the chemical shift between the peak of Pi and that of α-ATP [5–7].

Renal blood flow

Renal blood flow (RBF) in the left renal artery was measured continuously by a small-diameter flow transducer (FI-0057, Nihon Kohden, Tokyo, Japan) connected to an electromagnetic flow-meter (MFV-1200, Nihon Kohden). We determined absolute zero flow through the renal artery before an experiment by completely occluding the artery distal to the probe with forceps for 2–3 s; baseline drift was negligible. Renal blood flow was measured to an accurary of ± 15% [8].

Experimental protocol

Hemorrhagic shock was induced by withdrawing blood from the left carotid artery into a reservoir unil mean arterial pressure (MAP) reached 20 mmHg. This blood pressure was maintained by transferring blood in and out of the reservoir for 30 min. Then, the total blood shed in th reservoir was transfused to the rat via the right femoral vein over 5 min. Ulinastatin (50 000 U·kg^{-1}; UTI, $n = 10$) or saline (0.9% NaCl; NS, $n = 10$) was administered continuously during the 30 min of hemorrhagic shock. Each group was divided into subgroups, ^{31}P-NMR measurement ($n = 5$) and RBF measurement ($n = 5$), respectively. To ensure stability of the experimental conditions, the observation of ^{31}P-NMR spectra and the measurement of renal blood flow were performed three times (10-min intervals) before inducing hemorrhage as control, three times (10-min intervals) during hemorrhagic shock, and four times (every 15 min) after transfusion.

Statistical analysis

All values are expressed as mean ± SD. Mean values of each group were compared by analysis of variance (ANOVA) and Student's *t*-test for paired values. A P value less than 0.05 was considered to be statistically significant.

Results

In our hemorrhagic shock model, MAP was maintained at 20 mmHg during shock. No RBF was detected during hemorrhagic shock. To compare the severity of the hemorrhagic shock in both groups, we measured bleeding volume and body weight. No significant difference between the groups was found in either the bleeding volume or body weight (Table 1), indicating that the severity of the hemorrhage was similar in both groups.

Figure 1 shows sequences of ^{31}P-NMR spectra obtained from rat kidney treated with UTI and NS during the control period before ischemia (bottom trace), and during ischemia (next 3 traces), and subsequent transfusion (top 6 traces). During hemorrhagic shock, the β-ATP signal rapidly decreased and disappeared after 20 min, and that of inorganic phosphate shifted to the right in the NS group. However, the β-ATP signal in the UTI group persisted during hemorrhagic shock.

Figure 2 shows the time course of MAP during the experiment. The UTI group exhibited higher MAP values throughout the recovery period with a final MAP of 94.4 ± 7.9 mmHg.

Figure 3 shows the time course of RBF during the experiment. The control value of RBF was 3.7 ml·min^{-1}·grams kidney weight^{-1} in both groups. No RBF was measured during hemorrhagic shock, but at 60 min after

Table 1. Bleeding volume and body weight in both groups

	Hemorrhagic shock + Saline ($n = 10$)	Hemorrhagic shock + Ulinastatin ($n = 10$)
Bleeding volume (ml)	5.9 ± 0.2	6.1 ± 0.6
body weight (g)	387.5 ± 8.5	387.5 ± 8.5

All value are mean ± SD.

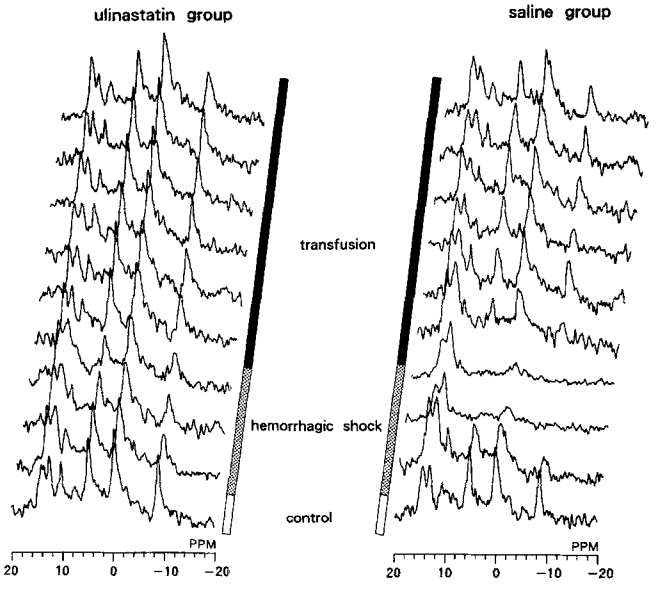

Fig. 1. Representative ^{31}P nuclear magnetic resonance (^{31}P-NMR) spectra during hemorrhagic shock (30 min) and after transfusion period (60 min) of ulinastatin and saline groups. Each spectrum was obtained by accumulation of 400 data points. The vertical axis shows the time course of the experiment, the abscissa shows the chemical shift in ppm. The peaks observed were, *from left to right*: phosphomonoesters, inorganic phosphate, phosphodiesters, and the three resonances of adenosine triphosphate (ATP)—γ, α, and β-phosphate

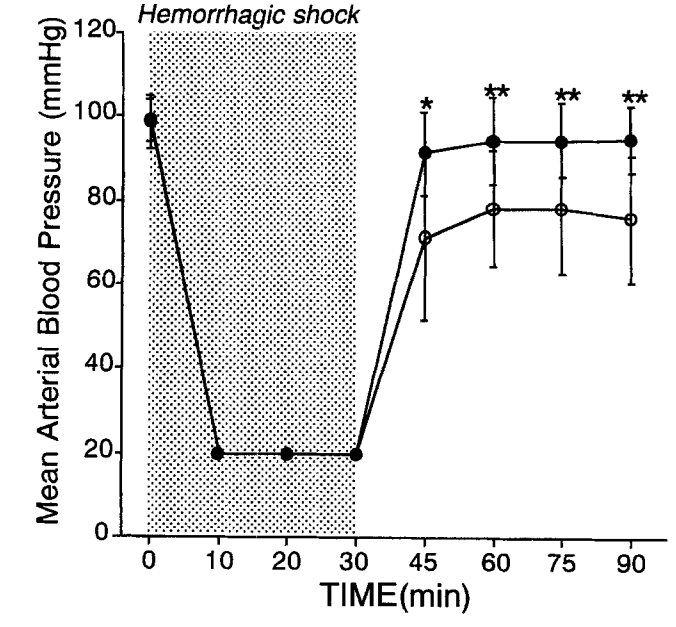

Fig. 2. Time course of changes in mean arterial blood pressure during hemorrhagic shock and transfusion period. All values are mean ± SD. UTI, ulinastatin group (*closed circles*, $n = 10$); NS, saline group (*open circles*, $n = 10$). $^{*}P < 0.05$, $^{**}P < 0.01$ UTI vs NS

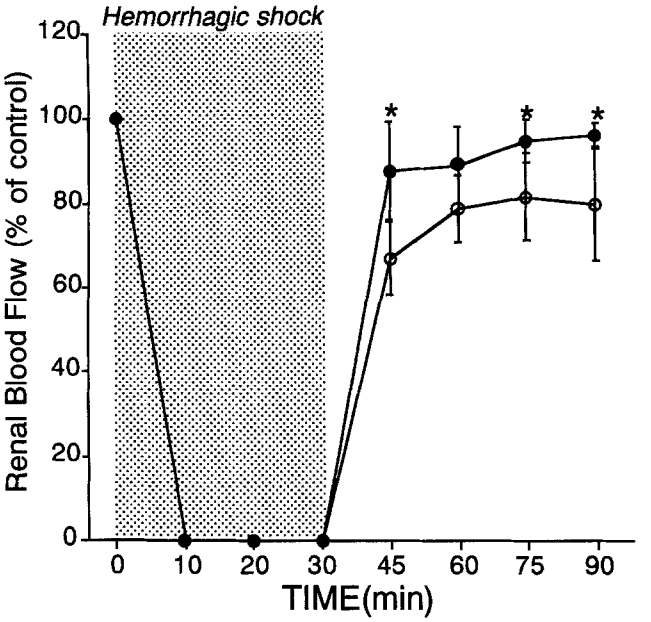

Fig. 3. Time course of changes in renal blood flow during hemorrhagic shock and transfusion period. All values are mean ± SD. UTI, ulinastatin group (*closed circles*, $n = 5$); NS, saline group (*open circles*, $n = 5$). $^{*}P < 0.05$, $^{**}P < 0.01$ UTI vs NS

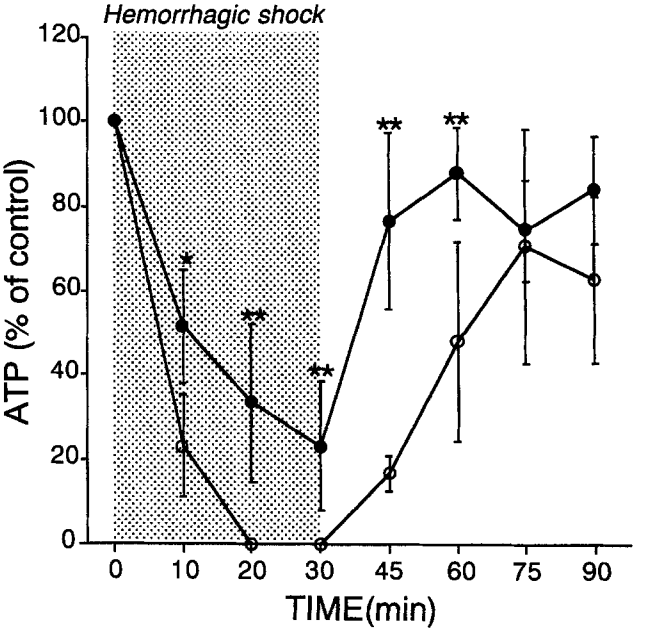

Fig. 4. Time course of changes in ATP during hemorrhagic shock and transfusion period. All values are mean ± SD. UTI, ulinastatin group (*closed circles*, $n = 5$); NS saline group (*open circles*, $n = 5$). $^{*}P < 0.05$, $^{**}P < 0.01$ UTI vs NS

transfusion it had returned to 79.5 ± 13.5% of control values in the NS group and 95.7 ± 3.1% in the UTI group. The UTI group exhibited significantly higher RBF values than the NS group after reperfusion.

Figure 4 shows the time course of the changes in ATP during the experiment. In the NS group, ATP was

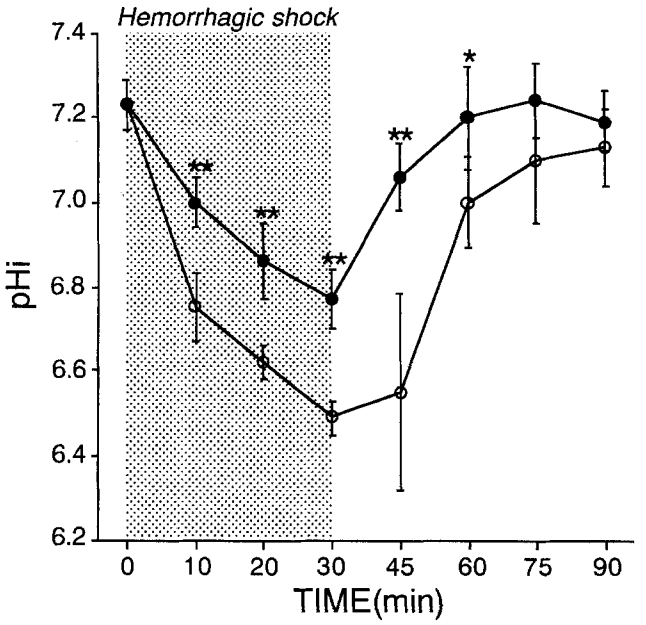

Fig. 5. Time course of changes in intracellular pH (pHi) during hemorrhagic shock and transfusion period. All values are mean ± SD. UTI, ulinastatin group (*closed circles*, $n = 5$); NS saline group (*open circles*, $n = 5$). $^{*}P < 0.05$, $^{**}P < 0.01$ UTI vs NS

rapidly depleted during hemorrhagic shock and was 62.5 ± 19.6% of the control value at 60 min after transfusion. However, 23.2 ± 15.1% of ATP was still detected at 30 min of hemorrhagic shock and 83.8 ± 12.4% of the control value at 60 min after transfusion in the UTI group. The UTI group exhibited significantly higher ATP values than the NS group during the experiment.

The changes of pHi are shown in Fig. 5. In the NS group, pHi showed severe acidosis (pHi: 6.49 ± 0.04 from 7.23 ± 0.06) at 30 min of hemorrhagic shock and 7.13 ± 0.09 at 60 min after transfusion. In the UTI group, pHi was 6.77 ± 0.07 at 30 min, which was not as acidic as that in the NS group, and finally returned to 7.19 ± 0.07. The UTI group exhibited significantly higher pHi values than the NS group during the experiment.

Discussion

Hemorrhagic shock leads to a release of lysosomal enzymes, extreme depletion of ATP, accumulation of lactic acid, and the decrease of tissue pH [9]. Because tissue viability depends on adenine nucleotides [10,11], several investigators have attempted to inhibit ATP depletion or accelerate its restoration to improve the survival rate after hypoxic damage [12–15]. It is well accepted that ischemia caused by circulatory deficiency is important in the pathogenesis of cell injury involving the disruption of lysosome and zymogen granules during shock. Thus, these subcellular alterations cause the release of lysosomal enzymes and the formation of toxic substances by these enzymes, resulting in further tissue injury during shock [16–18]. In this regard, it can be proposed that an enzyme inhibitor possibly attenuates the development of shock through the inhibition of lysosomal enzyme.

Ulinastatin was extracted and purified from fresh human urine by the method of Proksch and Routh [19]. It is an acid glycoprotein (molecular weight 67 000) which is known to inhibit trypsin, α-chymotrypsin, lipase, amylase, elastase, and carboxypeptidase [20]. Previously, ulinastatin was reported to improve survival time and hemodynamic status in experimental shock [4]. However, cellular and metabolic effects of ulinastatin on various organs have not been fully studied and its anti-shock mechanisms in hemorrhagic shock have not yet been resolved. After intravenous administration of ulinastatin, its renal concentration increases and almost all of the administered drug will be stored in the kidney tissue. Therefore, the kidney is the most suitable test organ for ulinastatin investigations. In this study, the effects of ulinastatin on renal energy metabolism were investigated in hemorrhagic shock using ^{31}P-NMR.

The application of ^{31}P-NMR to provide a noninvasive assessment of the phosphorous metabolism of living cells is well established [21,22]. Real time values for ATP, Pi and pHi were obtained from the ^{31}P-NMR.

In this study, ATP was completely depleted within 20 minutes in the NS group. In the UTI group, however, 20% of the ATP was still maintained at 30 min of hemorrhagic shock and pHi was not as low as in the NS group. After transfusion of the shed blood, MAP, RBF, ATP, and pHi in the UTI group were higher than those in the NS group. These results confirmed the beneficial effects of ulinastatin on metabolic aberration during and after hemorrhagic shock.

There is a close correlation between lysosomal function and ATP metabolism [23–25]. The uncoupling of oxidative phosphorylation in mitochondria is caused by lysosomal enzymes, which results in a depression of phosphorylation. An study revealed that the lysosomal enzymes released during anoxia and low flow states could destroy the structure, and thus the function, of mitochondrial membranes [26]. Mela et al. [27] suggested that released lysosomal enzymes in ischemic tissues directly caused an alteration in mitochondrial energy-linked functions. This implies that drugs with membrane-stabilizing or enzyme-inhibiting effects might improve the energy metabolism of the hypoxic organs.

According to this theory, our results suggest that ulinastatin can maintain the energy metabolism of the

kidney by lessening the mitochondrial damage caused by hemorrhagic shock.

Ulinastatin also inhibited the decrease of pHi during and after shock in our study. Depletion of ATP leads to a marked reduction in oxidative phosphorylation, and ischemic cells begin anaerobic glycolysis with the accumulation of lactate, concomitantly generating hydrogen ions, which decreases pHi. In the UTI group, the membrane channels could transport these hydrogen ions out of the cell because of the utilization of the ATP that was still maintained during and after hemorrhagic shock. Consequently, the decrease in pHi was not severe in the UTI group.

Another factor believed to be involved in the damage of mitochondria during ischemia and reperfusion is free radical-induced membrane lipid peroxidation [28]. Yoshikawa et al. [29] suggested that ulinastatin may be oxygen radical scavenger. Ulinastatin might diminish mitochondrial damage during and after hemorrhagic shock and this may involve the scavenging of free radicals. Further studies will be necessary to determine this.

Ulinastatin restored MAP, RBF, ATP, and pHi after hemorrhagic shock in our studies. This result suggests that ulinastatin improved the energy metabolism in the shocked kidney, which might mean that ulinastatin could protect the mitochondrial function from hemorrhagic shock due to its membrane-stabilizing effect.

References

1. Beard EL, Hampton JK Jr (1963) Effect of trauma on rat serum proteolytic activity. Am J Physiol 204:405–407
2. Aasen AG, Gallimore MJ, Ohlsson K, Amundsen E (1978) Alterations of plasmin activity, plasminogen levels and activity of antiplasmins during endotoxin shock in dogs. Haemostasis 7:164–169
3. Herlihy BL, Lefer AM (1974) Selective inhibition of pancreatic proteases and prevention of toxic factors in shock. Cire Shock 1:51–60
4. Ohnishi H, Suzuki K, Niho T, Ito C, Yamaguchi K (1985) Protective effects of urinary trypsin inhibitor in experimental shock, Jpn J Pharmacol 39:137–144
5. Gadian DG, Radda GK, Richards RE, Seeley PJ (1979) ^{31}P NMR in living tissue: the road from a promising to an important tool in biology. In: Shulman RG (ed) Biological applications of magnetic resonance. Academic, New York, pp 463–535
6. Seo Y, Murakami M, Watari H, Imai Y, Yoshizaki K, Nishikawa H, Morimoto, T (1983) Intracellular pH determination by a ^{31}P-NMR technique. The second dissociation constant of phosphoric acid in a biological system. J Biochem 94:729–734
7. Adam WR, Koretsky AP, Weiner MW (1986) ^{31}P-NMR in vivo measurement of renal intracellular pH: effects of acidosis and K^+ depletion in rats. Am J Physiol 251:F904–F910
8. Ozawa H, Takeda S, Ogawa K, Kanai H, Okino H (1978) Research for errors of blood flow measurement in electromagnetic flowmeters (in Japanese). J J M B (Japanese Journal of Medical Electronics and Biological Engineering) 16[Suppl]:224
9. Vogt MT, Farber E (1968) On the molecular pathology of ischemic renal cell death. Am J Pathol 53: 1–24
10. Collins GM, Taft P, Green RD, Ruprecht R, Halasz NA (1977) Adenine nucleotide levels in preserved and ischemically injured canine kidneys. World J Surg 2:237–243
11. Warnick CT, Lazarus HM (1977) Adenine nucleotides during organs storage. Transplant Proc 9:1575–1577
12. Crowell JW, Jones CE, Smith EE (1969) Effect of allopurinol on hemorrhagic shock. Am J Physiol 216:744–748
13. Cunningham SK, Keaveny TV (1978) Effect of xanthine oxidase inhibitor on adenine nucleotide degradation in hemorrhagic shock. Eur Surg Res 10:305–313
14. Fernando AR, Armstrong DMG, Griffiths JR, Hendry WF, O'Donghue EPN, Perrett D, Ward JP, Wickham JEA (1976) Enhanced preservation in the ischaemic kidney with inosine. Lancet 1:555–557
15. Siegel NJ, Avision MJ, Reilly HF, Alger JR, Shulman RG (1983) Enhanced recovery of renal ATP with postischemic infusion of ATP-$MgCl_2$ determined by ^{31}P-NMR. Am J Physiol 245:F530–F534
16. Lefer AM (1973) Blood-borne humoral factors in the pathophysiology of circulatory shock. Circ Res 32:129–136
17. Blattberg B, Levy MN (1965) Detection of reticuloendothelial-depressing substance in shock. Am J Physiol 209:71–74
18. Clowes GHA, Farrington GH, Zuschneid W, Cossette GR, Saravis C (1970) Circulating factors in the etiology of pulmonary insufficiency and right heart failure accompanying severe sepsis (peritonitis). Ann Surg 171:663–678
19. Proksch GJ, Routh JI (1972) The purification of the trypsin inhibitor from human pregnancy urine. J Lab Clin Med 79:491–499
20. Muramatu M, Mori S, Matsuzawa Y, Horiguchi Y, Nakanishi Y, Tanaka M (1980) Purification and characterization of urinary trypsin inhibitor, UTI_{68}, from normal human urine, and its cleavage by human uropepsin. J Biochem 88:1317–1329
21. Gadian DG, Radda GK (1981) NMR studies of tissue metabolism. Annu Rev Biochem 50:69–83
22. Shulman RG (1983) NMR spectroscopy of living cells. Sci Am 248:86–93
23. Malbica JO, Hart LG (1971) Effect of adenosine triphosphate and some anti-inflammatory agents on a purified lysosomal fraction having high acid phosphatase and labile β-glucoronidase activity. Biochem Pharmacol 20:2017–2026
24. Wilkinson JH, Robinson JM (1974) Effect of ATP on release of intracellular enzymes from damaged cells. Nature 249:662–663
25. Hallak GJ, Wilkinson JH (1977) Action of adenosine phosphates on the release of intracellular lactate dehydrogenase from human and rat lymphocytes. Enzyme 22:361–369
26. Mellors A, Tappel AL, Sawant PL, Desai ID (1967) Mitochondrial swelling and uncoupling of oxidative phosphorylation by lysosomes. Biochim Biophys Acta 143:299–309
27. Mela L, Miller LD, Bacalzo LV, Olofsson K, White RR (1973) Role of intracellular variations of lysosomai enzyme activity and oxygen tension in mitochondrial impairment in endotoxemia and hemorrhage in the rat. Ann Surg 178:727–735
28. Fiskum G (1985) Mitochondrial damage during cerebral ischemia. Ann Emerg Med 14:810–815
29. Yoshikawa T, Murakami M, Seto O, Yoshida N, Takemura T, Tanigawa T, Miyagawa H, Morita Y, Tainaka K, Yokoe N, Sugino S, Kondo M (1988) Protective effects of a protease inhibitor, ulinastatin, on endotoxin shock and its scavenging effect on oxygen-derived free radicals (abstract in English). Masui (Jpn J Anesthesiol) 37:969–974