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Role of Extracellular ATP in Hepatic Hemodynamics and Glucoregulation

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ROLE OF EXTRACELLULAR ATP

IN

HEPATIC HEMODYNAMICS AND GLUCOREGULATION

by

Joong-Woo|Lee

A Dissertation Submitted to the Faculty of the Graduate School

of Loyola University of Chicago in Partial Fulfillment

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VITA

Joong-Woo Lee, son of Ryu-Jong Lee and Ik-sun Shon, was born on February 4, 1943 in Kyong-Joo, Korea. He attended secondary and high school in Seoul. Undergraduate studies were carried out at Yonsei University in Seoul, Korea. In February of 1966, he graduated with a Bachelor of Science degree in Biology.

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The author is married to Young-Za Ryu and they have three sons, Han-Sung, Han-Kuk and Han-Chul.

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CHAPTER I

INTRODUCTION

The regulation of circulating blood glucose levels by hepatic mechanisms is critical for life. Adequate glucose production by the liver depends on proper circulatory hemodynamics as well as neuroendocrine regulation. The main processes of glucose delivery into the blood are glycogenolysis in the fed state and gluconeogenesis in the fasted state. The rates of hepatic glycogenolysis and gluconeogenesis are determined by many factors—hormones, hepatic blood flow, oxygen supply to the liver and intracellular levels of substrate as well as adenine nucleotides. The major function of intracellular ATP is as a free energy supply for most active processes including muscle contraction, active transport of substances through the biological membranes, and biosynthesis of cellular components.

In addition, extracellular ATP and related adenylate affect both vascular resistance and tissue metabolism. Adenosine and adenine nucleotides are potent vasodilators in vascular beds including the skeletal muscle, skin, intestinal vessels and the coronary circulation. On the other hand, a vasoconstrictor response to adenine compounds has been reported in various tissue preparations, viz, ATP increased the tension of canine saphenous vein strips, aortic strips and human umbilical arterial strips. ATP also contracted the isolated perfused renal artery. Therefore, the vascular response to ATP depends

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on the tissue preparation under study. However, the exact mechanisms for ATP-induced changes in vascular resistance are obscure.

Exogenous ATP produces hyperglycemia *in vivo* and in the perfused liver. In histochemical and electrophysiological studies, purine nucleotides, especially ATP were proposed as a neurotransmitter in various mammalian organs. A purinoreceptor-mediated glucose output for the ATP-induced hyperglycemia has been proposed by Clemens et al. (84) but the mechanism of hepatic glucose output is unknown.

Prostaglandins are recognized to play regulatory roles in smooth muscle tone and cell metabolism. There are many kinds of prostaglandins and the responses to prostaglandins depend on the animal species, the tissues, and prostaglandin type. Ca^{++} ion mobilization may be involved in prostaglandin action on smooth muscle. Cellular metabolism is also affected by prostaglandins due to changes in adenylate cyclase activity. Altough some controversy exists, many investigators have demonstrated that prostaglandin E stimulates adenylate cyclase in the hepatocyte. Many stimulants release prostaglandins--such as drugs, neural, and hormonal stimulation. ATP is also a potent stimulator of prostaglandin release from a number of tissues including liver, heart, lung, spleen and fat pad. Therefore, ATP-induced changes in vascular resistance and hepatic glucose production may involve a role for prostaglandins. If prostaglandins are involved in the ATP-induced changes in vascular resistance and hepatic glucose output, then indomethacin, a prostaglandin synthesis inhibitor, should attenuate ATP actions.

The overall purposes of present research were 1) to analyze the effects of exogenous ATP on the hepatic circulation in the perfused liver using measurements of both portal vein and hepatic arterial resistances and hepatic capacitance, 2) to characterize ATP action on liver carbohydrate metabolism including glucose and lactate production in the perfused liver or isolated hepatocyte preparation as well as oxygen consumption and K^+ movements in the perfused liver, and 3) to elucidate the mechanism of ATP on hepatic hemodynamics and liver metabolism using indomethacin treatment.

CHAPTER II

REVIEW OF RELATED LITERATURE

A. Experimental Models for the Study of Liver Metabolism and Hemodynamics

1. VARIOUS TECHNIQUES FOR THE STUDY OF LIVER METABOLISM

Various experimental models have been used for the study of liver metabolism--such as in vivo studies, perfused liver preparations, liver slice techniques, isolated hepatocyte preparations, liver homogenates, and isolated subcellular preparation. Although an in vivo study is physiologically the most ideal, the host of interactions with other organs complicates interpretation of specific liver functions. The liver slice technique is appropriate for qualitative elucidation of constituent functions and metabolic pathways; however, liver slices-- although they consist mainly of intact cells--have proved unsuitable for certain studies because essential components of liver metabolism are greatly changed by slicing. According to Krebs et al. (222), liver slices lose most of their nucleotides during incubation--for example, in the case of ATP, more than 70% is lost in 10 minutes of slice incubation. The rates of gluconeogenesis and ketone body formation are also much lower in liver slices than in the perfused liver and were accounted for by loss of ATP from the liver slices during incubation (221, 292).

The perfused liver preparation has been widely employed in quantitative studies of liver metabolism. The first liver perfusion was conducted by Claude Bernard (27) in 1855 in the course of his classical observations on glycogen breakdown in the dog. After his initial study many investigators perfused the liver in different species and with a variety of techniques. In early investigations mainly large mammalian livers were used for perfusion. Trowell (333) first perfused the rat liver for a study of urea synthesis from ammonia. In his study the liver was perfused in a retrograde direction, namely, oxygenated saline was pumped into the vena cava and exited from the portal vein. He also reported that the liver perfusion preparation excelled in function over liver slices. Subsequently Miller et al. (248) developed a perfusion-aeration apparatus and an operative procedure for the perfusion of the rat liver with oxygenated blood. Miller et al. (248) then employed their model to establish the dominant role of the liver in the biosynthesis of the plasma proteins. The apparatus and operative technique developed by Miller have been extensively used by many investigators for study of liver processes, including carbohydrate, protein and lipid metabolism, hormone action, membrane transport and ion fluxes.

Since the liver consists of 60% hepatic parenchymal cells by number and the parenchymal cells actually contribute over 87% to the total liver volume (155), isolated liver cell preparations are widely used to study liver metabolic functions. In the early development of methods for the isolation of parenchymal cells, physical methods for

instance, mechanical dispersion were employed (4, 39, 196, 204). However, the physical methods have not been widely accepted because of defects of cell damage and low metabolic rate (29, 31). Consequently Howard et al. (184) isolated hepatocytes with an enzymatic technique which incubated liver slices with collagenase and hyaluronidase. They obtained structually and functionally intact hepatocytes. A disadvantage of this technique was the low yield of only about 5% of the original tissue. Berry and Friend (30) introduced the enzymatic technique using liver perfusion rather than incubation of slices. They used calcium-free Hanks' solution containing 0.05% collagenase and 0.1% hyaluronidase and obtained more than a 50% yield of intact hepatocytes. According to Krebs et al. (222), the perfused liver and isolated hepatocyte preparations have almost the same performance in oxygen consumption, glucose production, urea synthesis and ketone body formation. Isolated hepatocytes also retain adenine nucleotides (176, 222) and K^+ ions (18) and exhibit appropriate responses to hormones (201). Therefore, the isolated hepatocyte preparation is one of the favored models for the study of liver metabolism.

2. PERFUSION MEDIUM AND PERFUSION FLOW FOR ISOLATED LIVER MODELS

In general, there are two kinds of liver perfusion medium--erythrocyte containing medium and erythrocyte free-medium. Erythrocyte containing medium has the advantage that due to a high oxygen carrying capacity, it allows much lower flow rate to achieve adequate oxygenation and thus avoids hypoxic injury. Usually the perfusion flow rates in models using rat whole blood or with red blood cell containing medium

are lower than those in models using red blood cell free medium (113, 119, 140, 197, 214, 248, 295, 310). However, in some experiments the value of red blood cell containing medium is limited because 1) red blood cells consume glucose and produce lactate (151), 2) red blood cells are hemolyzed during perfusion and release K^+ ions (10, 307), 3) it is difficult to elucidate porphyrin metabolism by the liver perfused with red blood cells (298) and 4) in models using a non-recirculating system (once-through perfusion), large volumes of perfusion medium are required. These considerations favor the use of red blood cell free medium in various experimental protocols.

If red blood cell free-medium is used, the flow rate must be incresed several times greater than the physiologic portal vein flow in order to insure sufficient oxygen supply to the liver. An inappropriate low perfusion may produce cell injury. According to Schmucker and Curtis (297), a low perfusion flow rate of 15 ml/min (approx. 1.3 ml/gm/min) with KRB resulted in extensive hepatocyte injury suggestive of hypoxia. In contrast, high perfusion flow of 72 ml/min (approx. 6.3 ml/gm/min) caused sinusoid distension and severe endothelial cell injury. Thus it is important to maintain an adequate perfusion flow to prevent both hypoxia and endothelial cell injury. Some investigators maintained low liver temperature to reduce oxygen demand (159, 323). In addition, Schmucker et al. (298) reported that hyperbaric oxygenation protected the liver from hypoxia and endothelial cell injury at near physiologic hepatic portal vein flow rates.

Several groups have reported the use of certain inert

fluorocarbons which have high solubility characteristics for oxygen and carbon dioxide in perfusion systems. According to Triner et al. (331), the liver perfused with fluorocarbon FC-47 emulsion with bicarbonate buffer had higher oxygen extraction and greater gluconeogenesis. Goodman et al. (151) compared three different perfusion media i.e. erythrocyte-free KRB buffer, erythrocyte-KRB buffer (at 15% hematocrit) and fluorocarbon (FC-47)-KRB buffer emulsion, with respect to their ability to maintain a viable perfused rat liver preparation. At flow rates of 30 ml/min gluconeogenesis, oxygen consumption, lactate and ketone body production and hepatic ATP concentration were the same with all three buffer preparations. However, at a relatively low perfusion flow (20 ml/min or less) the fluorocarbon-KRB and the erythrocyte-KRB buffer maintained better function than the erythrocyte free buffer due to their greater oxygen delivery. They concluded that fluorocarbon-KRB buffer had actually identical ability in their metabolic behavior to livers perfused with erythrocyte-containing buffer. Thus, fluorocarbon-KRB has certain advantages: it permits lower perfusion flow rates than erythrocyte-free medium with identical functions and it prevents inherent problems in using red blood cells such as consuming glucose and producing lactate etc. However, further studies of metabolic and histological alteration have to be accomplished to exclude any deleterious effect of fluorocarbon emulsions such as hepatotoxicity and uptake of these particles by liver cells (81).

3. ANALYSIS OF HEPATIC HEMODYNAMICS

The hepatic circulation has a unique vascular arrangement of dual

blood supplies consisting of the portal vein and hepatic artery. The portal vein collects blood from pancreas, stomach, spleen, small intestine and large intestine; thus it carries absorbed nutrients, hormones and toxins to hepatic parenchymal, and reticuloendothelial cells. The hepatic artery which arises from the celiac artery carries oxygen-enriched blood to the liver. The portal vein provides the liver with 67-80% of its blood supply, while the hepatic artery contributes the remaining 20-33% of hepatic blood flow (156). Hepatic blood flow varies with animal species; it ranges from 104 in human, 110 in dog, 134 in cat and 150 ml/min/100gm liver in rat (156, 158).

In the hepatic lobes the large portal vein branches repeatedly into small vessels and finally are connected with the sinusoids. The hepatic artery also branches and eventually the hepatic arterioles also join the sinusoids. After circulation through the sinusoids, the blood paths merge into the central vein and finally reach the inferior vena cava tributaries.

The hepatic sinusoids are specialized capillaries. They are on average 330 um long and 9-12 um wide--but this width can enlarge to 30 um when the sinusoidal pressure increases (72). The wall of sinusoids consist of a discontinuous basement membrane, endothelial cells, and Kupffer cells. It has varying sized fenestrations of 100-200 nm in diameter. Because of these fenestrations, sinusoids have high permeability to substances, even to relatively high molecular weights of 250,000 (65). Sinusoidal porosity allows perfusion of the space of Disse easily possible even with low intersinusoidal pressure (2-3 mmHg).

Portal vein pressure ranges from 5-10 mmHg, while mean hepatic arterial pressure is about 100 mmHg (156, 158). This indicates that the portal vein circulation has a low pressure-low resistance character, while the hepatic artery has a high-pressure-high resistance nature. Therefore, small changes of portal vein pressure cause large changes in flow. There are several sites which produce vascular resistance in the liver. The wall of portal venules and hepatic venules as well as the hepatic arterioles contain smooth muscle that modifies vascular resistance and also controls the liver capacitance. McCuskey (241) reported the presence of sphincters consisting of contractile endothelial cells located at the junctions of sinusoids with portal venules (inlet spincters), with central venules (outlet sphincters) and intersinusoidal sinusoids (sphincters of intersinusoids). These spincter systems also contribute – to changes in hepatic vascular resistance.

Hepatic vascular resistance is regulated by various stimuli including hormones, drugs and nerve stimulation. Hepatic nerves, particularly sympathetic nerves, exert major effects on the hepatic circulation. In contrast, parasympathetic nerves only sparsely innervate the hepatic vascular beds. Stimulation of sympathetic nerves increases the resistance of portal vein, hepatic vein and hepatic artery, consequently it causes net decreases in hepatic capacitance. Since the liver contains a large volume of stored blood, changes in liver capacitance may contribute to redistribution of blood into the pulmonary and systemic circulations.

There are reciprocal relationships between hepatic portal venous flow and hepatic arterial flow. When a branch of the portal vein was occluded, hepatic arterial flow to that area increased and when portal venous blood flow increased, hepatic arterial flow decreased (157, 165). This response may serve to maintain total hepatic blood flow and sinusoidal perfusion pressure. However, the mechanism of this reciprocal relationship of flow has not yet been elucidated.

B. Characteristics of Adenine Nucleotides

1. BIOCHEMICAL CHARACTERISTICS OF ATP

Adenosine triphosphate (ATP) was first discovered in mammalian muscle extracts by Fiske and Subbarow (128). A chemical structure was established by Lythgoe and Todd (234). ATP was synthesized by Baddiley et al (14). ATP consists of an adenine, a ribose, and a triphosphate unit. At a pH of 7.0 ATP is a highly charged anion and sodium salts are stable in aqueous solution for months at $-15^{o}C$ but for only 1 week at $4^{o}C$ (22). In solution ATP slowly decays forming 5′-adenylic acid (AMP). ATP is an energy-rich molecule because it contains two phosphoanhydride bonds (P-O-P). 7.3 Kcal/mole of free energy is liberated when ATP is hydrolyzed to ADP and orthophosphate (Pi) or hydrolyzed to AMP and pyrophosphate (PPi) (226). Biological systems use the free energy from ATP hydrolysis for such active processes as muscle contraction, biosynthesis of carbohydrates, proteins, nucleic acids and lipids and active uptake of many substances through the biological membrane.

ADP and AMP as well as ATP also play important roles in intracellular functions including glycogenolysis, glycolysis and mitochondrial respiration. Furthermore, the balance of these adenine nucleotides in the cytosol has often a more important influence than the actual concentration of each nucleotide because of their opposite allosteric activatory or inhibitory activity. ATP, ADP and AMP are interconvertible and usually the sum of their concentrations in the living cells remains relatively constant. Atkinson (12) established the concept of energy charge ([ATP] + 1/2[ADP]/[ATP] + [ADP] + [AMP]) which reflects the cellular energy balance between the energy generating processes and energy utilizing processes. According to Ozawa (269), the energy charge of the liver cells decreases under various cell conditions such as in endotoxicosis, in hypoxia and in hemorrhagic shock. In these states hepatic intracellular ATP _levels decrease and AMP levels increase.

2. MOVEMENTS OF ATP ACROSS THE PLASMA MEMBRANE

It is controversial if ATP crosses cell plasma membranes. Glynn (150) reported that ATP is impermeable to the plasma membrane; however, many investigators have reported that ATP does indeed cross the plasma membrane. Buchthal et al. (43) observed that ATP caused twitch-like or short tetanus-like contraction and caused birefringence of isolated frog skeletal muscle. In this study they suggested that ATP permeates the membrane of the muscle fiber. Chaudry and his associates have provided strong evidence of ATP movements across the plasma membrane in various tissues by using radioisotope labelled adenine compounds. Chaudry and

Gould (76) reported that soleus muscle takes up ATP from the external medium. In their study ^{14}C-labelled ATP and ADP were found in the muscle and the data ruled out the possibility that ATP arose by synthesis from taken up adenosine. Chaudry et al. (80) have also demonstrated the uptake of ATP by liver and kidney slices, and they confirmed that this intracellular ATP was due to the movement of ATP itself from medium and not due to the resynthesis of ATP from its breakdown products. In further studies they have shown ATP uptake by rat soleus muscle using dual labelled ATP (^{32}P-ATP and ^{14}C-ATP). They also proposed that the transport of ATP into the cell would be a carrier-mediated process (74).

Similar results were obtained in the perfused rat kidney (339) and kidney cortex slice preparations (239). ATP added in the medium increased ATP content in the kidney tissues. Sharma and Eiseman (305) demonstrated ATP uptake by heart tissue; ATP treated rats following hemorrhagic shock have high content of ATP in their cardiac tissue. Pant et al. (270) have demonstrated that ATP movements across the squid axonal membrane and reported ATP (γ-^{32}P-ATP) in the extracellular space is utilized in the process of intracellular phosphorylation after transport into the axoplasm. ATP movements across the plasma membrane have been reported in the lymphoma cell (13). When ATP was added to the incubation medium of intact mouse lymphoma cells, the cyclic AMP level was enhanced with adenosine transport inhibitor. They interpreted that ATP enters the cell and serves as substrate for adenylate cyclase.

On the other hand, release of ATP into the plasma from various

cells has also been reported. Forrester and Lind (137) reported that arterial and venous blood of a human subject had 0.19 ug/ml and 0.7 ug/ml of ATP respectively. Forrester (136) measured the rate of appearance of ATP in the blood during exercise as approximately 7.5-10.5 ug/min. He suggested that ATP detected in the venous effluent is derived from active skeletal muscle. The release of ATP from isolated heart cells has also been demonstrated (139). It was found that 0.34 ± 0.22 uM of ATP/mg protein was released by heart cells maintained in the oxygenated condition, while 1.28 ± 0.41 uM of ATP/mg protein was released by cells under hypoxic conditions. Other tissues may also be sources of ATP in the plasma, including red blood cells (20, 133), vascular endothelial and smooth muscle cells (276), and nervous cells (183). In particular, the release of ATP from purinergic nerves as a neurotransmitter has been well recognized (52, 55, 58, 59, 321). Thus, on the basis of the above described studies, the release and subsequent uptake of extracellular ATP by the various cells may be normal physiological processes which are perturbed in various pathophysiologic states.

C. Physiological Role of Extracellular ATP

1. ATP AND VASCULAR RESISTANCE

Adenosine and adenine nucleotides (AMP, ADP and ATP) are potent vasodilators in most vascular beds. In 1929 Drury and Szent-Gyorgyi (107) first reported that dog carotid arterial pressure decreased following injection of adenosine and AMP. This vasodilator activity has

subsequently been demonstrated in various vascular beds, including coronary (23, 28, 97, 108, 132, 349), skeletal muscle (132, 216) human hand and forearm (110), forelimb (141), hindlimb (326), skin (132), ear (23) and intestinal vessels (90, 132, 161). Many investigators have attempted to correlate adenosine and adenine nucleotides to local regulation of blood flow but the hypothesis is accepted mainly in the coronary circulation (28; 162).

The relative vasodilator potency of the different adenosine compounds has been reported. Windbury et al. (348) determined the relative coronary dilator activity in molar potency values as follows; ATP 100, ADP 95, AMP 28, adenosine 25 and adenine inactive. Similar results were reported by Fleisch and Weger (129); for the cat and dog, ATP was a hundred times stronger than AMP as a dilator of the peripheral vessels. In isolated canine femoral arteries, the relaxation response to adenine nucleotides were ordered as follows: ATP = ADP > AMP = adenosine (246). However, according to Gillespie (148), adenine is most active in coronary vasodilation; the order of activity of the adenine compounds is adenosine > AMP > ATP. Kalckar and Lowry (203) failed to find any potency differences between three adenine nucleotides on rabbit arterial pressure by injection of equimolar amounts of AMP, ADP and ATP. Thus, quantitative comparison of the dilator activity for adenosine, AMP, ADP, and ATP is not clearly defined.

The magnesium salt of ATP (ATP-$MgCl_2$) as well as the sodium salt was used in some experiments, because there is some evidence that the ATP in the cell is present in ATP-Mg complex (32). Duff et al. (110)

compared vasodilator activity of magnesium salt with that of the sodium salt. While both substances were potent vasodilators, the dilator effect of the magnesium salt was greater than that of sodium salt.

A vasoconstrictor response to adenine compounds has also been reported in various tissue preparations. According to Ismail and Hems (193), adenosine caused vasoconstriction in perfused rat liver. In the renal circulation adenosine, AMP, and ADP increased renal vascular resistance (166, 185, 268, 329). Hrdina et al. (185) demonstrated vasoconstrictor effects of ATP on isolated perfused rat renal arteries. The contractile effect of ATP was sensitive to Ca^{++} concentration in the perfusion medium. With low Ca^{++} concentration in the medium, ATP had very little activity or was inactive. However, Scott et al. (300) reported that injection of adenosine into the renal artery increased renal vascular resistance, while injection of ATP decreased renal vascular resistance. Verhaeghe (338) demonstrated vasoconstrictor effects of adenosine nucleotides in the isolated vein. ATP and ADP but not adenosine increased basal tension of unstimulated (relaxed) dog saphenous vein strips. In contracted strips as induced by acetylcholine or norepinephrine, low concentrations ($10^{-6} - 10^{-5}$M) of ATP or ADP caused relaxation, whereas high concentrations ($10^{-4} - 10^{-3}$M) of both adenine nucleotides caused further contraction. Alpha-adrenergic blockade with phentolamine did not alter the contraction induced by ATP and ADP but removal of Ca^{++} from the incubation medium prevented the contraction induced by both adenine nucleotides. Similar results were reported in the isolated aortic strips (143). ATP caused a

contraction of a relaxed strip, whereas it caused relaxation in contracted aortic strips. Burnstock et al. (60) reported variations in effects of ATP in animal species. ATP produced relaxation or some biphasic response of the rabbit portal vein whereas ATP caused only contraction in the guinea-pig portal vein. Therefore, the responses to ATP or adenine compounds depend on the tissue preparation under study, the dose of agent employed, and the contractile state of the test preparation.

2. ATP AS A NEUROTRANSMITTER

Burnstock and his associates have pursued a purinergic neurotransmission hypothesis. Burnstock et al. (53, 54) observed a transitory relaxation and hyperpolarization during nerve stimulation in guinea-pig taenia coli smooth muscle after blocking both adrenergic and cholinergic nerve transmission with guanethidine and atropine. They confirmed the presence of non-adrenergic, non-cholinergic nerves in various organ systems including the gastrointestinal tract, urinary bladder, esophagus, trachea, lung and cardiovascular system (48, 56, 58, 59, 64, 144). In further studies, substances such as catecholamines, 5-hydroxytryptamine, histamine, prostaglandins, several amino acids, some polypeptides, and purine compounds were tested to identify the transmitter in non-adrenergic, non-cholinergic nerves of various smooth muscle. By a systematic series of experiments, ATP, a purine nucleotide, was recognized as the possible neurotransmitter (55, 61). ATP was then shown to satisfy the criteria which are necessary for definition of a substance as a neurotransmitter: properties of

synthesis, storage, release, inactivation, ability to mimic response to nerve stimulation, and existence of receptor on the effective tissue.

Regarding synthesis and storage, Su et al. (321) demonstrated uptake and resynthesis of purine derivatives by guinea-pig taenia-coli. When the taenia coli preparations which included nerve terminals were incubated with [3H] adenosine, tissue uptake of adenosine occurred, and approximately 60% of [3H]-adenosine was converted into and retained as [3H] ATP. In an ultrastructural study of non-adrenergic and non-cholinergic nerve endings, large opaque vesicles which are not affected by 6-hydroxydopamine (used as a marker to identify catecholamine-containing vesicles) were identified (291). Histochemical study has also demonstrated an ATP-rich microsomal fraction in the non-adrenergic, non-cholinergic nerve endings (86). Burnstock (49) offered the term "purinergic nerve" for this non-adrenergic, non-cholinergic nerve which utilizes ATP as the principal neurotransmitter.

Su et al. (321) demonstrated ATP release by nerve stimulation . [3H] ATP resynthesized from [3H]-adenosine was released into the medium by stimulation of nerve containing both guanethidine and atropine. Burnstock et al. (59) further reported direct evidence for ATP release from purinergic nerves in the guinea-pig taenia coli and bladder. While a large amount of ATP was released by purinergic nerve stimulation from guinea-pig taenia coli, there was no significant increase of ATP release during direct muscle stimulation which developed similar responses.

Exogenously applied ATP mimicked mechanical and

electrophysiological responses to purinergic nerve stimulation. According to Jager and Schevers (195), ATP produced hyperpolarization and relaxation in guinea-pig taenia caecum and purinergic nerve stimulation also caused hyperpolarization and smooth muscle relaxation. In addition, $\beta - \gamma$-methylene ATP (APPCP) which is resistant to degradation to AMP and adenosine are 100-fold more potent than ATP and caused phasic contractions which mimicked an atropine-resistant response to nerve stimulation (42).

ATP action decays rapidly after application which indicates that tissues have an efficient ATP inactivation mechanism. Ectonucleotidases such as nucleoside triphosphatase, nucleoside diphosphatase, and 5'-nucleosidase as well as Mg^{++}-activated ATPase are found in various tissues including vascular endothelial cells, vascular smooth muscle cells, red blood cells and plasma (88, 138, 188, 272, 274, 275). The ectonucleotidase system degrades ATP to ADP, AMP, and finally to adenosine; the adenosine and some of adenine nucleotides are then uptaken by nerve or smooth muscle (321).

Burnstock (50) have proposed two types of purinergic receptors—P_1 and P_2—in the effective smooth muscle. P_1 purinergic receptors are most sensitive to adenosine and are blocked by methylxanthines. P_2 purinergic receptors are most sensitive to ATP and are blocked by quinidine, 2-substituted imidazolines and apamin. The mechanisms by which ATP binding to the receptor produces smooth muscle activation is still not well-known. Burnstock (52) has suggested that ion movements and/or prostaglandin release by receptor activation may be possible

mechanisms of smooth muscle contraction or relaxation.

3. ATP ACTION ON CELL METABOLISM AND ION MOVEMENT

Exogenous ATP affects glucose metabolism. Levine (228) demonstrated hyperglycemic effects by administration of adenine nucleotides in vivo. Both ATP and 3′,5′-cyclic AMP administration increased blood glucose levels and reduced rat liver glycogen contents. Similar effects have been observed by Filkins and Buchanan (126). ATP-$MgCl_2$ (10 umoles, iv) produced hyperglycemia in control rats and prevented endotoxin-induced hypoglycemia. Levine (228) failed to demonstrate hyperglycemia in perfused liver by ATP (1mM). In contrast, Clemens et al. (84) reported that ATP-$MgCl_2$ ($>5 \times 10^{-6}$M) stimulated glycogenolysis in the isolated perfused rat liver. Furthermore, at a low concentration of ATP-$MgCl_2$ (10^{-6}M), gluconeogenesis was increased whereas at concentrations over 5×10^{-5}M gluconeogenesis was decreased (84). In a further study Clemens et al. (83) observed increments of glycogenolysis and reductions of gluconeogenesis in isolated rat hepatocytes at an ATP-$MgCl_2$ concentration of 10^{-4}M. It was proposed that the stimulation of glycogenolysis is mediated by purinergic receptor on the hepatocyte and that inhibition of gluconeogenesis is mediated via adenosine. Inhibition of gluconeogenesis by ATP (2.5mM) was also observed in the rat kidney-cortex slices and isolated perfused rat kidney (339).

ATP reduced renal metabolism as evidenced by Maxild (239) who reported that externally added ATP inhibited oxygen consumption, p-

amino-hippurate (PAH) accumulation and ^{14}C-glucose degradation in the cortical slices of the rabbit kidney. He also found an elevation of ATP concentration in the cortical slices with exogenous ATP (0.1-10mM). He interpreted that the reductions of glucose degradation and oxygen consumption are presumably due to allosteric inhibition of glycolysis and mitochondrial respiration via increased level of ATP in the tissue. In contrast, Weidemann et al. (33) reported that glucose uptake by kidney cortex slices was stimulated by added ATP; but oxygen consumption by kidney slices was not significantly affected by ATP. Thus effects of ATP on glucose degradation and oxygen consumption by the kidney are controversial. However, Clemens et al (84) reported that ATP (10^{-4}M) decreased oxygen consumption in the isolated perfused rat liver.

Various stimuli including hormones, drugs and nerve stimulation cause changes in cell membrane permeability. Exogenous ATP also increased cell membrane permeability to several ions such as Na^+, K^+ and Ca^{++} in various tissues. Rozengurt et al. (293) reported that externally added ATP (0.5 mM) produced ^{86}Rb (an index of K^+ movement) efflux in the cultured 3T6 mouse cell line. Similar effects on K^+ efflux have been observed in isolated hepatocytes (46) and in erythroleukemia cells (69) where extracellular ATP stimulated K^+ efflux and Na^+ influx. Indirect evidence for increased K^+ permeability (P_K) by ATP comes from studies of changes in membrane potential. ATP produced hyperpolarization in guinea-pig liver slice (200) and the guinea-pig taenia caecum (195). Many investigators have suggested the possibility that the increase in P_K by ATP is a consequence of a rise in the

intracellular Ca^{++} ion. ATP increased Ca^{++} uptake in various cells including rat mast cells (91), isolated intestinal epithelial cells (213) and Ehrlich ascites-tumour cells (23). Dahlquist et al. (93) reported that exogenous ATP induced a prompt increase in Na^+ influx and K^+ efflux in the rat mast cells and this K^+ efflux was prevented by blocking Ca^{++} influx by the presence of $LaCl_3$. Burgess et al. (46) demonstrated that A23187, a divalent cationophore, increased K^+ efflux in the isolated hepatocytes. In this study they also observed K^+ efflux by addition of ATP into the medium. These reports suggest that ATP-induced K^+ efflux is Ca^{++}-dependent process.

4. THERAPEUTIC USES OF ATP

In 1964 Talaat et al. (328) first introduced ATP therapy in experimental studies of shock and reported that infusion of ATP increased survival rates in hemorrhagic shock. Beneficial effects of ATP for the survival rates and recovery from organ failure have been proved in various shock models. $ATP-MgCl_2$ treatment increased survival rates after hemorrhagic shock (103, 217, 305), endotoxin shock (126, 142), in burn (352), following hepatic ischemia (180, 181), and insulin induced seizure deaths (125). Treatment with ATP also increased survival time in a lethally hypoxic condition such that mice that received ATP (i.p.) had a significant longer survival time in 5% O_2-95% N_2 mixture (220).

ATP enhanced recovery from acute renal failure. Siegel et al. (309) reported that $ATP-MgCl_2$ improved tubular function and inulin

clearance (GFR) 24 hours after 30 minutes of bilateral renal artery occlusion, and the degree of GFR was directly related to the quantity of ATP-MgCl$_2$. Lyton et al. (235) also demonstrated improvement in renal function using ATP-MgCl$_2$ in the preserved and then transplanted dog kidney. ATP treated groups maintained lower serum creatinine and blood urea nitrogen levels, higher GFR and a greater osmolar clearance than untreated dog. ATP infusion increased renal cortical levels of ATP after prolonged ischemia (147).

Many investigators have indicated that ATP improves organ function after hepatic ischemia. Chaudry et al. (75) demonstrated restoration of liver cell function and blood flow with ATP. They measured clearance of indocyanine green (ICG) at 3 hours following hepatic ischemia. ICG clearance was markedly depressed by ischemia but the ATP-MgCl$_2$ treated group had almost same ICG clearance value as the sham-operated animals. In a further study, the effects of ATP-MgCl$_2$ on mitochondrial function and hepatic ultrastructure were undertaken. ATP-MgCl$_2$ treated group maintained high mitochondrial function--high ATP level, energy charge and respiratory control ratio--at 3 hours following reflow (266). Improved mitochondrial function by ATP following hepatic ischemia was also observed by Chaudry et al. (78). Three hours after hepatic ischemia and reflow, hepatic mitochondrial Ca^{++} and free fatty acids (FFA) levels increased and mitochondrial nucleotide translocase activity was depressed. However, ATP-MgCl$_2$ infusion resulted in lower FFA and Ca^{++} levels and restored nucleotide translocase activity to approximately 85% of normal. Chaudry et al. (78) suggested that

improved organ function with ATP may be mediated via improvement in mitochondrial function. In addition to liver tissue, ATP content in cultured myocardial cells, renal tissue and skeletal muscle after ischemia or hemorrhagic shock were also increased by ATP infusion (79, 147, 346).

Cell membrane transport of Na^+ and K^+ was depressed during hemorrhagic shock (21), but ATP-$MgCl_2$ restored normal membrane function. According to Ohkawa et al. (265), hepatic ischemia caused cell membrane dysfunction which led to cellular edema, high intracellular Na^+ content and low intracellular K^+ content; ATP-$MgCl_2$ treatment improved cellular edema and electrolyte homeostasis. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels in the plasma were increased sixty minutes after total hepatic ischemia in rats which represent hepatic cellular damage or cell membrane disturbance. ATP-$MgCl_2$ administration following hepatic ischemia reduced both SGOT and SGPT levels (180). Kraven et al. (216) also demonstrated that treatment of hemorrhagic shock rat with ATP-$MgCl_2$ returned altered cell membrane permeability toward normal.

Disturbances in glucose homeostasis which are characteristic of infection and endotoxicosis are prevented by ATP. Filkins and Buchanan (126) demonstrated that ATP-$MgCl_2$ treatment protected the depression in postendotoxin gluconeogenesis and reduced glucose utilization in vivo and by epididymal fat pads. Chang and Cuatrecasas (71) and Chaudry and Gould (77) observed that ATP inhibited glucose transport and utilization in the adipocyte and isolated soleus muscle. Similar results were also

obtained in a in vivo study (125). The ATP effect on glucose uses may be due to modulation of insulin receptor. Since insulin binding to its receptor was inhibited by ATP in fat cells, liver cells and placental membranes (89, 332).

In addition as reviewed recently by Chaudry (73), exogenous ATP-$MgCl_2$ has beneficial effects via improvements in blood flow, improvement in metabolic capacity, restoration of reticuloendathelial cell function, restoration of cell membrane function and correction of hormone imbalance

D. Hepatic Glycogenolysis

1. HORMONAL STIMULATION OF GLYCOGENOLYSIS

Hepatic glycogenolysis is stimulated by a variety of hormones, including catecholamines, glucagon, vasopressin, angiotensin II and oxytocin. Catecholamine-induced glucose output is mediated by two basic types of adrenergic receptor on the hepatocyte-- α- and β-adrenergic receptors (118, 179). Glycogenolysis as mediated through β-adrenergic receptors is associated with stimulation of adenylate cyclase and a subsequent increase in intracellular cyclic-AMP. It is widely accepted that c-AMP is the second messenger which initiates a series of intracellular enzymatic reactions including cascade style activation of protein kinases; phosphorylase kinase and phosphorylase to result in an increased glucose output from the hepatocytes (324).

Many adrenergic receptor agonists and antagonists have been used to elucidate the regulation of glycogenolysis by catecholamines. Mayer et al. (240) demonstrated that dichloroisoproterenol (DCI), a β-receptor antagonist, almost blocked the increase in blood glucose by epinephrine, norepinephrine and isoproterenol in the dog. In contrast to DCI, phenoxybenzamine, α-receptor antagonist, did not interfere with epinephrine-induced hyperglycemia in the dog. Newton and Hornbrook (263) reported that propranolol completely abolished the increased phosphorylase activity induced by both isoproterenol and epinephrine in the dog liver. It is generally accepted therefore that catecholamine-induced glucose output due to glycogenolysis is mediated via a β-receptor mechanism in the dog.

Several groups have, however, shown different results in rat models of catecholamine-induced glycogenolysis. Hutson et al. (187) demonstrated that epinephrine increased phosphorylase activity in isolated rat hepatocyte and phenoxybenzamine blocked epinephrine-induced phosphorylase activity but propranolol was not active. Similar results on α-adrenergic activation of hepatic glucose output in the rat have been observed in isolated rat parenchymal cells and in perfused rat liver (70, 117, 302). Ellis (114) reported that there are species differences in the glycogenolytic responses to catecholamines; catecholamine-stimulated glycogenolysis is mainly α-adrenergic receptor mediated in rat, mice, baboon and human whereas β-adrenergic receptors are dominant in dog and cat. It is also known that catecholamine-induced glycogenolysis is dependent on sex difference, thyroid function,

and aging (35, 237, 319). Recently Itoh et al. (194) demonstrated that the adrenergic mechanism for phosphorylase activation was converted from an α- to β-type during 8 hours culture of rat hepatocyte.

The dominant role for an α-adrenergic receptor mediated, cyclic-AMP-independent mechanism for catecholamine action in rat liver has been supported by many laboratories including Exton et al. (33, 117, 118). Several investigators have shown that α-adrenergic stimulation resulted in changes in Ca^{++} flux which coincided with glucose release. Blackmore et al. (33) demonstrated that infusion of phenylephrine increased Ca^{++} release from the perfused rat liver. This Ca^{++} release was blocked by phenoxybenzamine and was not affected by propranolol. Glucose output and phosphorylase activation by phenylephrine were impaired in isolated hepatocytes which were incubated in Ca^{++} free medium and it was increased by the ionophore A23187 (11, 209). Murphy et al. (254) also measured the changes in cytoplasmic free Ca^{++} level and phosphorylase $\underline{a}$ activity after addition of phenylephrine and found a very close relationship between intracellular Ca^{++} level and cyclic-AMP independent phosphorylase activity.

Several calcium-pools exist in the liver including mitochondria, endoplasmic reticulum, plasma membrane and extracellular Ca^{++}. It is not well understood which Ca^{++}-pool is the major source of the mobilized Ca^{++} by catecholamines but there is much evidence that α-adrenergic stimulation mobilizes Ca^{++} from mitochondria in the liver (34, 254). As described above the removal of Ca^{++} from incubation medium prevented activation of glycogen phosphorylase by catecholamines and ionophore

A23187 produced activation of phosphorylase. These findings indicate that Ca^{++} influx from the extracellular fluid may contribute to increase cytoplasmic Ca^{++} levels.

It is uncertain how catecholamines initiate Ca^{++} entry from the extracellular space or Ca^{++} release from intracellular stores. Michell (247) suggested that the hydrolysis of phosphatidylinositol by catecholamines is linked in some way with generation of signal for Ca^{++} movement. Epinephrine increased ^{32}P incorporation into phosphatidyl inositol and α-receptor antagonists (prazosin or phentolamine) blocked ^{32}P invotpotsyion, but propranolol did not (330). Exton (118) has proposed a more detailed hypothesis in which activation of α-adrenergic receptors lead to production of a hypothetical enzyme which open Ca^{++} gates in the plasma membrane and also another hypothetical intracellular messenger lead to Ca^{++} release from mitochondria or other intracellular Ca^{++} stores. Both of these processes result in increased cytoplasmic free Ca^{++} which then combines with the Ca^{++}-dependent regulatory protein, calmodulin, and this Ca^{++}-calmodulin complex regulates phosphorylase activity.

Glucagon is one of the most potent hepatic glycogenolytic hormones. In contrast to catecholamines, glycogenolysis by glucagon is principally and solely by a cyclic-AMP mediated process. The first step in initiation of a glucagon effect is hormone binding to the receptor. Although both glucagon and catecholamines stimulate adenylate cyclase in the liver, there are different receptors on the hepatocyte for each hormone. According to Newton and Hornbrook (263), propranolol blocked

completely the increased adenylate cyclase activity induced by epinephrine or isoproterenol but propranolol did not affect glucagon-stimulated adenylate cyclase activity. This indicates the existence of separate receptors on the liver cell membrane for the glucagon and catecholamines. However, beyond binding to the receptor and activation of adenylate cyclase, cyclic-AMP is the common mediator of glucagon and epinephrine effects (121). Namely, the glucagon-receptor complex activates adenylate cyclase which generates the second messenger, cyclic-AMP. Cyclic-AMP then increases phosphorylase in the liver cell by converting phosphorylase b to phosphorylase a via cyclic-AMP dependent protein kinase and phosphorylase b kinase (120).

Some hormones produce glycogenolysis in the liver which is not mediated by cyclic-AMP. Vasopressin increased glucose output in the perfused rat liver but it did not increase cyclic-AMP content in the liver (177, 215). Keppens and DeWulf (210) reported that vasopressin increased phosphorylase activity but it did not affect any protein kinase or phosphorylase kinase. According to Hems et al. (178), the glucose release by vasopressin was critically dependent on Ca^{++} in the medium. In Ca^{++}-free medium, vasopressin-stimulated glucose output did not occur. Angiotensin II and oxytocin have similar characteristics with vasopressin of a Ca^{++} dependent activation of phosphorylase without mediation by cyclic AMP or protein kinase (177, 178, 211). These findings indicate that glycogenolysis by vasopressin, angiotensin II, and oxytocin is related to an elevation of intracellular Ca^{++} similarly as α-receptor activation by catecholamines.

2. HYPOXIC STIMULATION OF GLYCOGENOLYSIS

Hepatic glycogenolysis is sensitive to the oxygen tension in the liver. As oxygen delivery to the liver or isolated hepatocyte is limited, hepatic glycogenolysis is accelerated (175, 197, 301). The mechanism of hepatic glycogenolysis during hypoxia is completely different from those stimulated by hormones. As described above, hormones--catecholamines, glucagon and vasopressin etc.--stimulate hepatic glycogenolysis via cyclic-AMP mediation or changes in intracellular Ca^{++}. However, hepatic glycogenolysis in the hypoxic condition is due mainly to changes in intracellular metabolite concentrations (179).

Two forms of phosphorylase--phosphorylase a and b--exist in the hepatocyte (179). In normal states, phosphorylase b is the main form which is subjected to allosteric regulations by ATP, AMP and glucose-6-phosphate. Glycogenolysis is accelerated by AMP and inhibited by ATP and glucose-6-phosphate. Therefore, in a normal state of sufficient oxygen tension, hepatic glycogenolysis is suppressed due to high levels of intracellular ATP and low level of AMP. However, in hypoxia hepatic intracellular ATP content decreases while intracellular AMP, inorganic phosphate (Pi) and ADP increases. AMP content in particular increased dramatically in hypoxic liver (122, 175). Therefore, hepatic glycogenolysis in hypoxia is due to direct activation of phosphorylase activity via stimulation by a high level of intracellular AMP and deinhibition by a low level of ATP (179).

Free glucose is the main product of glycogenolysis in hypoxia but the hypoxic liver also produces lactate from glycogen via anaerobic glycolysis. Wood and Krebs (351) demonstrated that in anaerobic conditions perfused rat liver produced both lactate and glucose. Similar results have been obtained in isolated hepatocytes in that Seglen (301) demonstrated glycogen breakdown and lactate production during hypoxia. He also observed inhibition of lactate production by glucogon.

E. Prostaglandin Action on Metabolism and Hemodynamics

1. PROSTAGLANDIN SYNTHESIS AND RELEASE

Prostaglandins are 20-carbon unsaturated oxygenated fatty acids that contain a cyclopentane ring. They appear to be present in every mammalian tissue and they cause a wide variety of physiological activities including smooth muscle contraction, aggregation of platelets, and alterations in tissue metabolism. Prostaglandin production begins when phospholipase A_2 liberates arachidonic acids from phospholipid in the plasma membrane. Prostaglandins are synthesized from arachidonic acid by cyclo-oxygenase and additional specific enzymes for each prostaglandin. There are several prostaglandin families which are classified according to their configurations and substituents on the cyclopentane ring. It has been found that there are more than 16 different prostaglandins which belong to 9 classes—they include PGA, PGB, PGC, PGD, PGE, PGF, PGG, PGH, and PGI. The major circulatory prostaglandins are PGE, PGF, PGA (thromboxane A), and PGI (prostacyclin)

series (206).

Prostaglandins are not extensively stored in tissues but rather are synthesized de novo and then released by suitable stimuli such as drugs, nerves and hormonal stimulation. Prostaglandin release has been demonstrated by chemical or nervous stimulation in rat phrenic nerve-diaphragm preparation (285), in rat epididymal fat pads (306), and in rat gastric mucosa (24, 284). The release of prostaglandins or prostaglandin like substances is markedly enhanced during renal ischemia (112, 119, 242), during nerve stimulation (112), norepinephrine infusion (112), bradykinin addition (243) and infusion of angiotensin analogs (259). Prostaglandin release has also been demonstrated in perfused spleen by catecholamines, splenic nerve stimulation and by mechanical vibration (98, 149). In addition, the release of prostaglandins was reported in many other tissues including guinea-pig lung by anaphylaxis (279), cat cerebral cortex by electrical stimulation and human cultured endothelial cells by histamine and bradykinin (1).

Adenine nucleotides are potent stimulators of prostaglandin release. According to Minkes et al. (252), ATP and ADP at concentrations of 1 x 10^{-7} to 10^{-6} moles stimulated release of prostaglandin like substances from hearts perfused with Krebs solution. Needleman et al. (260) also demonstrated prostaglandin release by ATP and ADP from various perfused organs--including rat liver, rabbit heart, spleen, kidney and fat pads. In addition, prostaglandin release with ATP or ADP was blocked by prostaglandin synthesis inhibitors.

There are many prostaglandin synthesis inhibitors and the responses to inhibitors vary greatly in different species and different organs. Flowers (130) reported the order of inhibitory potency of some aspirin-like prostaglandin synthesis inhibitor as follows: meclofenamic acid > indomethacin or niflumic acid > mefenamic acid > flufenamic acid > naproxen > phenylbutazone > aspirin or ibuproten. According to Flower et al. (131) the I_{50} of meclofenamic acid, indomethacin and aspirin was 0.1, 0.17 and 37 uM, respectively. The mechanism of prostaglandin synthesis inhibitor action is not clear, however, several hypothesis of their actions have been proposed. It includes a competition at the substrate or cofactor site (327), irreversible inactivation of the synthesis enzyme (286), and a chelating action (311).

2.   PROSTAGLANDINS AND METABOLISM

Prostaglandins affect cell metabolism either by modulation of the action of hormones or by direct action. The mode of action of prostaglandins is via changes in intracellular cyclic-AMP levels due to increased or decreased adenylate cyclase or phosphodiesterase activities. Prostaglandins E_1 and E_2 inhibited catecholamine-induced free fatty acid release in the dog (25, 26). A similar effect with prostaglandins has been observed using in vitro studies. PGF_1 and PGF_2 inhibited the release of free fatty acid and glycerol from isolated rat epidiymal fat pads as stimulated by nerve stimulation or catecholamine addition (317). Butcher et al. (63) demonstrated reduction of cyclic-AMP content by PGE_1 in isolated fat cells. Prostaglandins attenuated epinephrine, ACTH, glucagon and TSH-induced cyclic AMP levels. These

data indicate that the antilipolytic effect of prostaglandin is associated with a decrease in cyclic-AMP accumulation.

In contrast to adipose tissue, prostaglandins of the F-series increase cyclic AMP levels and increase cell activity in various tissues including human platelets (290, 350), rat anterior pituitary gland cells (354), bovine corpus luteum (238), rat erythrocytes (308) and the thyroid gland of dogs and mice (47, 124).

Prostaglandins also have effects on liver metabolism. Bohle and May (38) demonstrated that prostaglandin E_1 injection (i.p.) produced hyperglycemia in fasted rats. In an in vitro study, they observed glycogen depletion in liver slices by addition of PGE_1 into the incubation medium. A hyperglycemic effect of PGE_1 was also demonstrated in the perfused rat liver (227). According to Hansson and Samuelsson (167), when they injected ^3H-labelled PGE_1 into a mouse, the ^3H PGE_1 is taken up primarily by liver, kidney, and uterus. This suggests those organs have high affinity binding sites to take up the prostaglandin from the blood. Smigel and Fleischer (313) found the presence of PGF_1 receptor in plasma membrane of the rat liver. In a further study, they confirmed that only the plasma membrane of hepatocytes has an exclusively high affinity binding receptors, and other cell fractions such as nuclei, rough microsomes, Golgi complex or mitochondria have no high affinity receptors.

Although the interaction of prostaglandin receptors and adenylate cyclase has not yet been fully understood, many studies suggest that

prostaglandin binding to the receptor leads to increased adenylate cyclase activity in the hepatocyte. Many investigations have supported that prostaglandin E_1 increased adenylate cyclase activity in the rat liver cell (267, 288, 289, 325, 347, 353). In addition, Robertson et al. (289) reported down-regulation of PGE receptors. When a dimethyl analogue of PGE_2 was injected into the rat subcutaneously, the concentration of PGE receptors was significantly decreased with PGE-induced plasma membrane adenylate cyclase. Up-regulation of hepatic PGE receptors was also reported (288). However, some controversy exists in that Levine (229) reported that PGE_1 did not increase either hepatic cyclic-AMP activity or glucose output in the isolated perfused rat liver. Brass et al. (40) failed to demonstrate glycogenolysis by PGE_1 in the isolated hepatocytes. Furthermore, dimethyl PGE_2 inhibited glucagon-stimulated glycogenolysis. —

3.   PROSTAGLANDINS AND VASCULAR RESISTANCE

A prominant action of prostaglandins is regulation of vascular smooth muscle contraction. Different prostaglandins exert multiple hemodynamic changes in different preparations. The injection of PGE_1 or PGA_1 into the coronary artery increased coronary blood flow in anesthetized dogs (255, 258). Vasodilator effects of PGE_1 have also been observed in such vasculature as the renal artery, femoral artery and carotid artery. PGE_1 elicits a biphasic change in portal vein resistance—initial increased followed by a decrease (257). In contrast to PGE_1, $PGF_{2\alpha}$ increased vascular resistance in most vascular beds. Infusion of $PGF_{2\alpha}$ increased portal venous pressure (257), pulmonary

arterial pressure (8), renal vascular resistance (256) and decreased utero-ovarian blood flow (278).

Strong and Bohr (318) demonstrated in vitro that PGA_1, PGE_1, PGE_2 and $PGF_{1\alpha}$ caused contraction of isolated arterial strips from various rabbit arteries such as aorta, coronary artery, skeletal muscle artery, messenteric artery and renal artery. The prostaglandins also caused a biphasic response in canine small arteries with relaxation at low concentration and contraction at high concentration.

Ca^{++} ions are associated with the development of the contractile responses of smooth muscle to prostaglandins. Increased extracellular Ca^{++} ion concentration enhanced the response to prostaglandins and reduction of Ca^{++} concentration inhibited the prostaglandin responses (192, 271). According to Greenberg et al. (153), $PGF_{2\alpha}$ increased norepinephrine, tyramine or high KCl-stimulated ^{45}Ca uptake in canine mesenteric arterial, mesenteric venous and tibial arterial strips. $PGF_{2\alpha}$ also increased ^{45}Ca efflux from those vascular smooth muscle preparations. PGA_2, however, increased ^{45}Ca efflux from canine tibial but did not alter ^{45}Ca uptake, which may suggest PGA_2 increases intracellular Ca^{++} via release of Ca^{++} from intracellular stores (154). Ishizawa and Miyazaki (191) also reported that the contractile mechanism of PGE_1 in guinea-pig stomach is mainly dependent on release of intracellular bound Ca^{++} since verapamil markedly suppressed K^{+}-induced contraction but did not affect PGE_1-induced muscle contraction. Carsten (67) reported that PGE_2 and $PGF_{2\alpha}$ inhibited Ca^{++} binding to the sarcoplasmic reticulum of human uterine muscle and also

enhanced Ca^{++} release from the microsomal fraction.

The mechanism of prostaglandins action to increase Ca^{++} influx or Ca^{++} release from intracellular Ca^{++} stores is unclear. Due to similarity in the characteristics of prostaglandins and ionophores in Ca^{++} release from uterine microsomes, Carsten and Miller (68) proposed an ionophoretic action of prostaglandins. Anderson and Kohn (5) suggested however that prostaglandins may increase Ca^{++} permeability to the plasma membrane via activation of Ca^{++} channel or acting as Ca^{++} carrier. Thus, prostaglandins may transport Ca^{++} ions across the plasma membranes by forming a PG-Ca^{++} complex which is highly soluble in the lipid layer of the membrane.

Prostaglandin mediation of the contractile response to ATP has also been reported. Indomethacin (20-50 uM) blocked rebound contraction of guinea-pig taenia coli induced by exogenous application of ATP or by purinergic nerve stimulation (57, 205). Indomethacin (3-20 uM) also depressed the contraction induced by ATP in rabbit detrusor (99, 105) and in isolated human umbilical artery (127). On the other hand, Burnstock et al. (60) demonstrated that ATP caused relaxation in the rabbit portal vein strip and indomethacin potentiated the relaxation induced by ATP.

In summary, in reference to actions on vascular smooth muscle, prostaglandins are both stimulatory and inhibitory agents. The contractile responses to prostaglandins depend on the species of animal, the organ, the tissues and the prostaglandin type. Ca^{++} ions may be involved in prostaglandin action.

CHAPTER III

METHODS

A. Animals and Care

Male albino rats of the Holtzman strain (300 to 350 gm) were used throughout this study. The rats were supplied from the Holtzman Company (Madison, Wi.) and were acclimated to our care facilities for 7 to 10 days. All rats were housed in groups of three animals per cage with dimensions of 9.5" in length, 17" in width, and 7" in height. A 12 hour light-dark cycle (7:00 AM to 7:00 PM), ambient temperature of 22 to 26°C and 50% humidity were maintained. Rats were fed Wayne Lab Blox and tap water ad libitum. The nutrient composition of Wayne Lab Blox consists of 24.5% protein, 4.3% fat, 3.7% fiber, 7.8% ash, with minerals and vitamins added. The gross energy of this preparation is 3.92 Kcal/gm.

B. Preparation of Perfusion Solution and Apparatus

1. SOLUTIONS

    a. Krebs-Ringer bicarbonate buffer (KRB)

The perfusion medium was a standard Krebs-Ringer bicarbonate buffer solution except glucose was omitted. This solution contained (in mM) NaCl 117, KCl 4.7, $CaCl_2$ 1.91, KH_2PO_4 1.19, $MgSO_4$ 1.44 and $NaHCO_3$ 24.8. KRB was saturated with an O_2/CO_2 (95:5 v/v) gas mixture and the pH was adjusted to 7.4 at 36°C.

b. ATP, ADP, AMP and adenosine

ATP, ADP, and AMP (sodium salts 99%, Sigma) were prepared as stock solutions in a concentration of 50 mM in cold 0.9% NaCl. The pH was adjusted to 7.1 to 7.3 with 1 N NaOH. Adenosine was prepared likewise at a concentration of 10 mM.

c. Glucagon

2×10^{-5} M glucagon (Sigma) was prepared in saline as a stock solution. It was dissolved by adjusting the pH to 10 because of its poor solubility in acid and then re-titrated to 7.4 with 1 N HCl.

d. Indomethacin

2.8 mM indomethacin (Sigma) was prepared in KRB solution which contained 10% ethyl alcohol in order to facilitate dissolution.

e. ATP-MgCl$_2$ and ATP-NaCl

100mM of ATP, MgCl$_2$ and NaCl were prepared. The solutions, i.e., ATP and MgCl$_2$ or ATP and NaCl, were mixed in equal volumes. Thus, the concentration of ATP-MgCl$_2$ or ATP-NaCl after mixing was 50 mM respectively.

2. APPARATUS

A liver Perfusion-Aeration Apparatus from the MRA Corp. (M81-XL, 22" x 15" x 40") was used for all perfusions. The arrangement of the perfusion components is shown in Figure 1. The perfusate was pumped from a perfusate reservoir by a Masterflex roller pump (Cole-Parmer Instrument Co.). It initially passed through a silk mesh filter (mesh

number 97, Sargent-Welch) which was changed everyday. From the filter the fluid moved to the gas exchange tube where the fluid was saturated with humidified O_2/CO_2 (95:5) gas mixture. The perfusion medium was then passed through a second silk mesh filter by either another pump or a hydrostatic potential gradient. It then perfused the liver via the portal vein or hepatic artery. Flowmeters (Gilmont Instrument, Inc.) measured perfusion flow rates into the portal vein (Gilmont F-1300) and hepatic artery (Gilmont F-1200). Pressure transducers (Statham P23DC) measured portal vein perfusion pressure and hepatic arterial perfusion pressure which were recorded on Grass Polygraph (Model 79D). Changes in liver weight were measured by a force transducer (Grass FT03C). A pH meter (Beckman 76004) determined the pH of perfusate. Perfusate from the hepatic vein returned to the perfusate reservoir via a collection chamber. In this recirculating system, 130 ml of perfusion medium was recirculated every 2 minutes. The temperature in the perfusion cabinet and of the circulating medium was maintained at $36^{o}C$. A magnetic stirrer was placed below the floor of the cabinet to mix the perfusate in the perfusate reservoir. Several analyzing systems which were located outside of the perfusion cabinet were connected to the collection chamber. Figure 2 shows a schematic diagram of the analyzing system, including a K^+ ion analyzer, a glucose analyzer, a lactate analyzer, a thermometer, an oxygen monitor, a pressure transducer and a force transducer. All of the analyzed information was recorded on a Linear Record (Linear Instrument Corp. Model 1200) and a Grass Polygraph.

FIGURE 1

SCHEMATIC DIAGRAM OF LIVER PERFUSION SYSTEM

All devices in the diagram except the roller pumps were located in the lucite perfusion cabinet at a regulated temperature of $36^{\circ}C$. Arrows represent directions of movements of perfusate and gas mixture. HA : hepatic artery; HV : hepatic vein; L : liver; PV : portal vein.
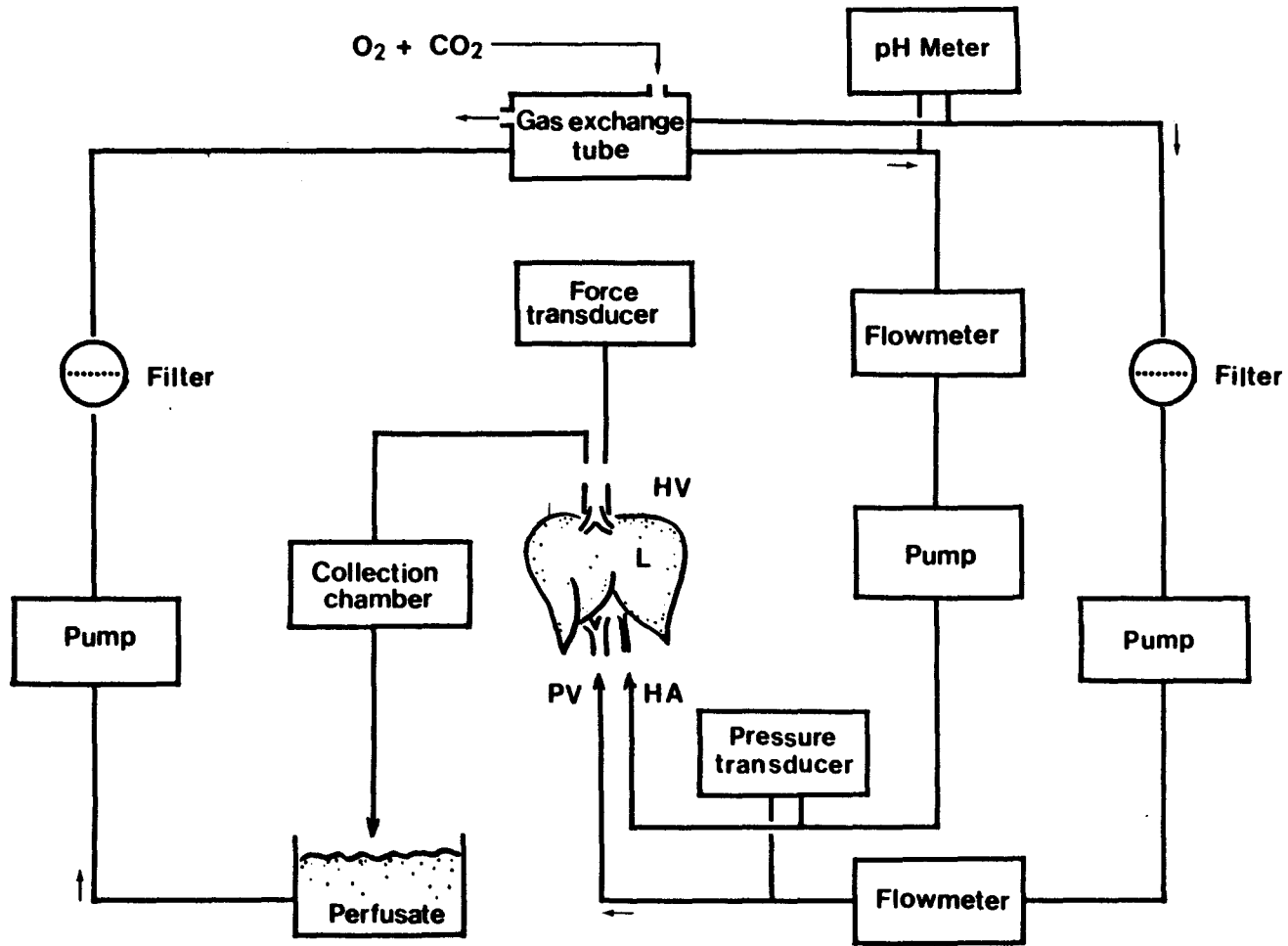
FIGURE 2

SCHEMATIC DIAGRAM OF THE ANALYZING SYSTEMS

All analyzers except the flow meter were located outside of the perfusion cabinet. Oinput and Ooutput represent oxygen tension of influent and effluent of perfusate to or from the liver respectively.

—

Flowmeter

O input

O output

Collection Chamber

Pressure Transducer

K^+ Ion Analyzer

Glucose Analyzer

Thermometer

Lactate Analyzer

Oxygen Monitor

Force Transducer

Recorder
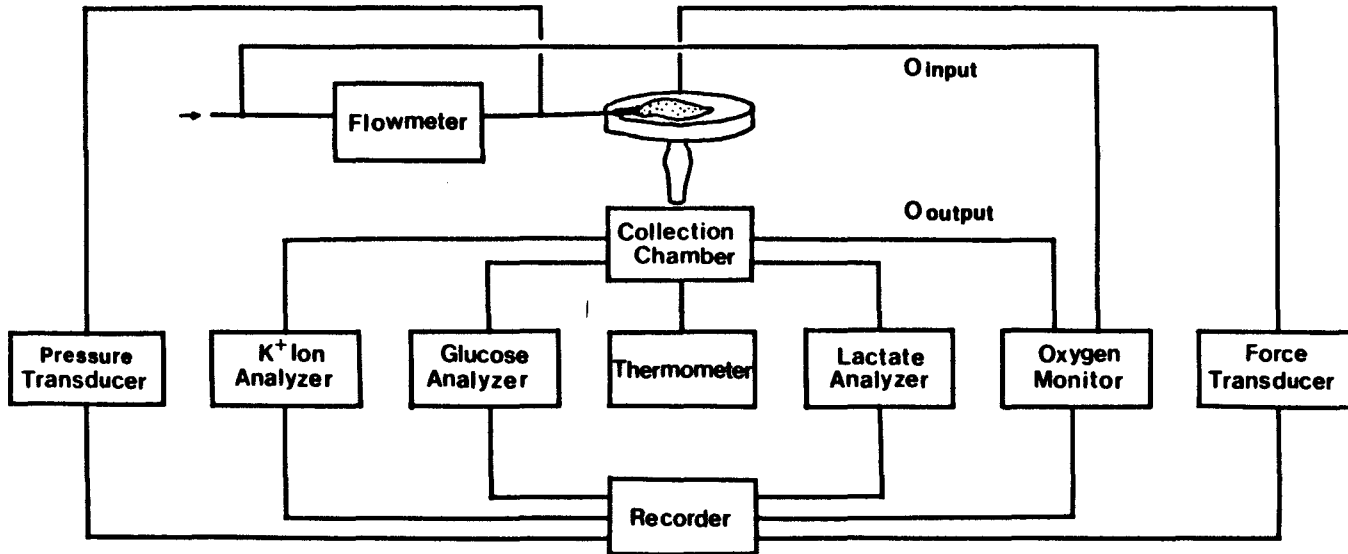
C. Operative Technique and Perfusion Procedure

1. OPERATION

    a. Perfusion through portal vein

Most rats were used at 10:00 AM in the well-fed state. The rat was anesthetized with pentobarbital sodium (30 mg/Kg) via the dorsal vein of the penis. The abdomen was opened through a mid-line incision (10 to 12 cm). A mid-transverse incision to the left and right of the midline (6 to 7 cm) was then made, avoiding the large lateral blood vessels. The intestines were then placed to the animal's left, so that the liver, portal vein, right kidney and inferior vena cava were exposed. The inferior vena cava and portal vein were isolated and 500 USP units of heparin were injected i.v. After ligating the abdominal vena cava above the renal vein, a PE-260 polyethylene catheter was inserted into the portal vein and tied in place with silk suture (size 3-0). The liver was then rapidly flushed using about 20 ml of 0.9% saline. The thorax was opened by a transverse incision just above and along the line of the diaphragm and by a longitudinal cephalad incision. A PE-280 polyethylene catheter, cut off at a 45° angle to form a moderately sharp tip, was inserted and secured in the thoracic vena cava via penetration of the right atrium. The liver was rapidly excised, transferred onto a liver platform, covered with saline-moistened gauze and placed in the perfusion chamber. The elapsed time between portal cannulation and restoration of flow was within 4 minutes.

b. Perfusion through hepatic artery

After opening the abdomen, the coeliac artery and its branches--the hepatic artery, superior pancreatico-duodenal artery and gastric artery--were isolated. All arteries which branched from the coeliac artery except the hepatic artery were ligated and a PE-50 polyethylene catheter was inserted and secured in the coeliac artery. The portal vein was then cannulated as described above.

2. PERFUSION TYPES

The liver perfusions were carried out in three ways as follows:

a. Perfusion through the portal vein with a constant pressure (28 cmH$_2$O).

b. Perfusion through the portal vein with a constant flow (64 $\pm$ 0.4 ml/min) using a roller pump.

c. Perfusion through both the portal vein with constant pressure (28 cmH$_2$O) and the hepatic artery with constant flow (3.1 $\pm$ 0.4 ml/min) using a roller pump.

D. Measurements of Various Parameters in the Isolated Perfused Liver

1. MEASUREMENTS OF GLUCOSE AND LACTATE PRODUCTION

Glucose was measured on a Yellow Springs Instrument Model 26 Glucose Monitor. The instrument is designed for the continuous monitoring of glucose using a glucose oxidase enzyme-hydrogen peroxide

probe. Samples were withdrawn continuously from the collection chamber of the liver perfusion apparatus with a thin polyethylene tubing (PE-10) and delivered to the reaction block of the monitor. Briefly, the principle of glucose determination by the glucose monitor is as follows. The tip of the glucose probe is covered by a three layer membrane. The outer membrane is composed of a polycarbonate material with relatively large pore size of 0.03 u which allows diffusion of small molecules (glucose, lactate, oxygen, hydrogen peroxide, water and ions) but excludes enzyme protein. The inner membrane is a cellulose acetate material with much smaller pore size that excludes even glucose but still allows hydrogen peroxide, oxygen, water, ions etc. to permeate. Between these two membranes immobilized glucose oxidase exists in a thin layer of glutaraldehyde resin. Glucose in the sample diffuses through the outer membrane and then is converted to gluconic acid and hydrogen peroxide under glucose oxidase by reaction 1.

1. $\text{D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$
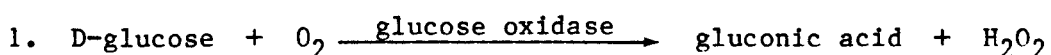
The hydrogen peroxide then diffuses through the inner membrane and is oxidized at a platinum anode by reaction 2.

2. $H_2O_2 \longrightarrow 2 H^+ + O_2 + 2e^-$
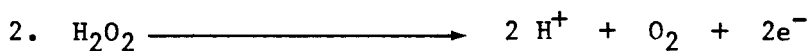
The current thus generated is directly proportional to the glucose concentration in the sample.

Lactate concentration was also measured on YSI Model 26 Monitor by exactly the same method and electrode principle but with a different

enzyme membrane and buffer. The probe consists of immobilized L-lactate oxidase and flavin adenine dinucleotide (FAD) between the outer and inner membrane. Lactate produces pyruvate and hydrogen peroxide under lactate oxidase and co-factors by reaction 3.

$$3. \quad \text{Lactate} + O_2 \xrightarrow[\text{FAD}]{\text{L-lactate oxidase}} \text{pyruvate} + H_2O_2$$
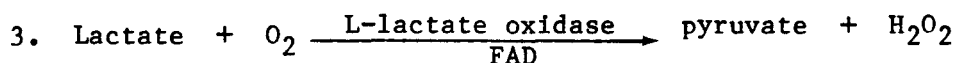
The hydrogen peroxide diffuses through the inner membrane, and generates a current when it is oxidized at the platinum anode by the same reaction as in the glucose determination (reaction 2).

Glucose and lactate production rates were calculated from glucose and lactate concentration changes of the perfusate as below:

Glucose production rate (umoles/gm/hr)

$= \Delta$ mg glucose/100 ml/5 min x 130 ml x 1000/180 umoles/mg x 60/5 x 1/gm

where; Δ mg glucose/100 ml/5 min: changes in glucose concentration in the perfusate for 5 minutes after addition of ATP.

130 ml: volume of total circulating perfusate.

1000/180 umoles/mg: conversion factor to calculate umoles of glucose from mg of glucose.

gm: liver weight after perfusion.

60/5: conversion factor from minute to hour.

Lactate production rate (umoles/gm/hr)

$= \Delta$ mg lactate/100 ml/5 min x 130 ml x 1000/90 umoles/mg x 60/5 x 1/gm

where; Δ mg lactate/100 ml/5 min: changes in lactate concentration.

1000/90 umoles/mg: conversion factor from mg of lactate to umoles.

The remaining terms of the expression were the same as in glucose calculation.

2. MEASUREMENTS OF OXYGEN CONSUMPTION

Oxygen tension was measured on Biological Oxygen Monitor (YSI Model 53). The oxygen probe was a Clark type polarographic electrode. The tip of the electrode is closed by a teflon FEP membrane with small amount of KCl solution. The membrane is permeable to gas (O_2) and allows them to enter the interior of the sensor. When a suitable polarizing voltage is applied across the probe oxygen will react at the cathode causing a current flow which is proportional to the oxygen pressure in the solution. Therefore, there is a linear relationship between external oxygen pressure and probe current. Two oxygen probes were employed--one at the site entering and the other at the site leaving the liver. In figure 2, O_{input} and O_{output} represent oxygen tension differences between the two probes indicates oxygen uptake by the liver. Oxygen tensions were generally measured at 30 seconds intervals e.g. after addition of ATP for 10 minutes. Oxygen consumption rates were calculated from oxygen uptake and perfusion flow as follows:

Oxygen amount in the solution: SO_2 (ul/ml)

= α x PO_2 (α : 0.024 ml O_2/ml of water/760 mmHg at 37°C)

= 0.024 ml O_2/ml of solution/760 mmHg x (760 x 95/100) mmHg

= 0.0228 ml O_2/ml of solution (=22.8 ul O_2/ml of solution)

Oxygen uptake by the liver: (ul/min)

= Oxygen amount in solution x Flow rate x Difference of saturation % /100

Oxygen consumption rate: (ul O_2/min/gm)

= Oxygen uptake by the liver x 1/Liver weight (gm)

3. MEASUREMENTS OF K^+ MOVEMENT

K^+ ion concentration in the perfusate was determined by an Orion Microprocessor Ionalyzer (Model 901). A K^+ selective electrode (Orion Model 9319) was used in combination with a single junction reference electrode (Ag/AgCl reference electrode, Orion Model 9001). The electrode consists of an electrode body and a sensing module. The replaceable sensing module contains an internal filling solution in contact with a porous plastic organophilic membrane. When the membrane is in contact with K^+ ion, an electrode potential develops across the membrane. This potential, which depends on K^+ ion concentration in solution, is measured against a constant reference potential of the ion analyzer. The measured potential corresponding to the K^+ ion concentration is described by the Nernst equation and it is converted to direct K^+ ion concentration by an internal computing program.

$E = E_o + S \log (A)$

where;   E   : measured electrode potential

E_o : constant reference potential

A   : K^+ ion concentration (in mM)

$$S \quad : \text{electrode slope}$$

The electrode was placed in the effluent flow passing through the collection chamber which was located just behind liver and it was calibrated by known K^+ standard solution before each experiment. Changes in K^+ ion concentration were read at 30 second intervals.

4. PERFUSION FLOW MEASUREMENT

The perfusion rate was measured by calibrated spherical float flowmeter (Gilmont Instrument, Inc.). Gilmont Flowmeter F-1300 (accuracy, $\pm$ 2%) was used for measurement of portal vein perfusion flow and Gilmont Flowmeter F-1200 was also used to adjust and to maintain constant perfusion flow through the hepatic artery. The real perfusion flow rate (ml/min) was calculated from scale reading on the flowmeter via a calibration chart.

5. PERFUSION PRESSURE MEASUREMENT

Portal vein and hepatic arterial perfusion pressures were measured by Statham pressure transducers (P23DC). To measure changes in the perfusion pressure of the portal vein, the perfusion flow was maintained at 64 $\pm$ 0.4 ml/min by a pump. Changes in hepatic arterial pressure were measured under constant flow (3.1 $\pm$ 0.4 ml/min). In this case, the liver was perfused through the portal vein simultaneously with constant perfusion pressure.

6. MEASUREMENTS OF CHANGES IN LIVER CAPACITANCE

After cannulation of portal and hepatic veins, the liver was transferred to a strain-gauge weighing device and perfused at a constant pressure. Changes in liver weight were measured with constant pressure (28 cmH_2O) by a force-transducer (Grass FT03C) and Grass Polygraph. After completion of the perfusion, the wet liver weights were determined on a Mettler top-loading balance (Model P 1200).

E. Measurements of Glucose and Lactate Production in the Isolated Hepatocytes

1. ISOLATION OF HEPATOCYTES

Hepatic parenchymal cells were isolated from fed rat livers by a modification of the method of Berry and Friend (30). The liver was isolated by the same method as described above and then perfused with Ca^{++} and glucose-free Hanks' (164) solution containing collagenase and hyaluronidase (Sigma). The perfusate contained (in mM): NaCl 137, KCl 5.36, Na_2HPO_4 0.34, KH_2PO_4 0.44, $NaHCO_3$ 4.17, $MgSO_4$ 0.41, $MgCl_2$ 0.49, and collagenase 0.05% and hyaluronidase 0.1% adjusted to pH 7.4. The liver was perfused in the recirculating system at a rate of 50 to 55 ml/min under a constant hydrostatic pressure of 28 cmH_2O. The enzyme digestion medium was oxygenated with 100% O_2 and liver temperature was maintained at 36°C. After approximately 20 minutes of perfusion, the liver was disaggregated within the capsule and was carefully removed from the perfusion box and placed in a 100 ml Nalgene beaker. After adding 50 ml of cold perfusion medium, the diaphragm and blood vessels

were removed. The digested liver was cut into very small pieces to aid the dispersion of the cells. In order to disperse the remaining tissue, the liver cell suspension was divided into two portions and each portion placed in a 250 ml Nalgene Erlenmeyer flask. After gassing with 100% O_2 for 1 minute, the liver cells were incubated for 15 minutes at 37°C in a Dubnoff metabolic shaker bath at 100 oscillations per minute. At the end of the incubation, the hepatocyte suspension was then sequentially filtered through three folds of sterile surgical gauze and 100-mesh silk screen into 50 ml Nalgene centrifuge tube in the ice box. Intact hepatocytes were separated from cellular debris, damaged cells, and Kupffer cells by centrifuging at 50 x g for 3 minutes at 4°C. The cell pellet was washed three times with buffer (two times with Ca^{++} and glucose-free Hank's solution and one time with glucose-free KRB buffer solution). The isolated hepatocytes were filtered again and finally resuspended in a 40 ml of cold glucose-free KRB buffer. Protein concentration of the hepatocyte suspension was determined by the method of Lowry et al. (1951) using a Beckman Spectrophotometer (Acta C3).

2. INCUBATION AND ASSAY

6 ml of the hepatocyte suspension was incubated at a concentration of 2.45 $\pm$ 0.25 mg of protein/ml in 25 ml Erlenmeyer flasks. Incubation was conducted at 37°C in a Dubnoff metabolic shaker bath at 60 oscillations/min and continuously gassed with water-saturated 95% O_2/5% CO_2 for 60 minutes. At 0, 10, 20, 30, 40, 50 and 60 min of incubation, 0.2 ml of incubation mixture were placed into Beckman microfuge tubes in ice, centrifuged in a Beckman 152 Microfuge for 30

seconds and frozen in the freezer. Glucose and lactate concentrations in the supernatant were measured using the glucose oxidase and lactate oxidase methods in the YSI 23A Glucose Analyzer and YSI 23L Lactate Analyzer respectively.

F. Statistical Analysis

Results are presented as mean $\pm$ standard error of the mean (Mean $\pm$ S.E.M.). The statistical significance of the data was determined by Student's t-tests for paired or unpaired observations. Significance was accepted at $p < 0.05$.

CHAPTER IV

RESULTS

A. Effects of ATP on Glucose and Lactate Production Rates in the
   Isolated Fed Perfused Liver

1. GLUCOSE PRODUCTION UNDER CONSTANT PERFUSION PRESSURE

Since the KRB solution used in these experiments initially
contained no glucose, the glucose concentration measured in the medium
prior to starting hepatic perfusion was zero. The glucose concentration
of the perfusate rapidly increased early in the perfusion probably due
to glycogenolysis; the rate however showed a saturation point after 20
min to 30 min (Fig. 3). Thirty minutes after perfusion ATP (26 ul of 50
mM) was added into the perfusate reservoir to achieve a concentration of
10 uM. 40 uM and 160 uM concentration of ATP were then achieved at 15
min and 30 min after the first addition of ATP.

As shown in Fig. 3 glucose concentration in the perfusate was
increased by addition of ATP and the magnitude of increase was
proportional to the ATP concentration. Fig. 4 depicts the effects of
ATP on glucose production rates (umoles/gm/hr) under constant portal
perfusion pressure. As the concentration of ATP in the perfusate was
increased from 0 (basal) to 10 uM, 40 uM and finally 160 uM, the glucose
production rates were significantly increased from 52.5 ± 3.5 (basal) to
86.5 ± 4.1 ($P<0.05$), 121.0 ± 7.1 ($P<0.001$) and 197.0 ± 17.4 ($P<0.001$),

FIGURE 3

RECORDING OF CHANGES IN GLUCOSE AND LACTATE LEVELS

DURING PERFUSION OF THE LIVER

The rat liver was perfused through portal vein with recirculating KRB solution under a constant perfusion pressure of 28 cmH_2O. The glucose and lactate levels were continuously and simultaneously measured and recorded. ATP was added into the perfusate reservoir at 30, 40, and 55 minutes of perfusion.
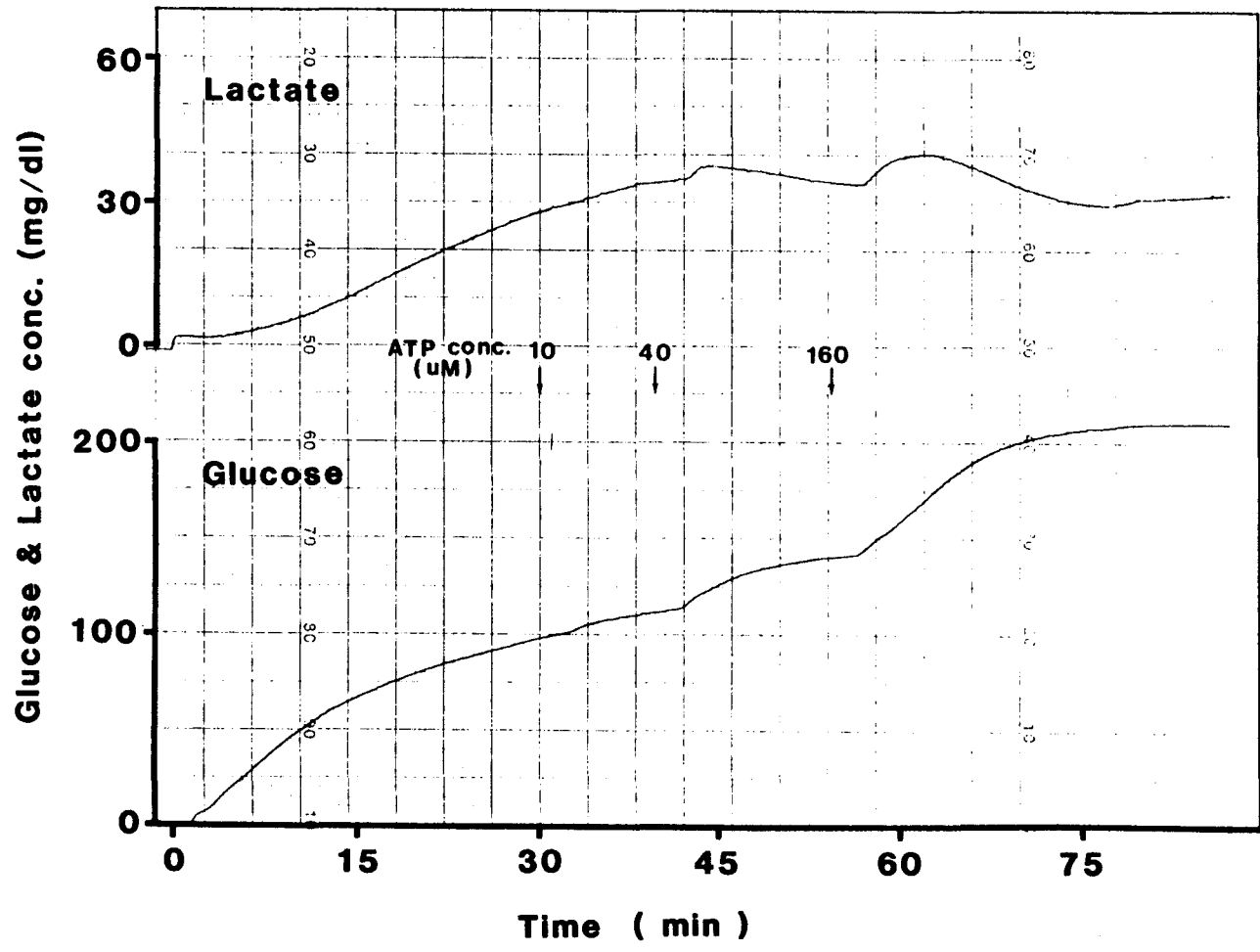
FIGURE 4

EFFECTS OF ATP ON GLUCOSE PRODUCTION UNDER CONSTANT

PERFUSION PRESSURE

Mean $\pm$ S.E.M. of glucose productin rates for control and ATP additions are presented. Number of experiments is indicated in parentheses. * denotes $P < 0.05$ and ** denotes $P < 0.001$ compared to control.
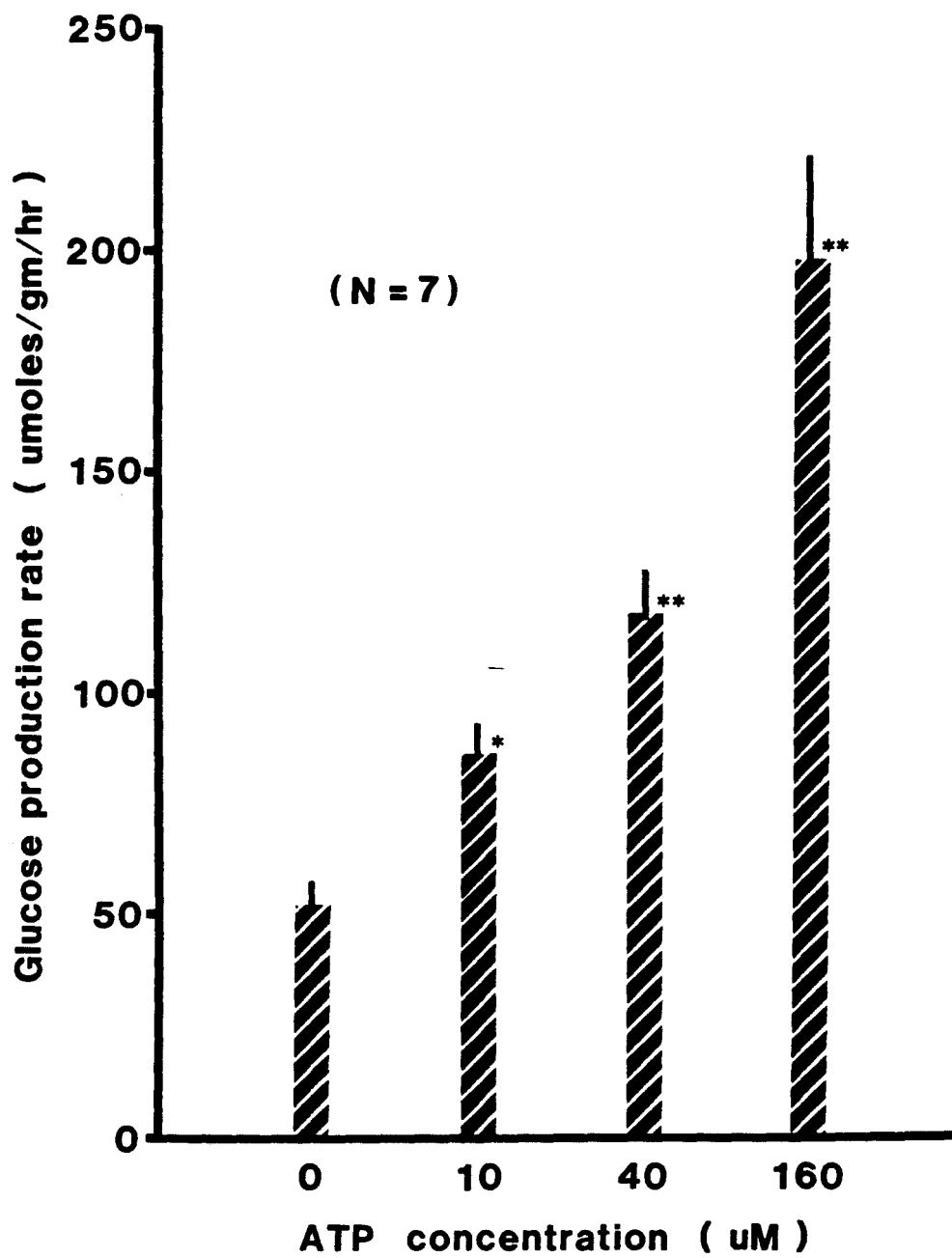
respectively.

2. GLUCOSE PRODUCTION UNDER CONSTANT PERFUSION FLOW

When ATP was added to the perfusate, perfusion flow was decreased. Thus, the reduction in perfusion flow might have affected glycogenolysis by limitation of oxygen delivery and therefore explain the effects of ATP on glucose production. To test the possibility that the glycogenolytic effect of ATP was due to the low perfusion flow, livers were perfused through the portal vein with a constant flow (64 ± 0.4 ml/min) and the effects of ATP on glucose production were observed. The results of the constant flow experiments are shown in Fig. 5. In general, glucose production rates in response to ATP additions under constant perfusion flow were lower than under constant perfusion pressure (Fig. 4 and 5). However, ATP still increased glucose production significantly and in proportion to its concentration i.e., control: 43.2 ± 3.4; 10 uM : 63.1 ± 3.7, $P<0.05$; 40 uM : 91.3 ± 7.9, $P<0.001$; and 160 uM ATP : 130.4 ± 11.7, umoles/gm/hr, $P<0.001$. These findings indicate that the low perfusion flow induced by ATP is not the sole factor which increased glucose production in the perfused liver.

3. LACTATE PRODUCTION UNDER CONSTANT PERFUSION PRESSURE

As shown in Fig. 3, lactate concentration in the perfusate was increased progressively during the perfusion. The rate of appearance of lactate was slow during initial 0 to 10 minutes of perfusion, then it increased progressively. The rate of lactate appearance was lower than that of glucose. Thirty minutes after perfusion three different

FIGURE 5

EFFECTS OF ATP ON GLUCOSE PRODUCTION UNDER CONSTANT

PERFUSION FLOW

Livers were perfused with constant flow of 64 ± 0.4 ml/min. Means $\pm$ S.E.M. of glucose production rates for control and ATP additions are presented. Number of experiments is indicated in parentheses. * denotes $P < 0.05$ and ** denotes $P < 0.001$ compared to control.
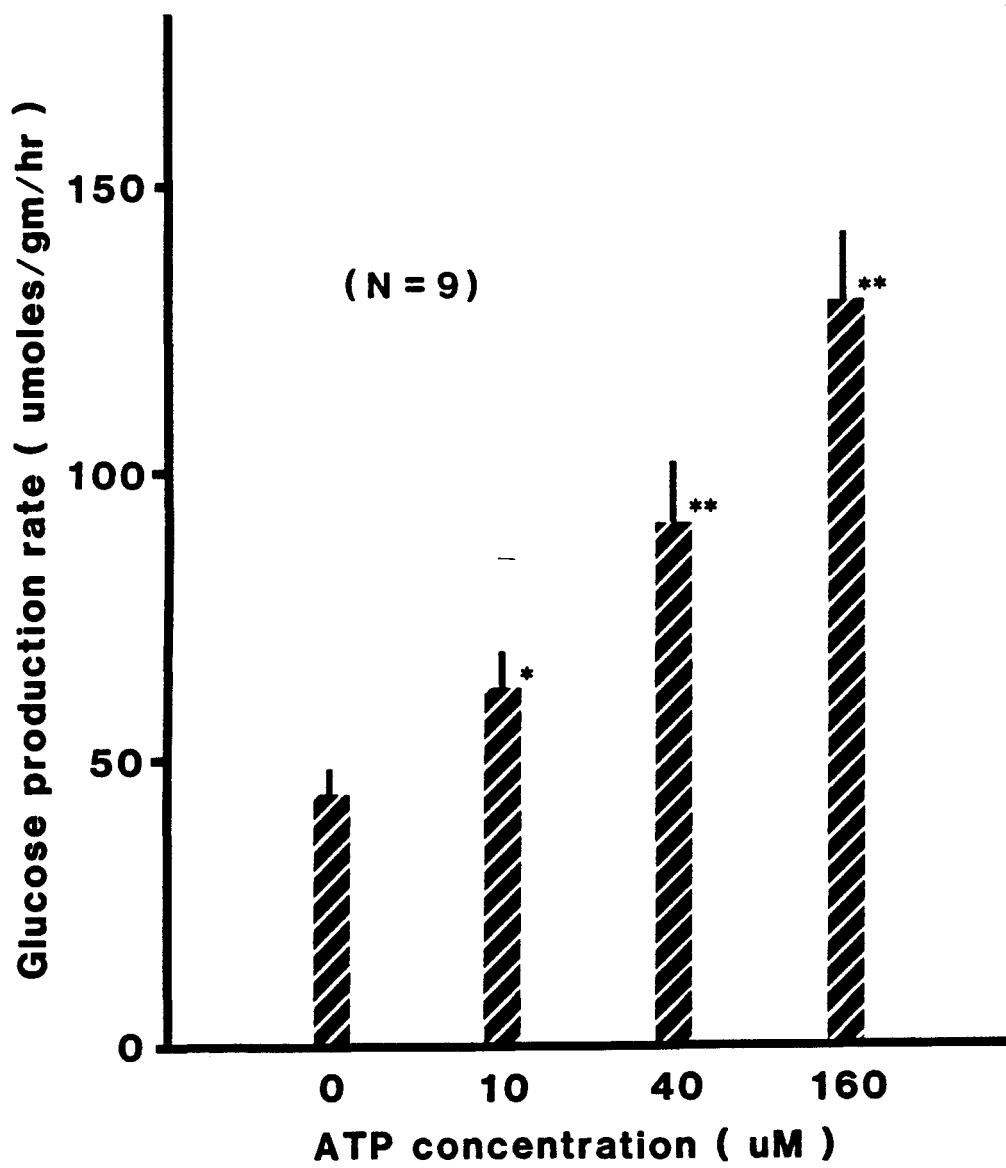
concentrations of ATP were added into the perfusate reservoir by the same means as in the section on glucose production (A-1). Lactate production was increased by the ATP added in proportional to its concentration. After reaching a peak, the concentration of lactate progressively declined. This may be due to use of the lactate for gluconeogenesis as a substrate by the liver. The pH of the perfusate was decreased by addition of ATP for a few minutes and then it was recovered. This may be due to an increase in lactate concentration. The rates of lactate production (umoles/gm/hr) by 0, 10, 40, and 160 uM ATP were 34.6 $\pm$ 4.9, 44.0 $\pm$ 8.6, 63.2 $\pm$ 3.5 (P<0.01), and 91.9 $\pm$ 4.6 (P<0.001) respectively (Fig. 6).

B. Effects of ATP on glucose and lactate production rates in the hepatocytes isolated from fed rat

1. GLUCOSE PRODUCTION RATE

In order to assess a direct effect of ATP on glycogenolysis in the liver, ATP was next evaluated in hepatocytes isolated from fed rat livers. During incubation of hepatocytes at $37^{\circ}C$, glucose production was measured both with and without ATP (50 and 200 uM) in the incubation medium. Glucagon ($10^{-6}M$) was also added to confirm whether this cell system responded to a known glycogenolytic agent. Fig. 7 illustrates the time course of glucose output of the isolated hepatocytes during incubation under the following conditions: presence of 50 uM ATP, 200 uM ATP, 10^{-6} M glucagon and glucagon ($10^{-6}M$) plus ATP (200 uM).

The effects of ATP on glucose production rates in the isolated

FIGURE 6

EFFECTS OF ATP ON LACTATE PRODUCTION IN THE PERFUSED LIVER

Livers were perfused under constant perfusion pressure of 28 cmH_2O. Means $\pm$ S.E.M. of lactate production rates for control and ATP additions are presented. Number of experiments is indicated in parentheses. * denotes $P < 0.01$ and ** denotes $P < 0.001$ compared to control.
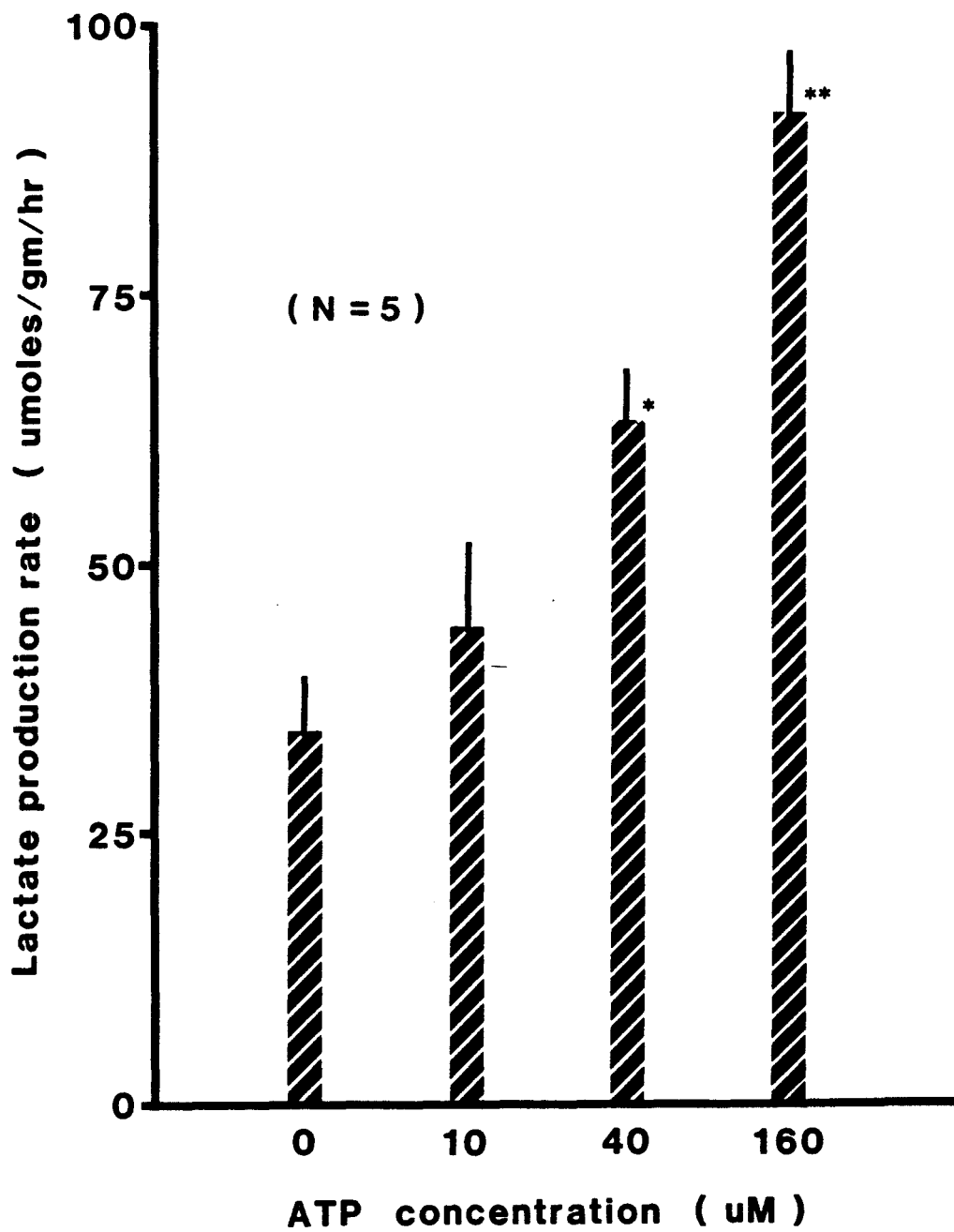
hepatocytes are presented in Fig. 8. Fifty uM ATP tended to decrease glucose production but the change was not significantly different from control (P>0.1); however, 200 uM ATP actually inhibited glucose production significantly (control : 0.575 $\pm$ 0.034; 50 uM : 0.530 $\pm$ 0.029; 200 uM: 0.475 $\pm$ 0.027 umoles/mg protein/hr, P<0.05). Glucagon (10^{-6}M), however, stimulated glucose production two fold as compared to controls. ATP inhibited glucagon-stimulated glucose output (10^{-6}M glucagon : 1.146 $\pm$ 0.059; glucagon (10^{-6}M) plus ATP (200 uM) : 0.802 $\pm$ 0.027, P<0.001). These results indicate that ATP does not directly increase glycogenolysis in the hepatic parenchymal cells and indeed may inhibit net glucose production.

2. LACTATE PRODUCTION RATE

Lactate production is generally considered as an indicator of glycogenolysis and subsequent glycolysis during hypoxic states. Therefore, the effects of ATP on lactate production were studied in isolated hepatocytes (Fig. 9). Lactate production increased linearly during incubation of control hepatocytes. The increase after 40 min subsequently diminished progressively. As shown in Fig. 10, exogenous ATP diminished lactate production (control : 0.434 $\pm$ 0.041, 50 uM : 0.361 $\pm$ 0.039, 200 uM ATP : 0.171 $\pm$ 0.031, P<0.001). Glucagon almost completely inhibited lactate production and ATP failed to alter the inhibitory response. These results also indicate that ATP has no direct stimulating effect on glycogenolysis in hepatic parenchymal cells.

FIGURE 7

TIME COURSE OF GLUCOSE PRODUCTION IN ISOLATED HEPATOCYTES

Hepatocytes were isolated from fed rat liver and incubated in KRB at 37°C. ATP and glucagon were added at 0 minutes of incubation time. Glucose concentration in the medium was measured at 10 minutes intervals for 60 minutes of incubation. Protein concentration in each flask was 2.45 mg/ml. Means $\pm$ S.E.M. are depicted.

FIGURE 8

EFFECTS OF ATP ON GLUCOSE PRODUCTION RATES IN THE

ISOLATED HEPATOCYTES

Means $\pm$ S.E.M. of glucose production rates for control, ATP, glucagon and glucagon plus ATP are presented. Number of experiments is indicated in parentheses. * denotes $P<0.05$, ** denotes $P<0.0005$ compared to control and *** denotes $P<0.001$ compared to glucagon addition.
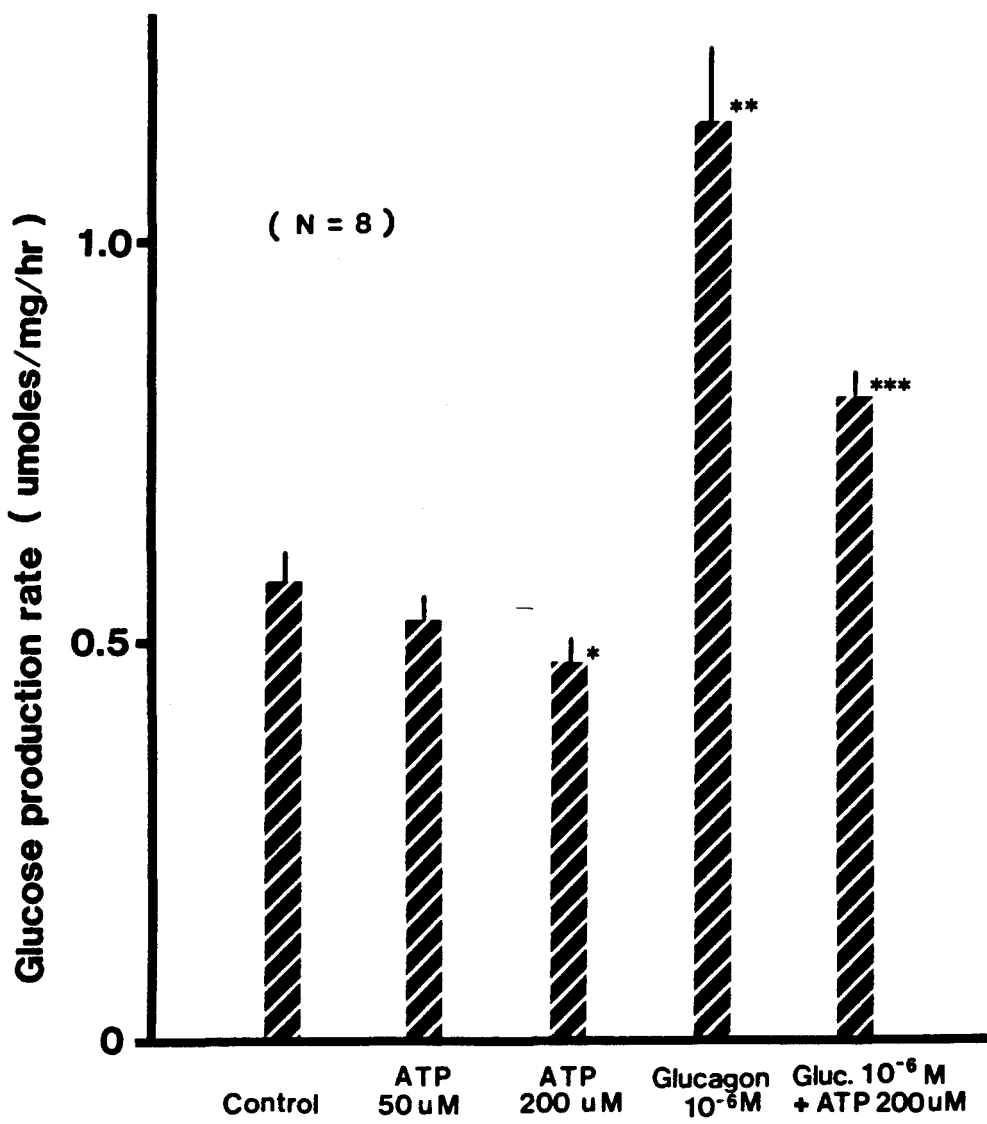
FIGURE 9

TIME COURSE OF LACTATE PRODUCION IN

ISOLATED HEPATOCYTES

Lactate concentration in the medium was measured in the same samples used to determine glucose production in Figure 7. Means $\pm$ S.E.M. are depicted.
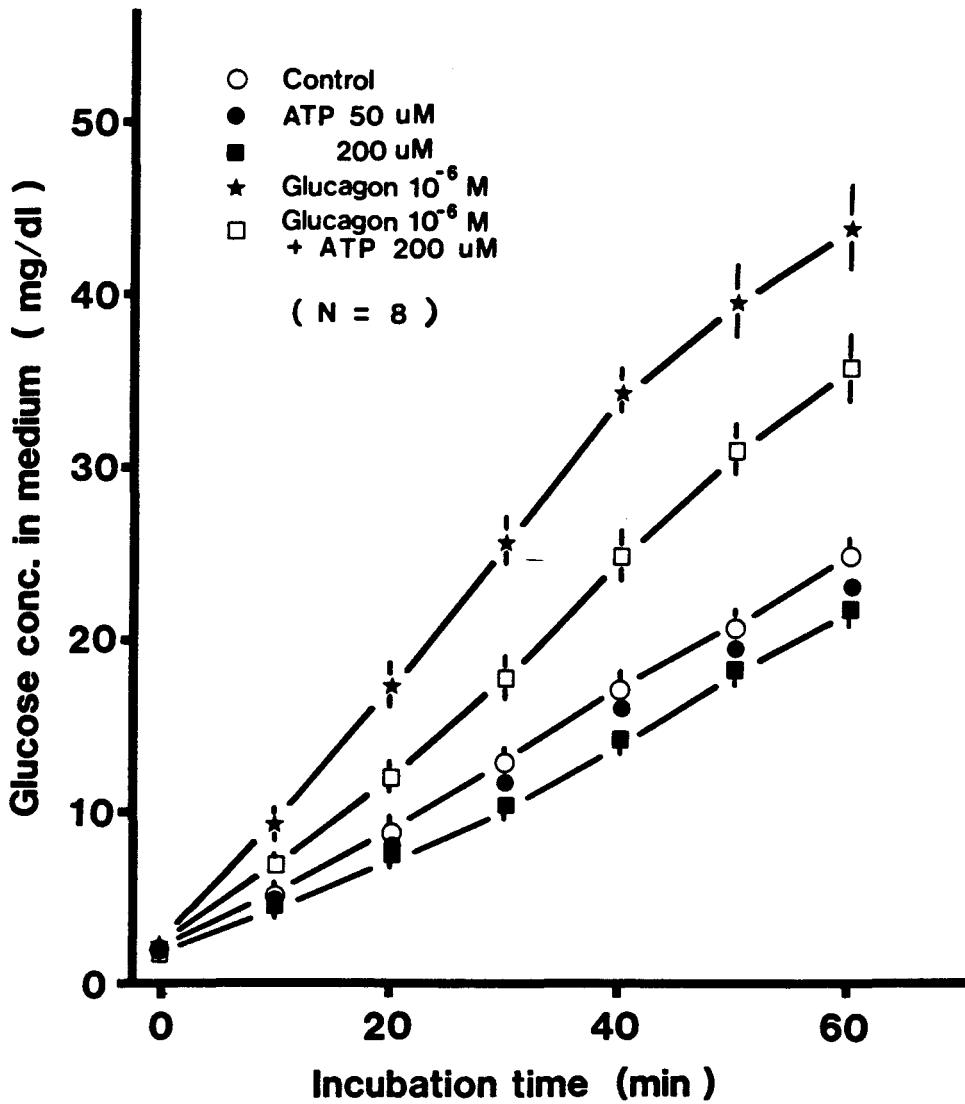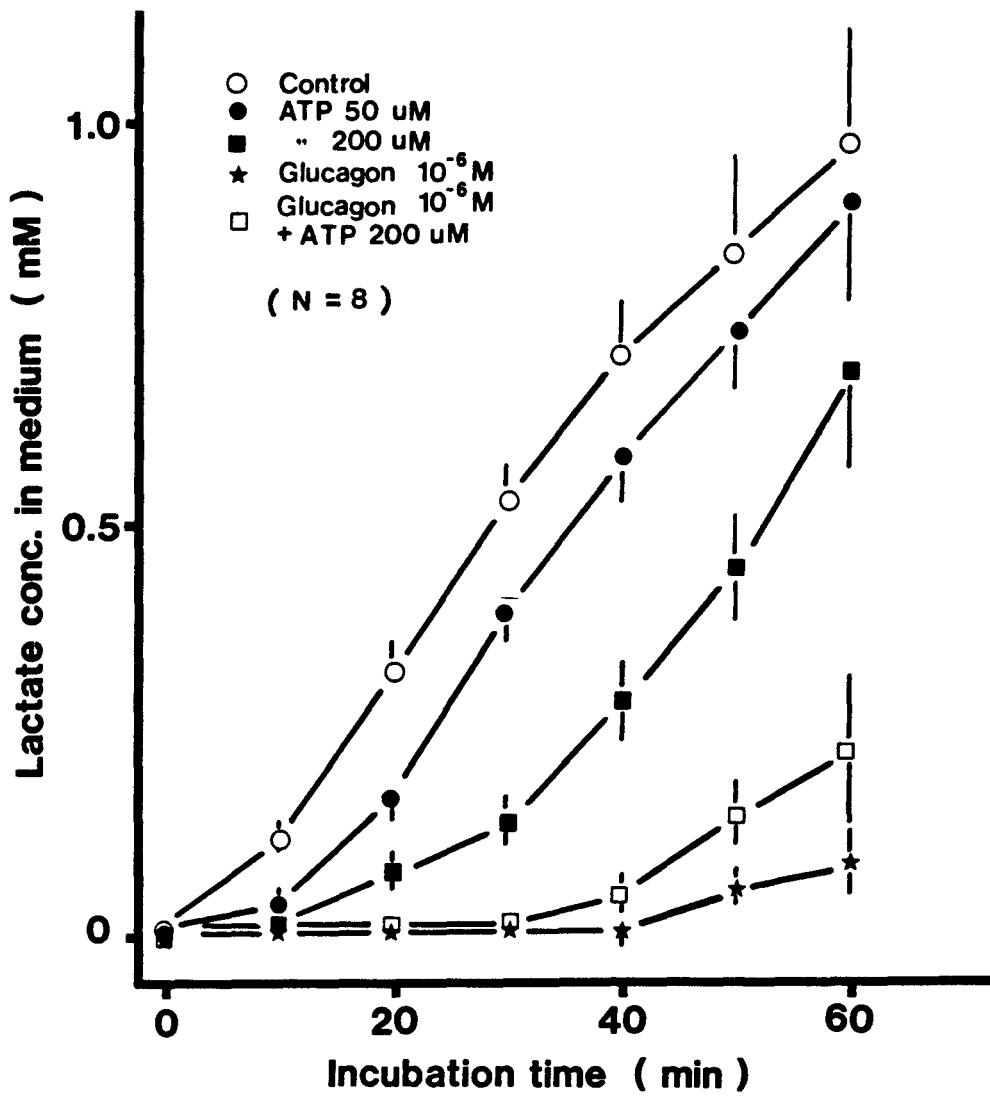
FIGURE 10

EFFECTS OF ATP ON LACTATE PRODUCTION RATES

IN THE ISOLATED HEPATOCYTES

Means $\pm$ S.E.M. of lactate production rates for control, ATP, glucagon, and glucagon plus ATP are presented. Number of experiments is indicated in parentheses. * denotes $P<0.005$, ** denotes $P<0.001$ compared to control and *** denotes $P>0.05$ compared to glucagon addition.
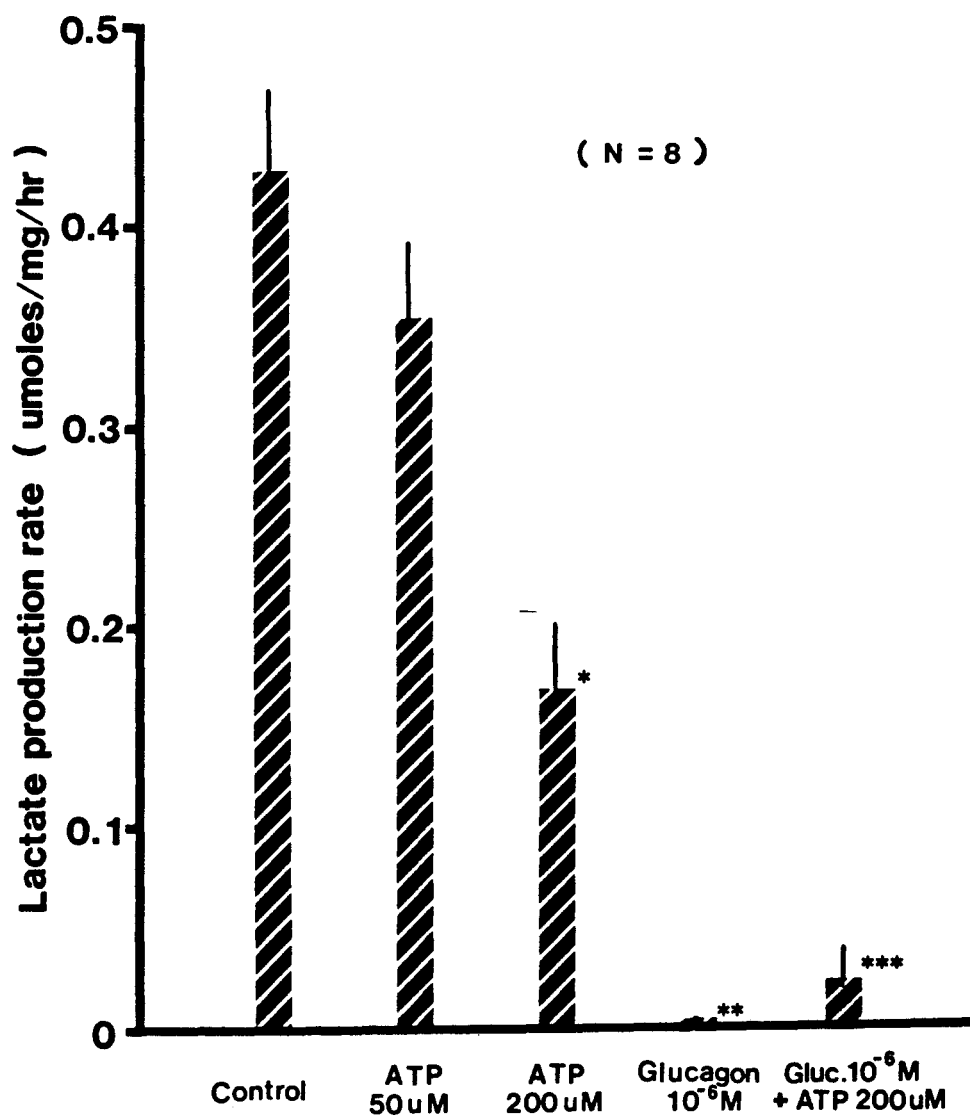
C. Effects of ATP on Oxygen Consumption in the Perfused Liver

It is possible that endothelial cell injury occurs at high perfusion flow, while the tissue falls into a hypoxic state at low perfusion flows (297). Therefore, it is important to maintain an adequate perfusion flow to prevent either cell injury or hypoxia particularly in erythrocyte-free perfusion system which requires high perfusion flow rate. Oxygen consumption of the perfused liver was calculated from perfusion flow and oxygen extraction measurements (see Methods Section, pp). As the perfusion flow increased from 25 ml/min to 65 ml/min, oxygen extraction (O_2 tension difference of the perfusate entering and exiting the liver) decreased. However, oxygen consumption rates increased as the perfusion flow increased; due to the fact that the percent increase in perfusion flow was higher than the decrease of oxygen extraction (Fig. 11). Therefore, in the present studies livers were perfused at a flow rate of 60 to 65 ml/min; this was the range at which oxygen consumption was saturated.

Fig. 12 presents hepatic oxygen extraction rates at varying doses of ATP. The bottom line (point a) represents the oxygen tension of the effluent during the perfusion period. Point b represents the oxygen tension of the influent at the same time. Therefore, the differences of oxygen tension between influent and effluent (points b - a) indicate oxygen extraction by the liver. ATP (10 uM) increased oxygen extraction. However, oxygen extraction at high concentrations of ATP (40 and 160 uM) exhibited a biphasic course with an early decrease in oxygen extraction followed by a later increase in oxygen extraction.

FIGURE 11

RELATIONSHIP BETWEEN PERFUSION FLOW AND OXYGEN

TENSION DIFFERENCE OR OXYGEN CONSUMPTION RATES

Portal vein perfusion flow was regulated by adjusting flow with a tubing clamp during a constant perfusion pressure of 28 cmH_2O. Means $\pm$ S.E.M. are depicted.
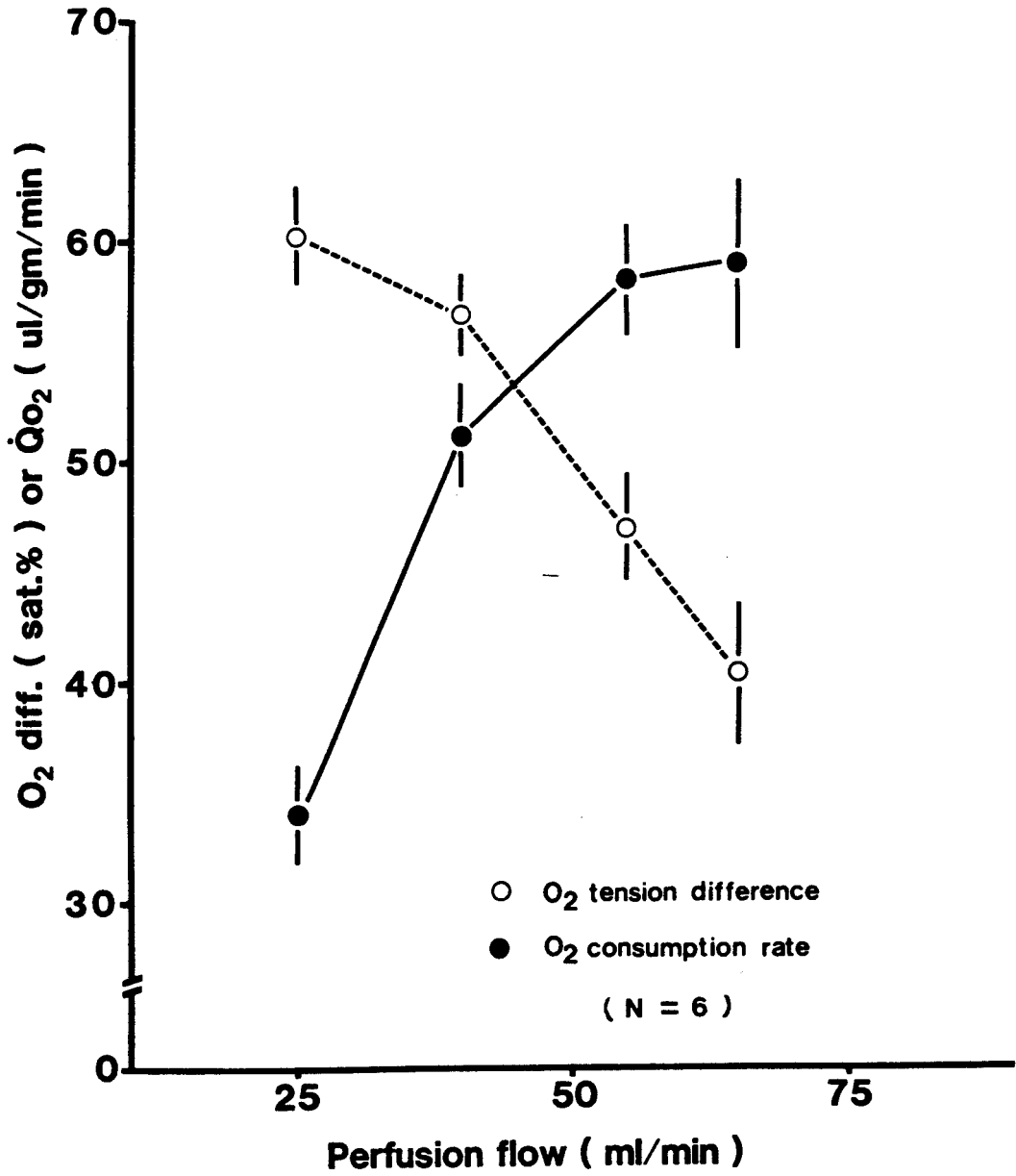
FIGURE 12

CHANGES IN OXYGEN TENSION IN THE PERFUSATE DURING

PERFUSION WITH ATP

Livers were perfused with oxygenated KRB under a constant perfusion pressure of 28 cmH_2O. Oxygen tensions in the influent and effluent perfusion fluid were monitored with two oxygen probes. Oxygen tension of the effluent was recorded continuously and that of influent was read at proper times using a probe-selection swtich. Point a represents the oxygen tension of the effluent and point b represents the oxygen tension of the influent at the same time.
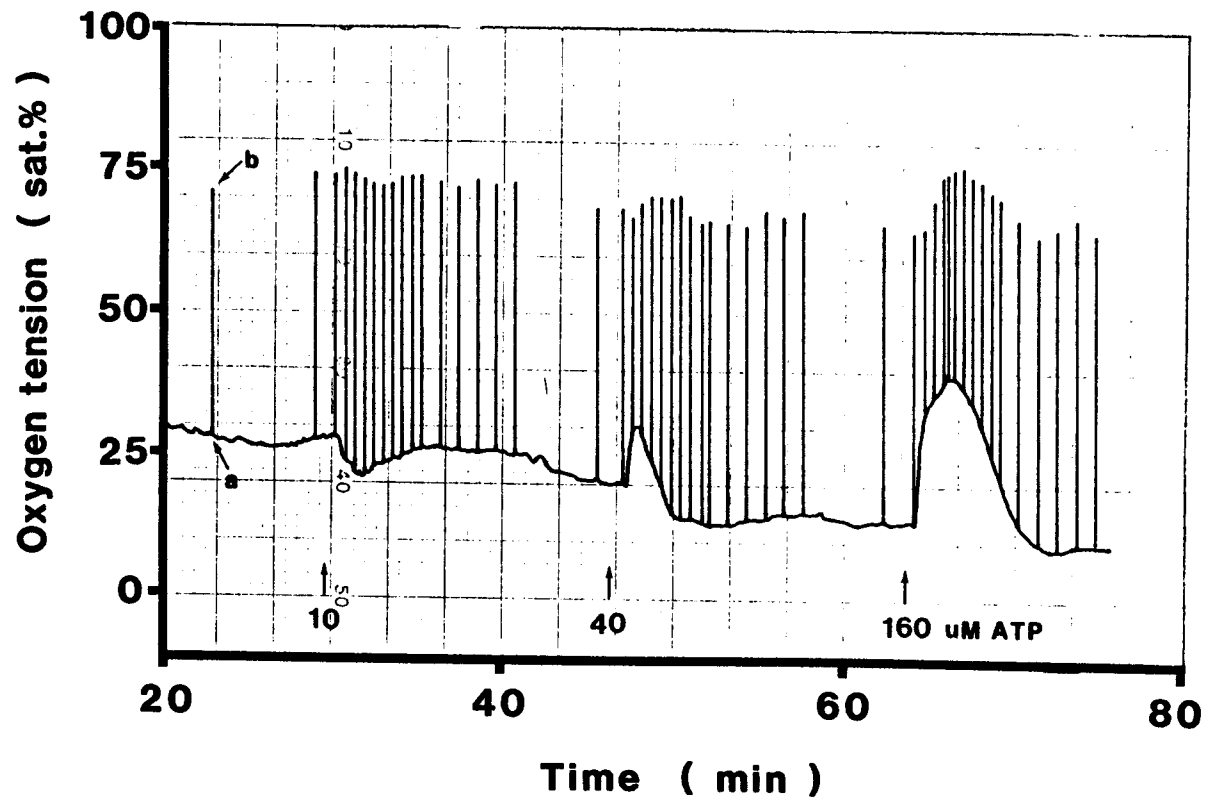
FIGURE 13

EFFECTS OF ATP ON OXYGEN CONSUMPTION RATE

Left figure (A) represents the effect of ATP on oxygen consumption rate in the perfused liver. Right figure (B) represents % changes in oxygen consumption rate by ATP. Each point represents mean $\pm$ S.E.M. from 6 liver.
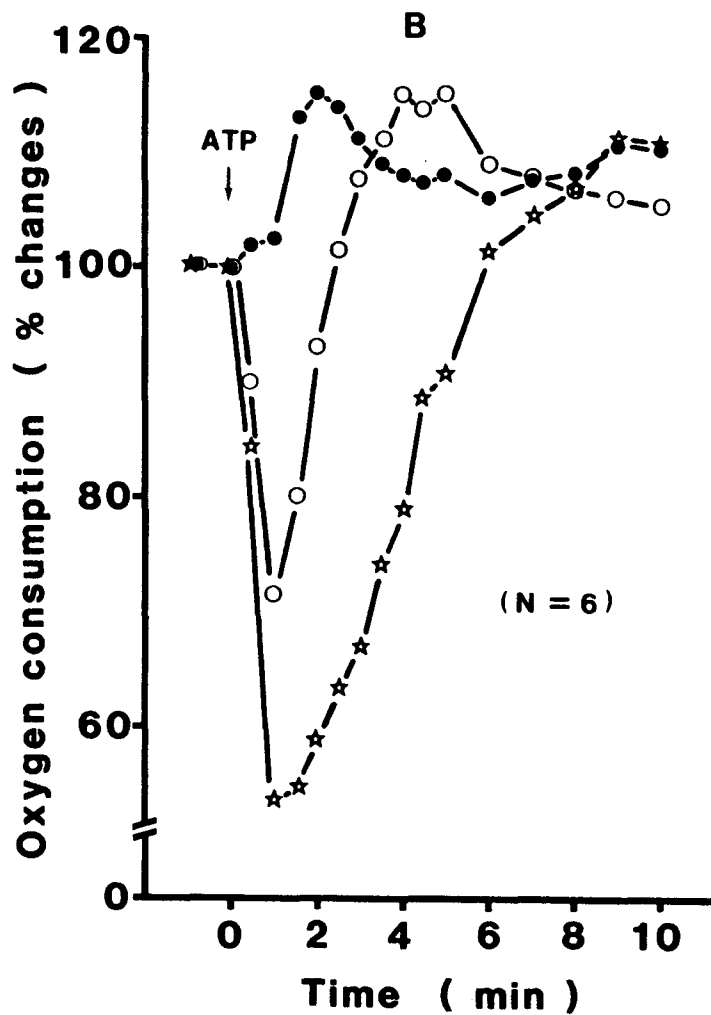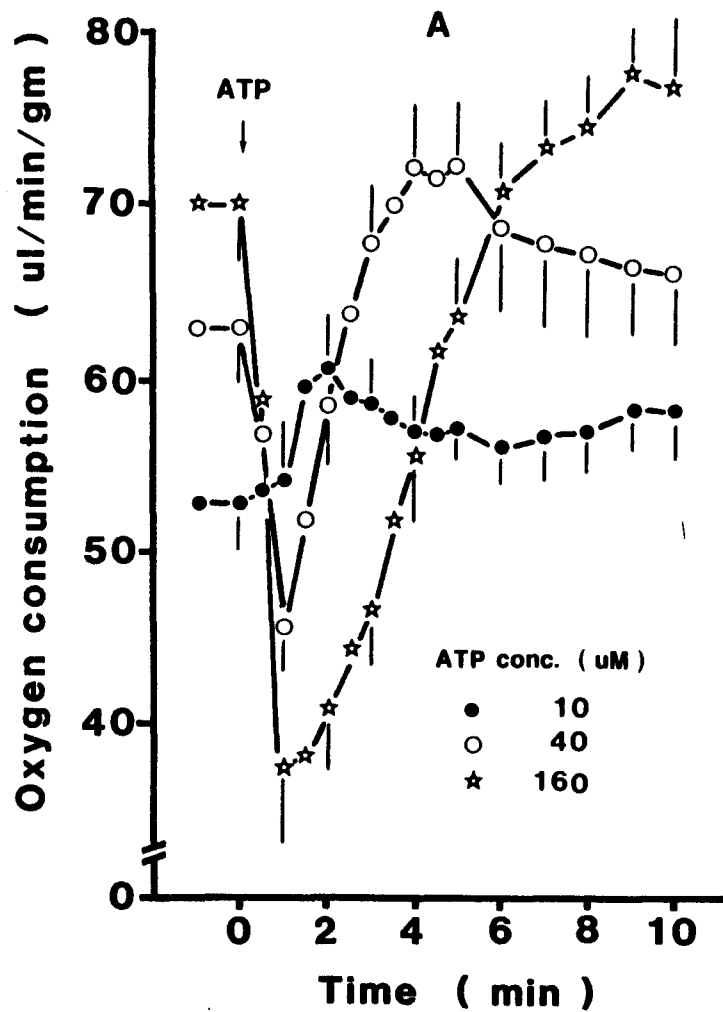
Fig. 13-A depicts the effects of ATP on oxygen consumption. Although ATP decreased perfusion flow, the increment of oxygen extraction after 10 uM ATP was relatively greater than the reduction of perfusion flow, such that the oxygen consumption rate was increased at low concentrations of ATP. However, high doses of ATP (40 and 160 uM) decreased both perfusion flow rate and oxygen extraction. Consequently 40 uM and 160 uM ATP decreased oxygen consumption in the early phase. Two to 5 min after ATP addition the perfusion flow recovered to the original level but oxygen extraction still remained increased. These factors caused a resultant high oxygen consumption in the late phase. Fig. 13-B presents percent changes in oxygen consumption by addition of ATP. The peak changes in oxygen consumption (in %) by 10 uM, 40 uM and 160 uM ATP were +15.2 ($P<0.05$), -39.5 ($P<0.05$) and -86.9 ($P<0.001$), respectively.

D. Effects of ATP on K^+ Movement in the Perfused Liver

A transient release and uptake of K^+ occur concomitantly with glucose output when the liver is stimulated by either hormones or other stimuli such as hypoxia (171, 294). K^+ concentration in effluents were measured at 30 sec intervals with a K^+ ion-sensitive electrode. Fig. 14 shows K^+ movement after addition of ATP. K^+ was apparently extracted by the liver during the first 1 min after addition of ATP (in 10 uM or 40 uM). The initial extraction was immediately followed by a K^+ release which reached a maximum at 4 to 5 min after ATP. The peak K^+ concentrations in mM of the perfusate after ATP were 5.06 ± 0.08, 5.33 ± 0.12 ($P<0.05$) and 5.62 ± 0.10 ($P<0.05$) at 10 uM, 40 uM and 160 uM ATP,

FIGURE 14

EFFECTS OF ATP ON K$^+$ MOVEMENT IN THE

PERFUSED RAT LIVER

K$^+$ was continuously measured in the effluent from the liver using ion-selective electrodes. After 30 minutes of perfusion 10 uM ATP was added. 40 uM and 160 uM ATP were added at 45 and 60 minutes of the perfusion. Each point represents mean $\pm$ S.E.M. from 6 livers.
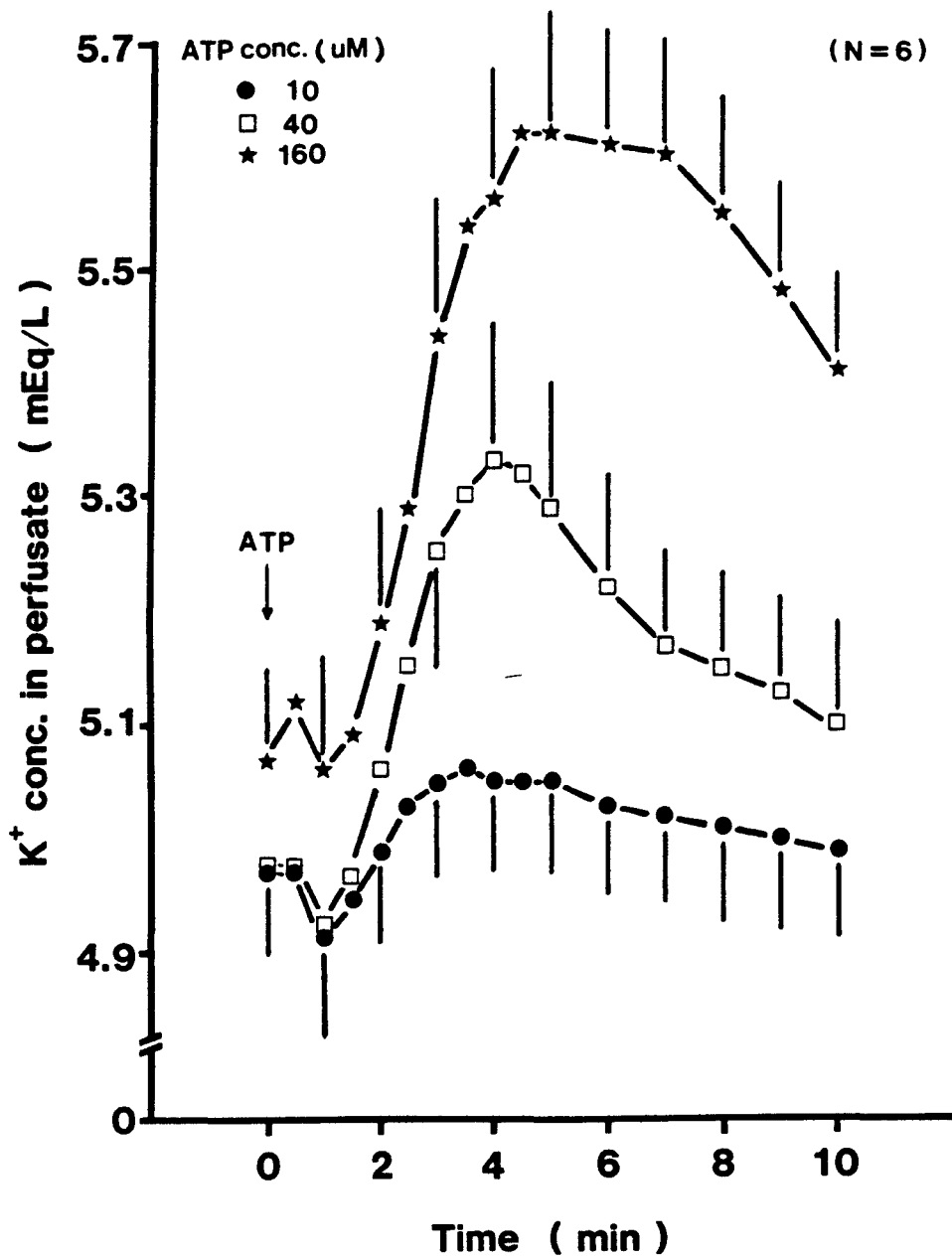
respectively. K^+ was subsequently taken up again, such that the K^+ concentration of the perfusate declined to the original level.

E. Effects of ATP on Hepatic Circulation in the Isolated Liver Preparation

1. ATP ON PORTAL VEIN PERFUSION FLOW

Changes in perfusion flow in the portal vein under constant perfusion pressure (28 cmH_2O) were measured. After a 30 min stabilizing period, 10 uM, 40 uM and 160 uM ATP were added into the perfusate and perfusion flows were determined at 30 sec intervals. As depicted in Fig. 15, ATP decreased perfusion flow in proportion to its concentration with the lowest values achieved at 1 - 1.5 min after ATP. The maximum reductions of perfusion flow were 16.8%, 27.3% and 43.7% at concentrations of 10 uM, 40 uM and 160 uM of ATP, respectively. The perfusion flow subsequently recovered to the original flow and the recovery time was directly proportional to the ATP dose.

2. ATP ON PORTAL VEIN PERFUSION PRESSURE

Fig. 16 depicts a representative record of the effects of ATP on portal vein pressure changes under constant flows of 64 $\pm$ 0.4 ml/min. Three different concentration of ATP (10, 40, and 160 uM) were added into the perfusate reservoir. ATP increased portal vein pressure significantly. ATP effects occurred within 30 sec and the portal vein pressure reached a maximum level at 1 or 1.5 minutes after ATP addition. The results of 8 similar experiments (Fig. 17) indicated

FIGURE 15

EFFECTS OF ATP ON PERFUSION FLOW VIA THE

PORTAL VEIN

Livers were perfused with KRB under a constant perfusion pressure of 28 cmH_2O. After 30 minutes of perfusion ATP was added into the perfusate reservoir. Each point represents mean $\pm$ S.E.M. from 14 livers.
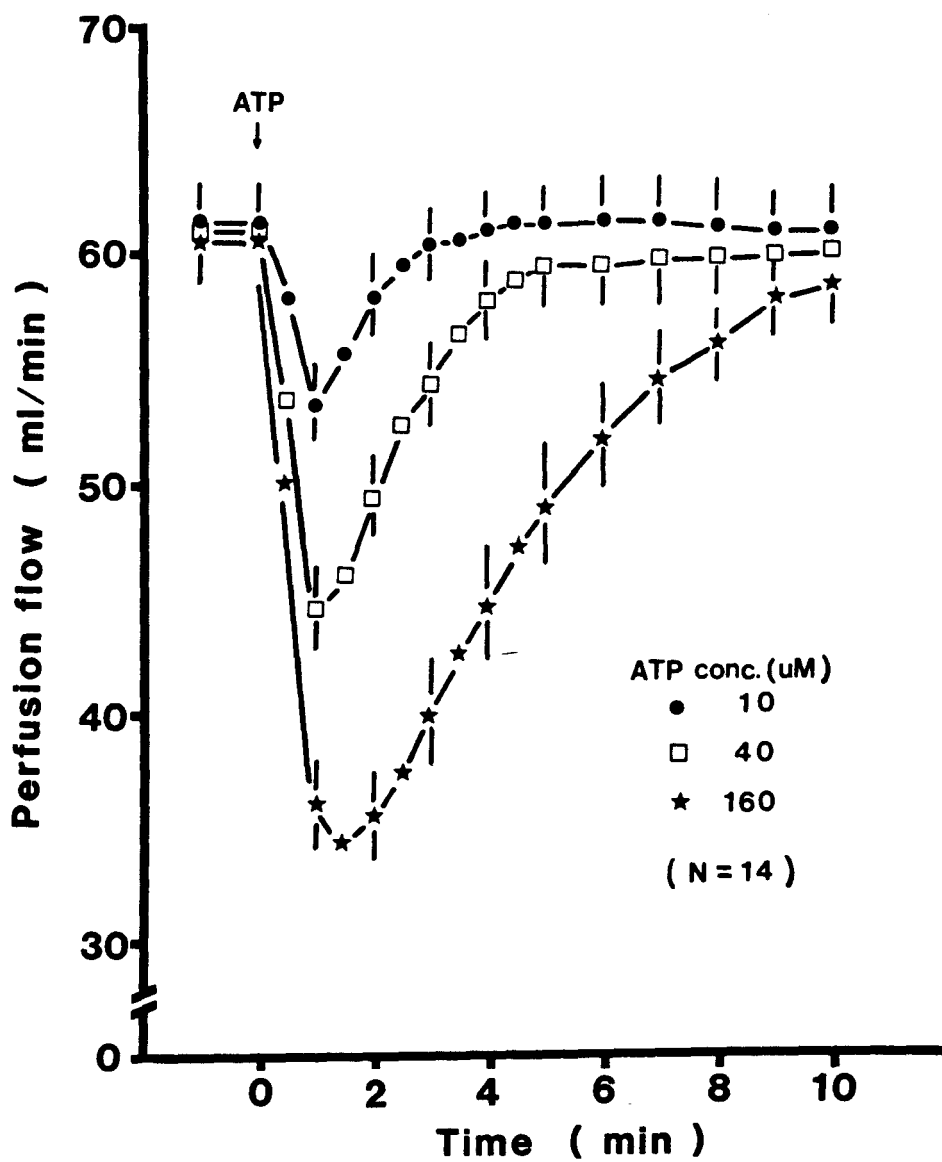
FIGURE 16

RECORDING OF CHANGES IN PERFUSION PRESSURE

IN THE PORTAL VEIN

Portal vein perfusion pressures were measured under a constant perfusion flow of 62 ml/min.   10uM ATP was added at 30 minutes of perfusion.   40uM and 160uM ATP were added at 40 and 50 minutes of perfusion.
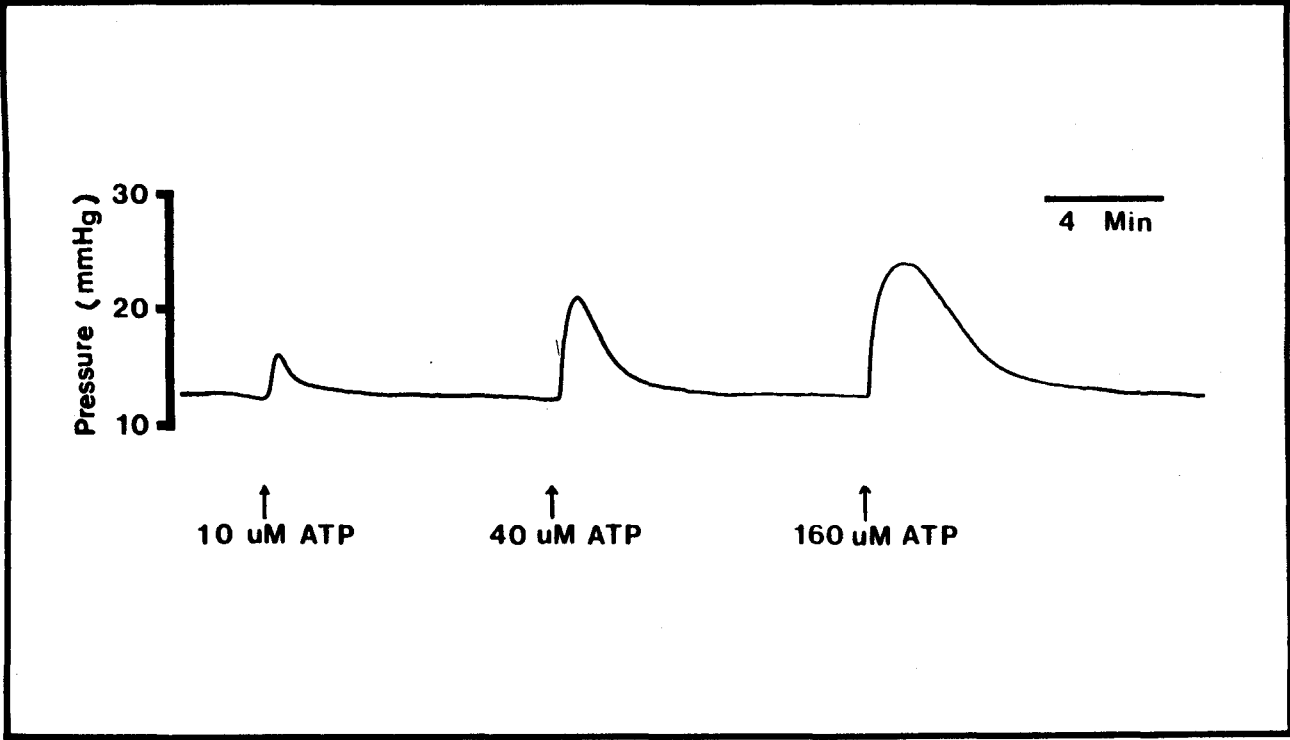
FIGURE 17

EFFECTS OF ATP ON PERFUSION PRESSURE

OF THE PORTAL VEIN

Each point represents means ± S.E.M. from 8 livers.
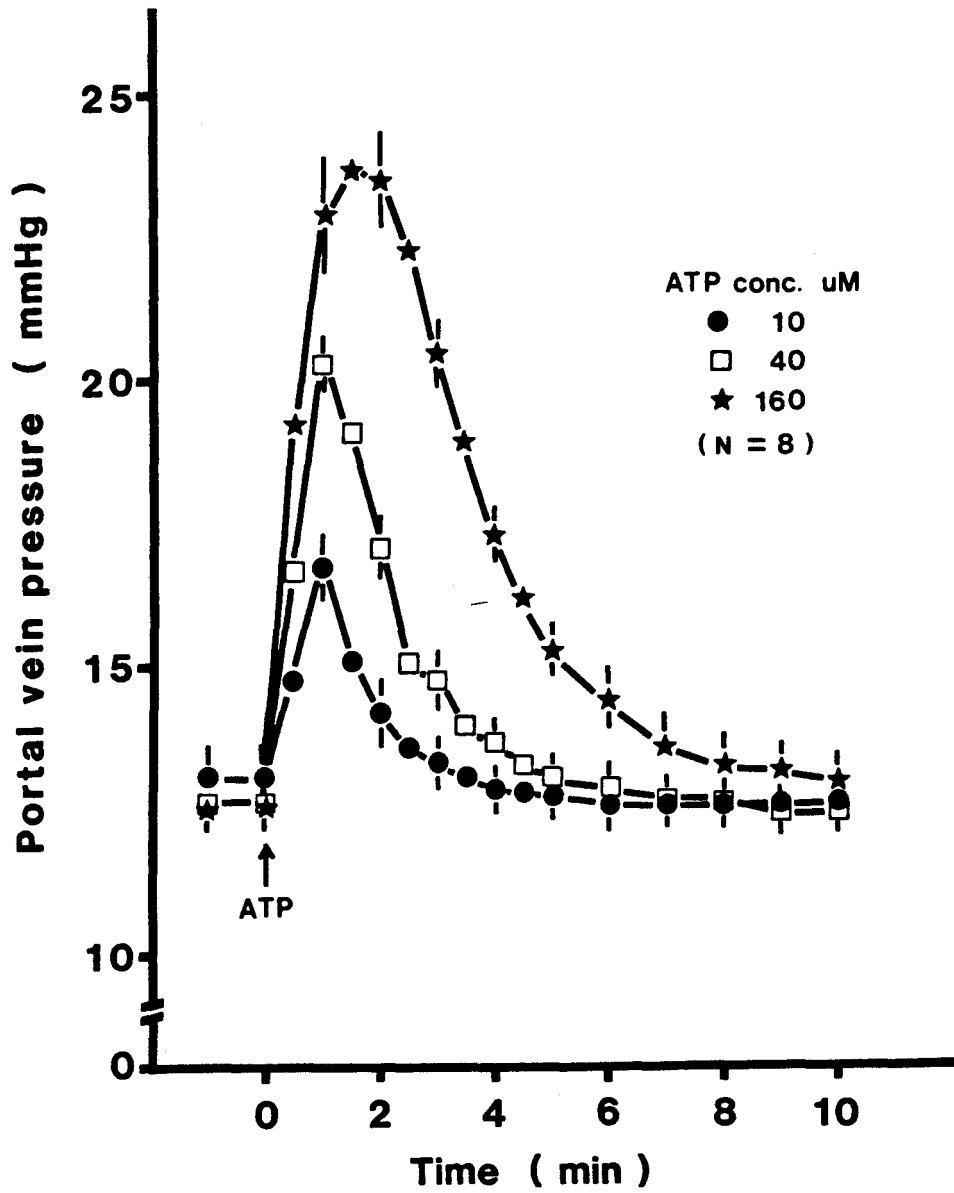
changes of portal vein pressure (mmHg) after ATP addition of 16.8 at 10 uM, 20.3 at 40 uM and 23.7 at 160 uM. At low concentration of ATP (10 uM) the increased portal vein pressure returned to control level within about 3 minutes but at higher concentration (40 and 160 uM) the recovery time was increased to 7 and over 10 min, respectively.

3. ATP ON HEPATIC ARTERIAL PERFUSION PRESSURE

Hepatic arterial perfusion pressure changes were measured under a constant arterial perfusion flow of 3.1 ± 0.4 ml/min. In addition livers were also perfused through the portal vein at about 60 ml/min flow. Hepatic arterial perfusion pressure was maintained about 77 mmHg before addition of either ATP or saline--as a vehicle control. Saline (0.5 ml) or ATP was injected into the inlet of the hepatic artery at 10 cm ahead of the liver. Fig. 18 shows a typical record of hepatic arterial pressure response to saline and ATP. Saline injection produced a brief hepatic arterial pressure change for a few seconds; this is probably an artifact of the saline injection. Two different amounts of ATP (5 umoles and 25 umoles) were then injected into the tubing in the same manner as for saline and the perfusion pressure changes were read at 20 sec intervals after addition of ATP. As ATP was injected through the hepatic artery, the injection artifact was followed by a prominent increase in hepatic arterial perfusion pressure. The magnitude of the hepatic arterial pressure increments and the duration of ATP action were proportional to the amounts of ATP added. As summarized in Fig. 19, 5 umoles of ATP increased hepatic arterial pressure from 77.1 ± 2.4 to 110 ± 8.4 mmHg ($P < 0.005$) at 20 sec after ATP injection. ATP (25 umoles)

FIGURE 18

RECORDING OF CHANGES IN HEPATIC ARTERIAL

PERFUSION PRESSURE

Hepatic arterial pressure was measured under a constant perfusion flow of 3.1 ml/min. Liver was perfused simultaneously through the portal vein with a constant perfusion pressure of 28 cmH_2O. Saline and ATP were injected into the inlet of hepatic artery at 10 cm proximal to the liver.
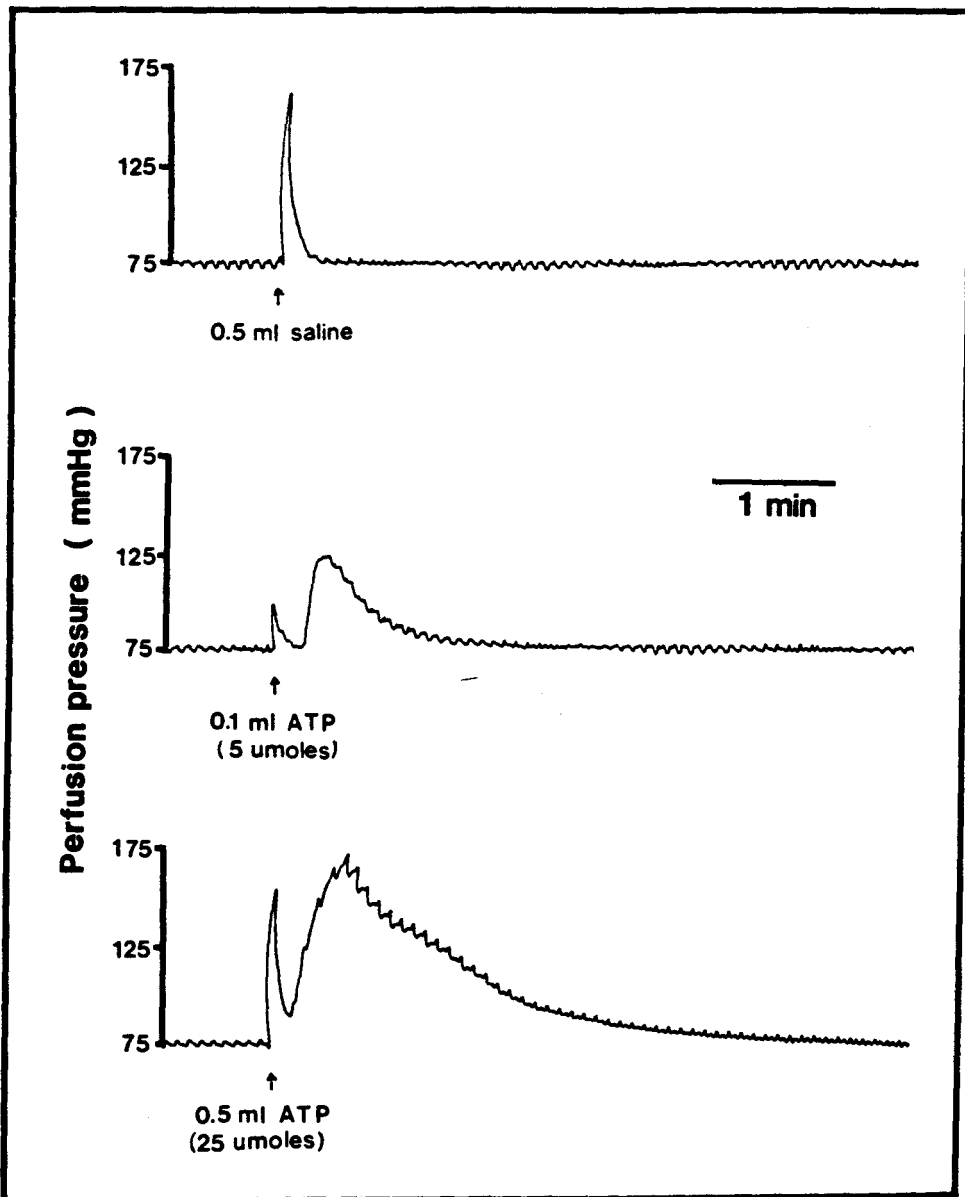
FIGURE 19

EFFECTS OF ATP ON PERFUSION PRESSURE IN THE

HEPATIC ARTERY

Each point represents mean ± S.E.M. for 7 livers.
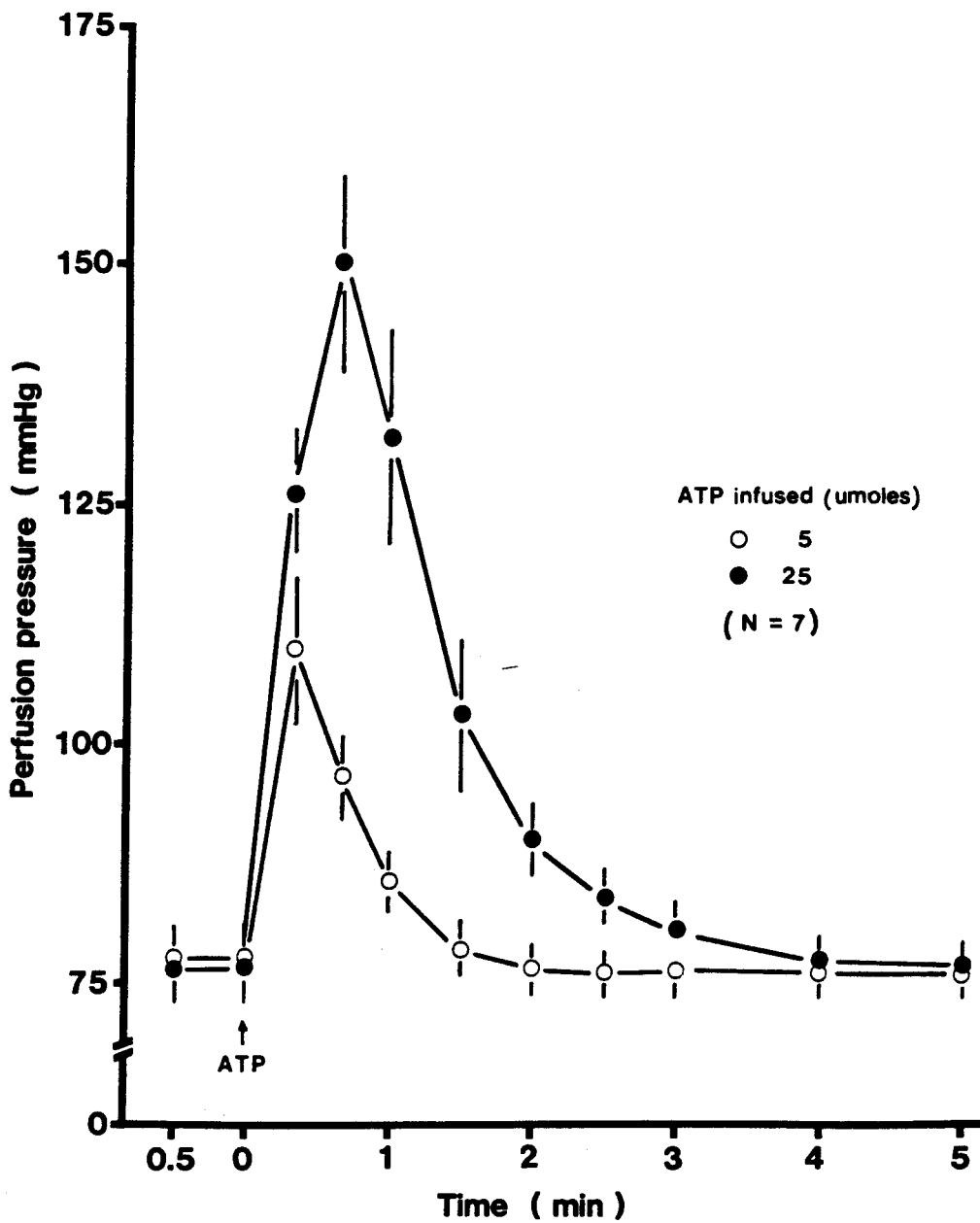
increased hepatic arterial pressure from a control of 76.9 $\pm$ 2.1 to 150.4 $\pm$ mmHg at 40 sec (P<0.001). Thus ATP increased the resistance of both the portal vein and hepatic arterial systems.

4. ATP ON LIVER CAPACITANCE

There are two major sphincteric sites in the hepatic circulation --i) inlet sphincters which are located at the junction of the sinusoids with the portal venules and ii) outlet sphincters which are located at the junction of the sinusoids with the central venules (241). The hypothesis of this study was that if ATP acted on the inlet sphincter region, it should decrease liver capacitance (weight); however, if ATP acted on the resistance of the outlet sphincter region, it should increase liver capacitance. To test the hypothesis liver weight changes were measured during perfusion. After a 30 min stabilizing period, ATP was added into the perfusate reservoir and liver weight changes were recorded. Fig. 20 presents an example of liver weight changes at three different ATP concentration. ATP transiently decreased liver weight in proportion to its concentration. Liver weights then recovered to original levels--the recovery period was proportional to the ATP dose. Results of seven experiments are summarized on Fig. 21. Liver weights reached minimum levels within 1 or 1.5 min after addition of ATP. ATP decreased liver weights from 12.5 $\pm$ 0.50 to 11.9 $\pm$ 0.52 at 10 uM (P>0.1); to 10.9 $\pm$ 0.48 at 40 uM (P<0.05); and to 9.9 $\pm$ 0.5 at 160 uMATP (P<0.01). Linear regression analysis (Fig. 22) was performed to determine any correlation between changes in perfusion flow (ml/min) and changes in liver weight (gm). The equation of the regression line was

FIGURE 20

RECORDING OF CHANGES IN LIVER WEIGHT DURING

PERFUSION

Liver weight was measured with a constant perfusion pressure of 28 cmH_2O. The force transducer was calibrated in the presence of the liver by adding or removing calibrated weights. ATP was added into the perfusate reservoir.
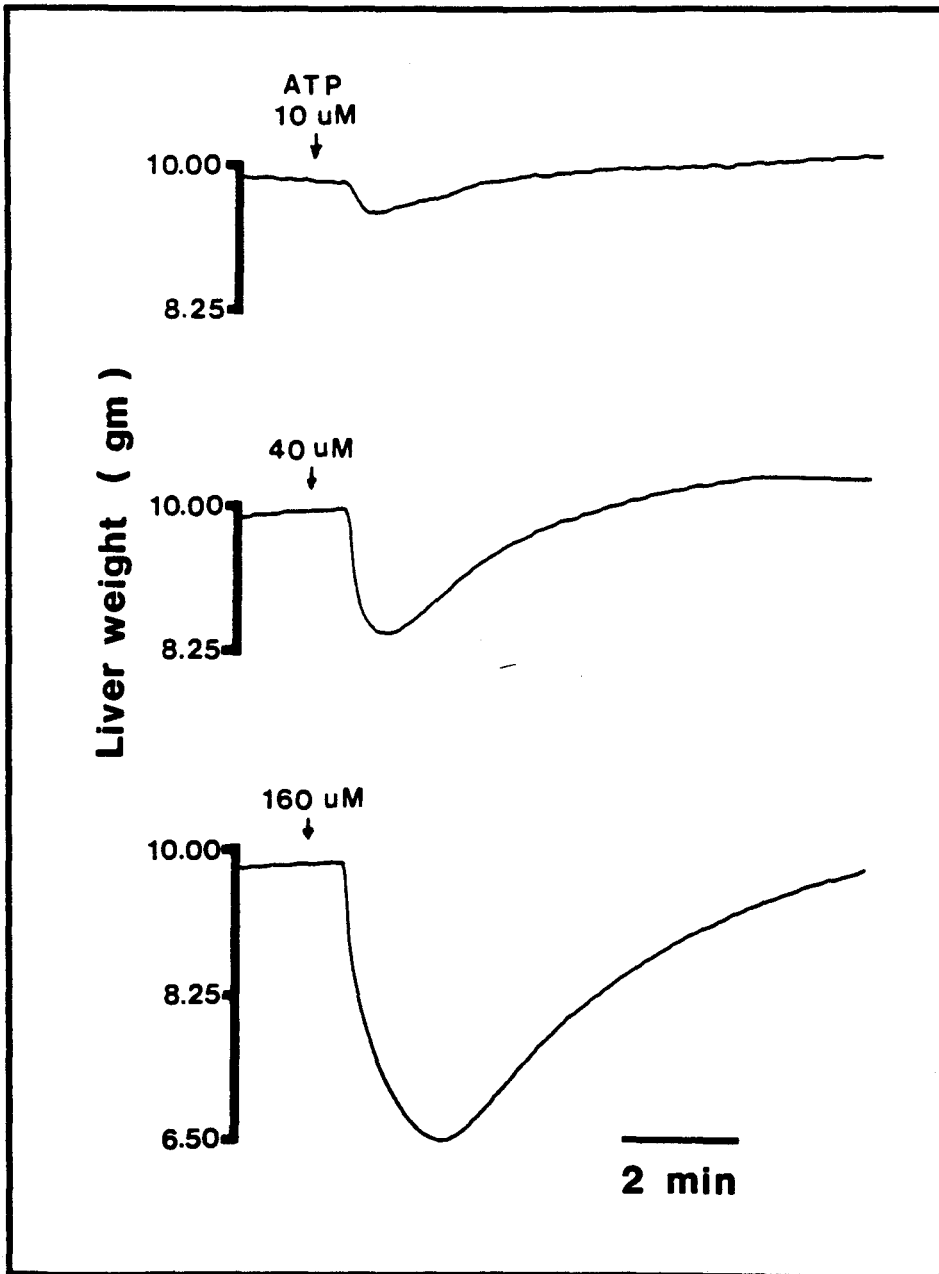
FIGURE 21

EFFECTS OF ATP ON LIVER CAPACITANCE

Each point represents mean $\pm$ S.E.M. of 7 livers.
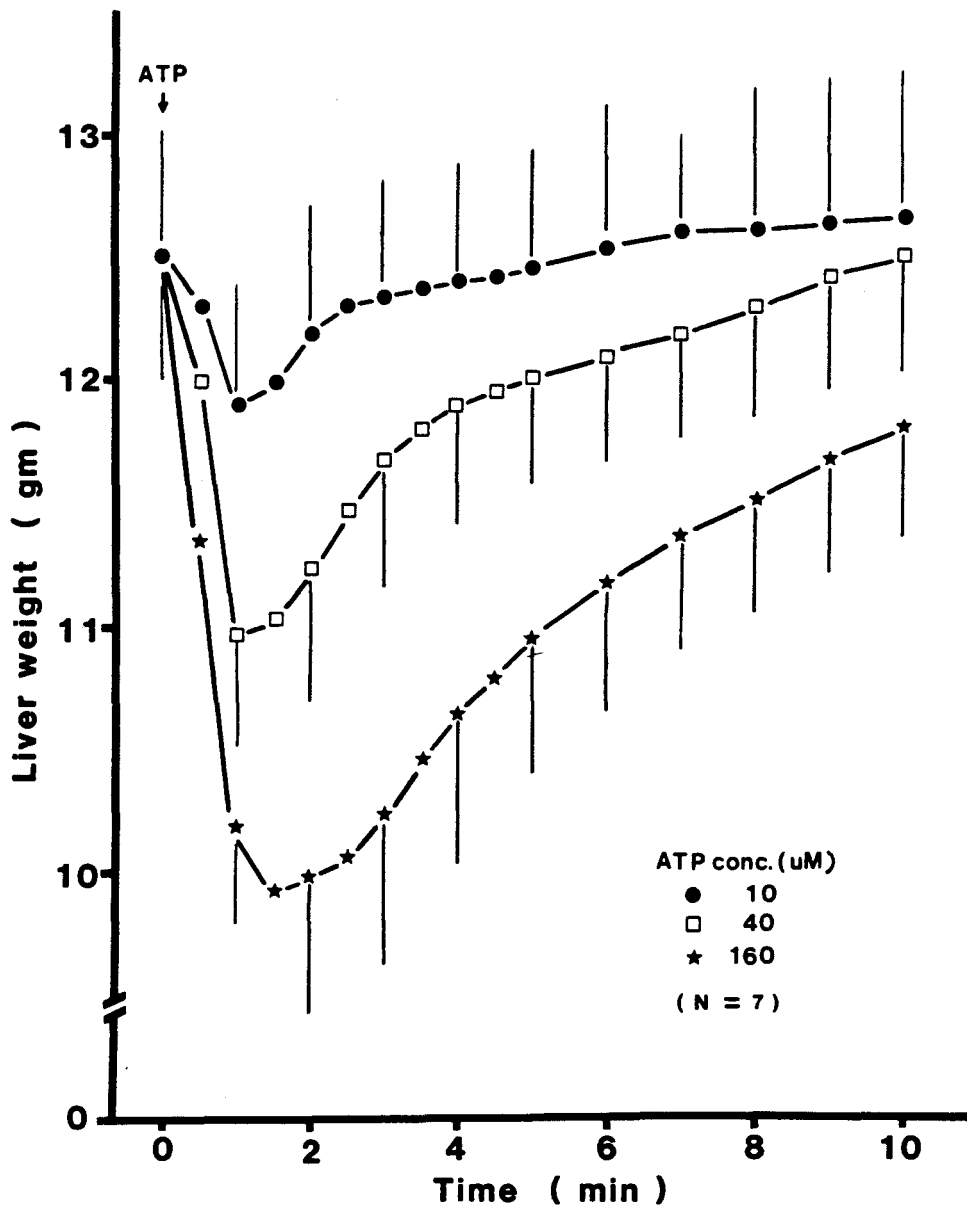
FIGURE 22

RELATIONSHIP BETWEEN CHANGES IN PERFUSION FLOW

AND CHANGES IN LIVER WEIGHT

Linear regression analysis indicates a significant correlation between changes in perfusion flow (ml/min) and changes in liver weight (gm) (r = 0.983, P<0.001).
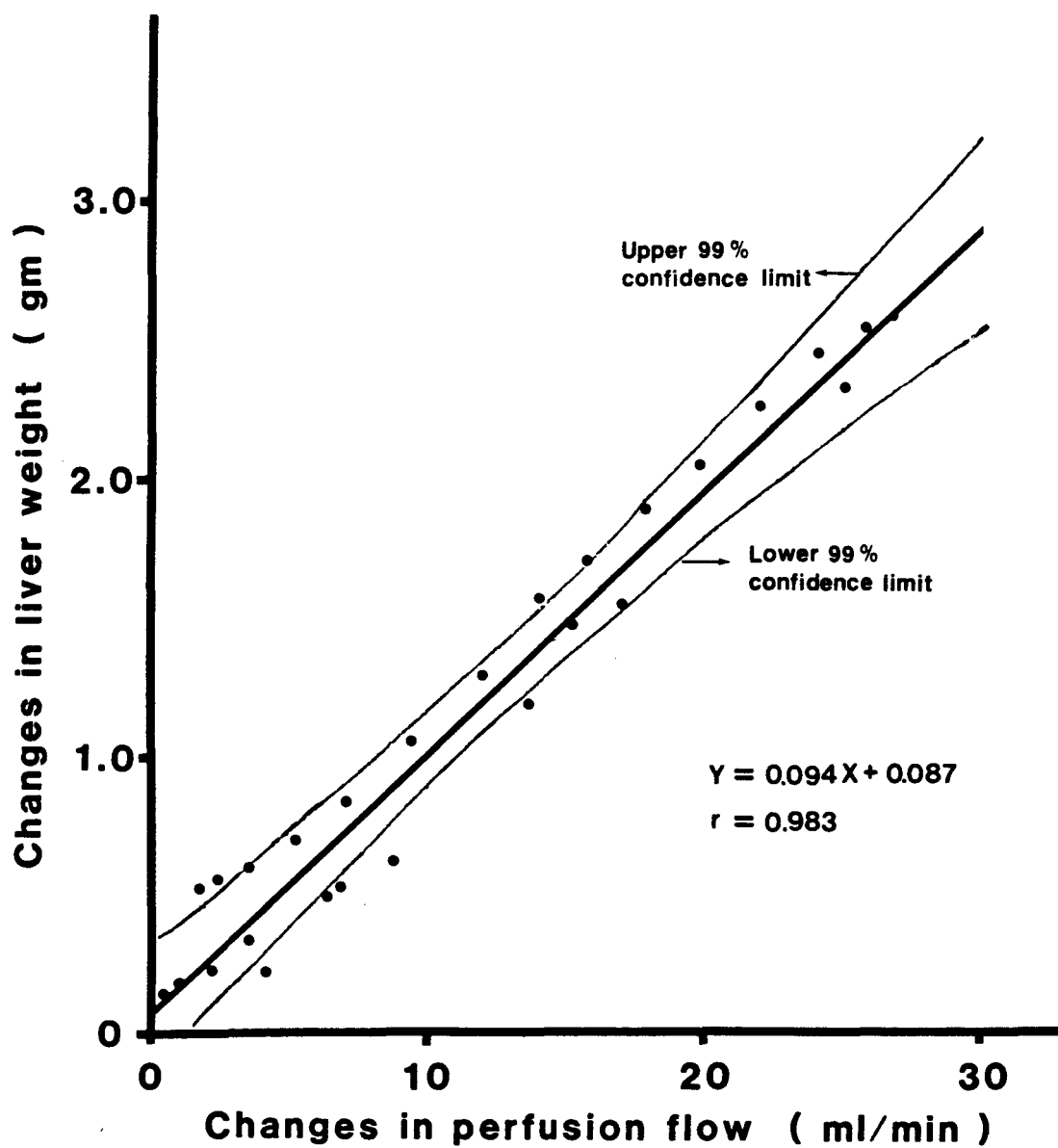
Y = 0.094 X + 0.087
r = 0.983

$y = 0.094 \ x + 0.087$. The correlation coefficient, r, was 0.983 and indicates a significant correlation ($P<0.001$) between changes in perfusion flow and changes in liver weight.

F. Comparison of Adenine Nucleotides and Adenosine on Perfusion Flow, Oxygen Consumption and Glucose Production

Perfusion flow changes were measured under a constant perfusion pressure of 28 cmH_2O and then 50 uM of adenosine, AMP, ADP and ATP, respectively were added into the perfusate. As depicted in Fig. 23, the magnitudes of the flow reductions were different. Adenine derivatives decreased perfusion flow in the following order: ATP > ADP > AMP = adenosine. Changes in perfusion flow at 1 min after addition of each agent were as follows: 63.1 ± 1.14 of control to 58.0 ± 1.39 ml/min for adenosine ($P<0.01$), from 64.0 ± 0.96 to 59.6 ± 1.09 ml/min for AMP ($P<0.01$), from 63.9 ± 0.67 to 47.6 ± 1.5 for ADP ($P<0.001$) and from 64.0 ± 0.96 to 44.4 ± 1.29 for ATP ($P<0.001$).

Adenosine and AMP did not significantly decrease oxygen consumption (ulO_2/gm/min) -- the changes were from 55.5 ± 3.1 to 51.6 ± 3.3 for adenosine ($P>0.1$), from 57.3 ± 2.5 to 54.6 ± 3.0 for AMP ($P<0.1$). However ADP as well as ATP significantly decreased oxygen consumption; the changes were from 58.2 ± 2.4 to 44.8 ± 2.9 for ADP ($P<0.005$) and from 56.5 ± 2.2 to 40.5 ± 1.7 for ATP ($P<0.001$).

Fifty uM of adenosine, AMP, ADP and ATP increased glucose production in the perfused liver. The glucose production rate changes (umoles/gm/hr) for each agent were from 51.8 ± 8.0 to 78.7 ± 8.5 for

FIGURE 23

COMPARISON OF ADENINE NUCLEOTIDES AND ADENOSINE

ON PERFUSION FLOW

Livers were perfused under a constant perfusion pressure of 28 cmH_2O. Each point represents mean $\pm$ S.E.M. for 12 livers.
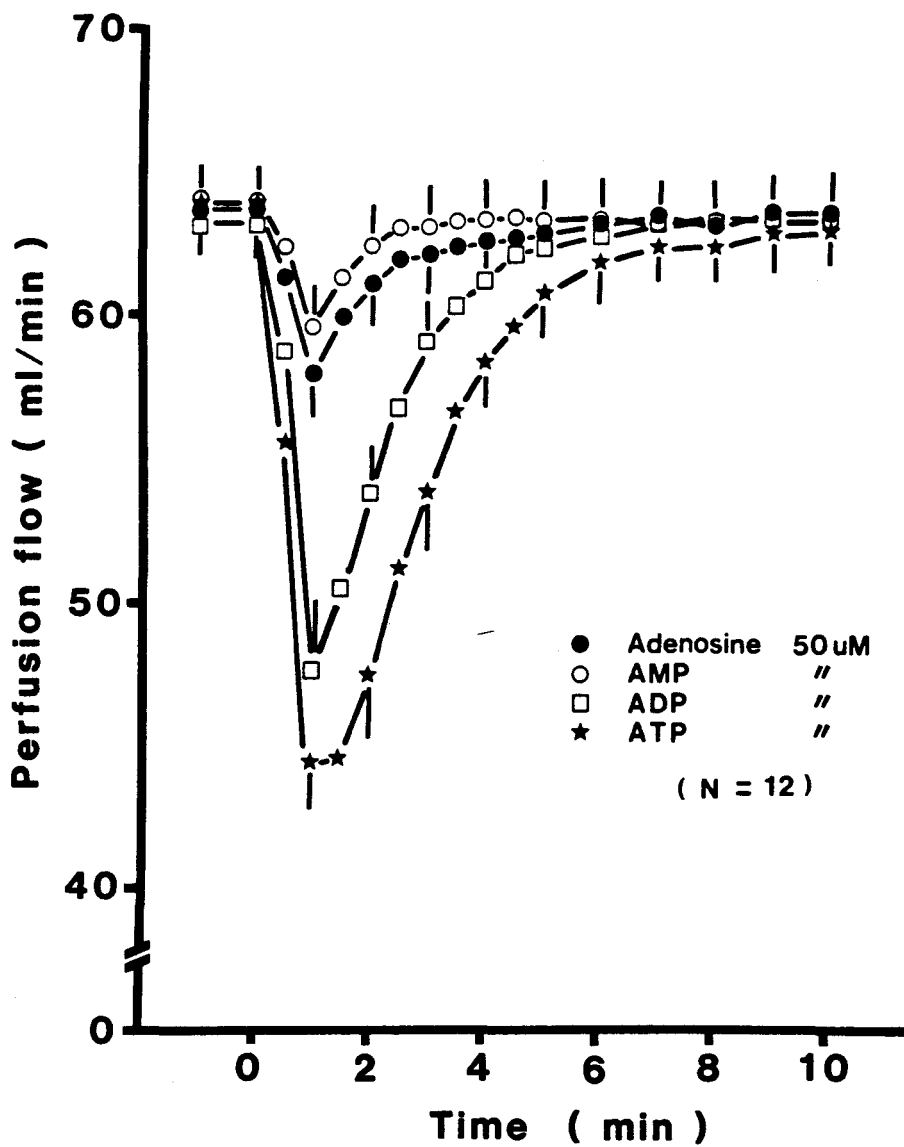
FIGURE 24

COMPARISON OF ATP-MgCl$_2$ WITH ATP-NaCl ON

PERFUSION FLOW

Livers were perfused under constant perfusion pressure of 28 cmH$_2$0. 0.26 ml of 50 mM ATP-MgCl$_2$ or ATP-NaCl$_2$ was added into the perfusate alternately. Each point represents mean $\pm$ S.E.M. for 8 livers.
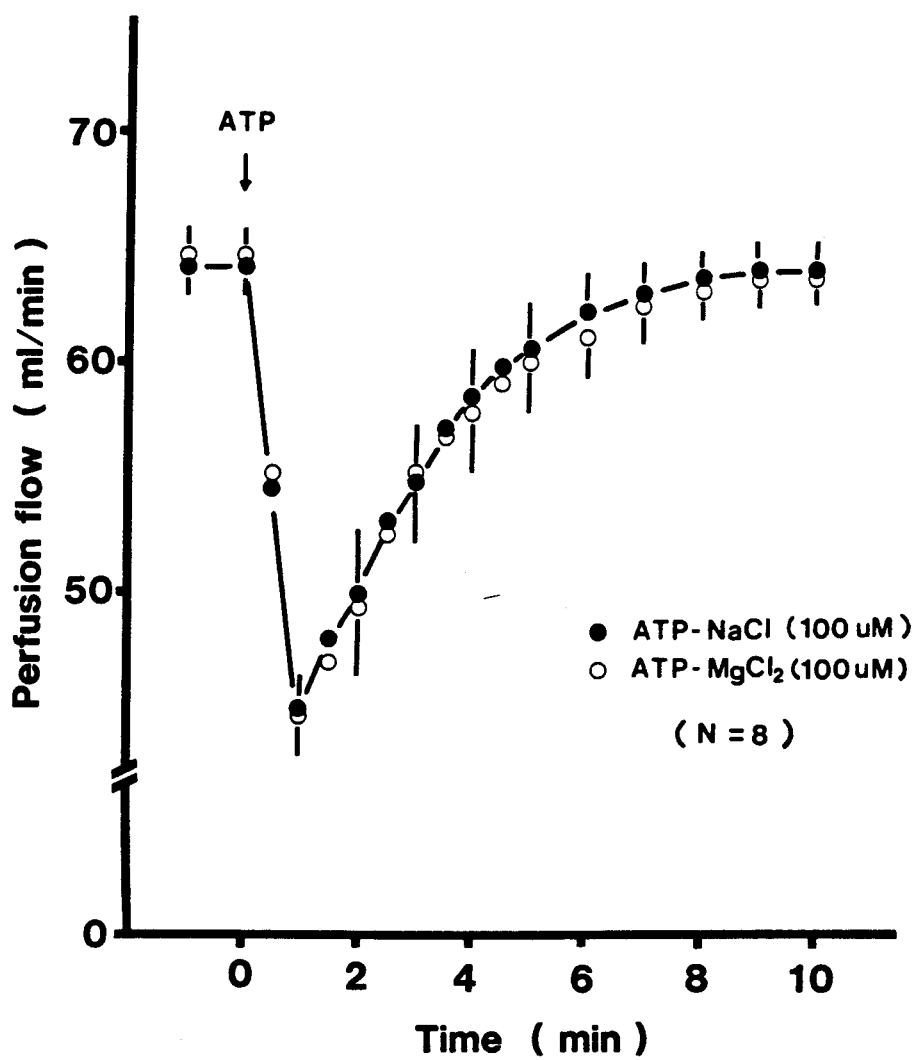
FIGURE 25

COMPARISON OF ATP-$MgCl_2$ WITH ATP-NaCl ON

OXYGEN CONSUMPTION RATE

Liver perfusion conditions and ATP addition were as described in Figure 24. Each point represents mean $\pm$ S.E.M. for 8 livers.
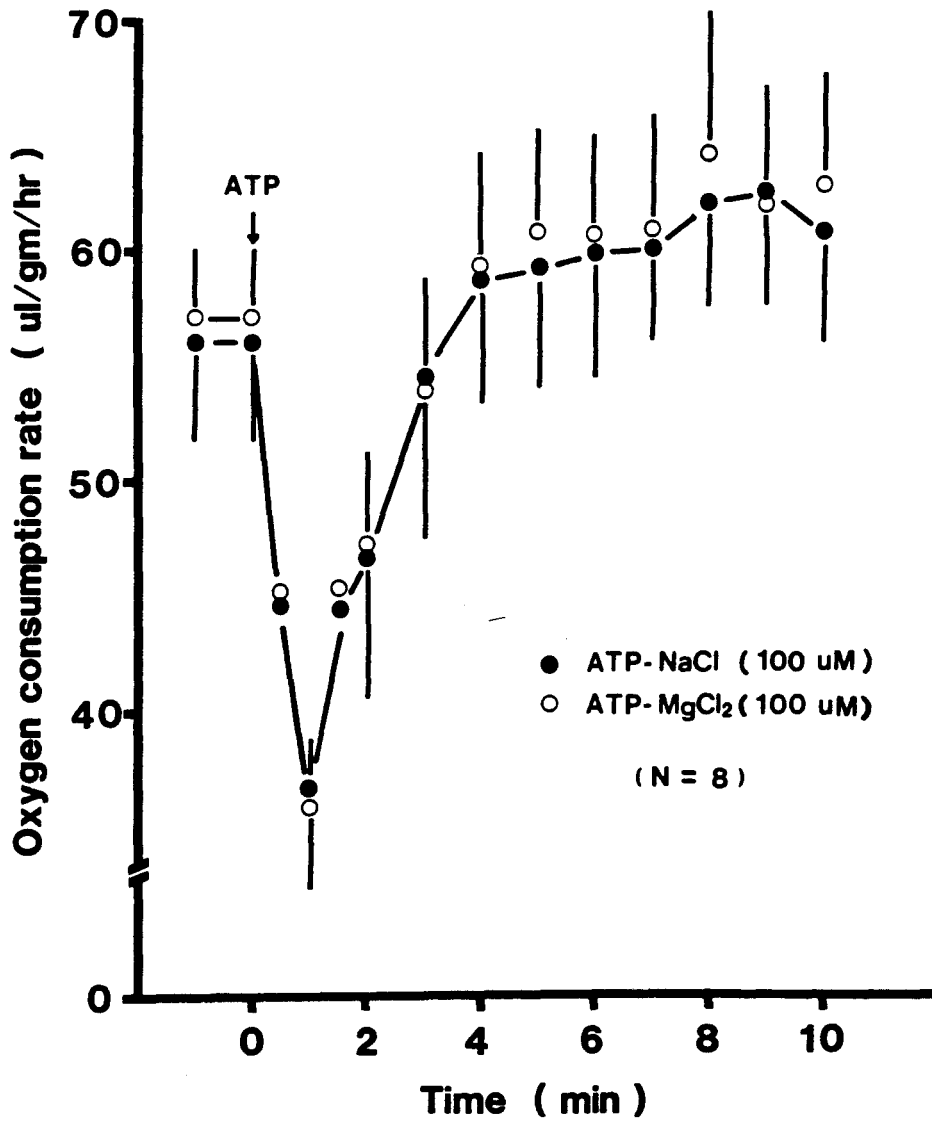
adenosine (P<0.05), from 49.8 $\pm$ 7.0 to 77.0 $\pm$ 10.7 for AMP (P<0.05), 55.5 $\pm$ 9.1 to 120.4 $\pm$ 7.0 for ADP (P<0.001) and from 58.4 $\pm$ 11.6 to 138.9 $\pm$ 11.6 (P<0.001) for ATP.

In addition, the effects of ATP-NaCl and ATP-MgCl$_2$ on perfusion flow and oxygen consumption were also compared. Changes in portal vein perfusion flow with a constant perfusion pressure (28 cmH$_2$O) were measured. One hundred uM ATP-NaCl or ATP-MgCl$_2$ was added into the perfusate and perfusion flows were determined at 30 sec intervals. As depicted in Fig. 24, both ATP-NaCl and ATP-MgCl$_2$ decreased perfusion flows with lowest values achieved at 1 min after ATP addition. Fig. 25 depicts the effects of ATP-NaCl and ATP-MgCl$_2$ on oxygen consumption rate in the perfused liver. A biphasic response to ATP occurred with a decrease in oxygen consumption initially and an increase later. Thus, these findings indicate that there is no difference between ATP-NaCl and ATP-MgCl$_2$ on either perfusion flow in the portal vein or oxygen consumption.

G. Effects of Indomethacin on ATP-induced Perfusion Pressure, Perfusion Flow and Glucose Production

In this study, portal vein pressure was maintained about 13 mmHg with constant perfusion flow. After a 30 min stabilization period, 20 uM ATP and then 20 uM indomethacin were added to the perfusate. Ten or 15 min after indomethacin treatment, the same amount of ATP (20 uM) was added again and portal vein pressure changes were compared with control responses to the first ATP addition (Fig. 26). Portal vein pressure

changes by prostaglandin $F_{2\alpha}$ (2 uM), a vasoactive prostaglandin, were also observed after the second addition of ATP. Comparison of the control group with the indomethacin treated group at 1 min after ATP addition showed that the portal vein pressure induced by 20 uM ATP was 18.7 $\pm$ 0.46 and 15.6 $\pm$ 0.25 mmHg, respectively (P<0.005) (Fig. 27). Prostaglandin $F_{2\alpha}$ also increased portal vein pressure (Fig. 27). Indomethacin also inhibited ATP-induced glucose production in the perfused liver. Indomethacin, however, by itself did not affect glucose production rate. Glucose production rate by 40 uM ATP was significantly reduced (P<0.05) from a control value of 125.4 $\pm$ 6.9 to 84.6 $\pm$ 11.6 moles/gm/hr in 50 uM indomethacin treated liver (Fig. 28).

FIGURE 26

RECORDING OF CHANGES IN PORTAL VEIN PRESSURE BY

ATP AND INDOMETHACIN

The liver was perfused with a constant flow of 62 ml/min. ATP was added into the perfusate at 30 minutes of perfusion and then at 40 indomethacin was added. 10 or 15 minutes after indomethacin, same amount of ATP was added.

Indomethacin, 20 uM

10 min

Portal vein pr.
(mmHg)

30

15

0

ATP
20 uM

ATP
20 uM

$PGF_{2\alpha}$
2 uM
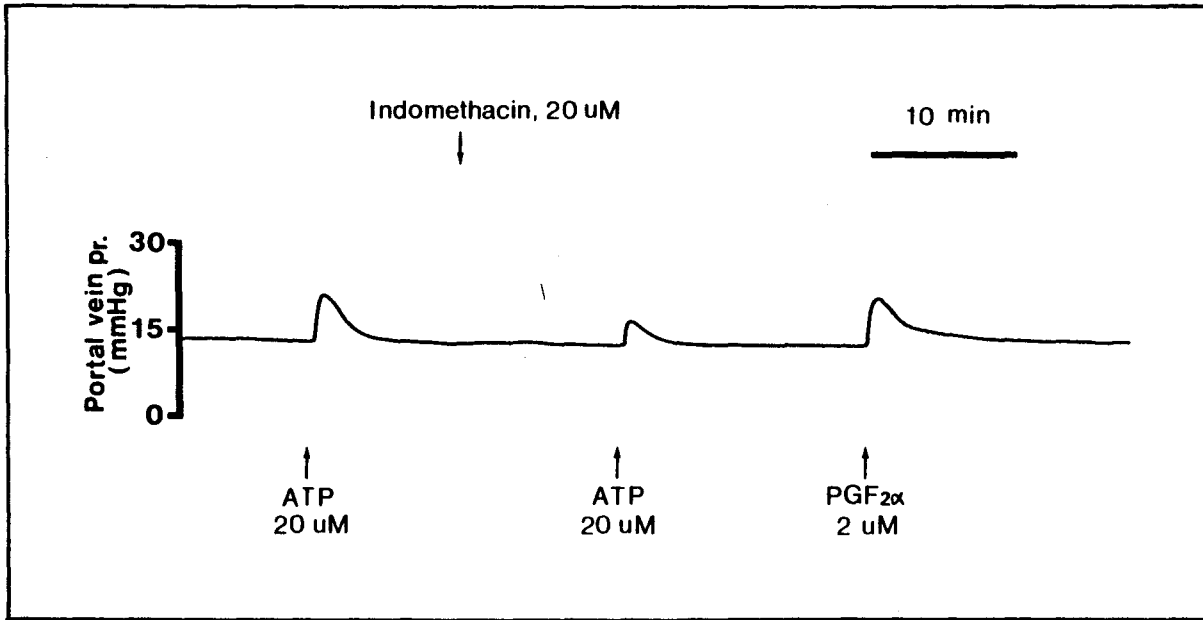
FIGURE 27

EFFECTS OF INDOMETHACIN ON PORTAL VEIN PRESSURE

INDUCED BY ATP

Each point represents mean $\pm$ S.E.M. for 7 livers.
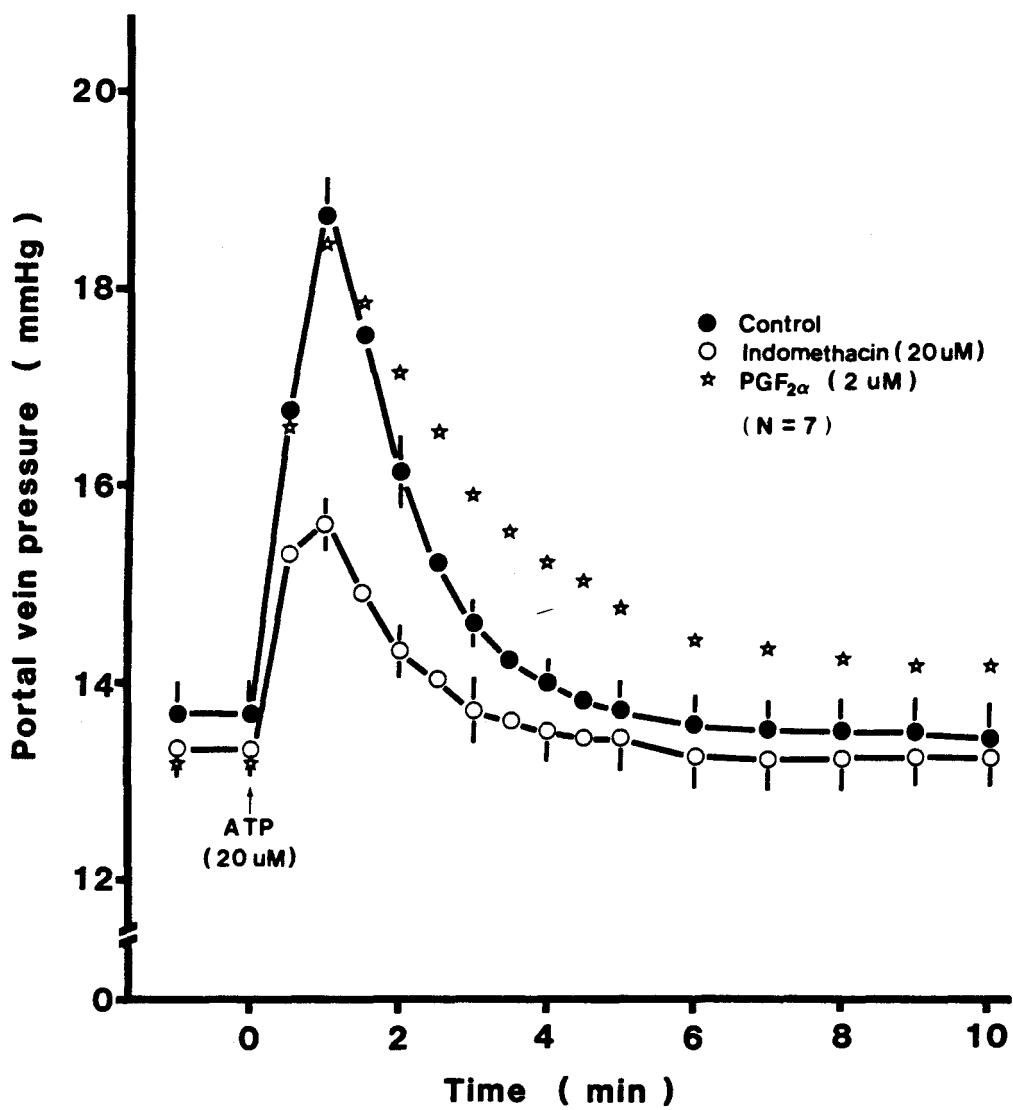
FIGURE 28

EFFECTS OF INDOMETHACIN ON ATP-INDUCED

GLUCOSE PRODUCTION

Livers were perfused under a constant perfusion pressure of 28 cmH_2O. Means $\pm$ S.E.M. of glucose production rate are depicted. * denotes $P<0.001$ compared to control and ** denotes $P<0.05$ compared to ATP addition.
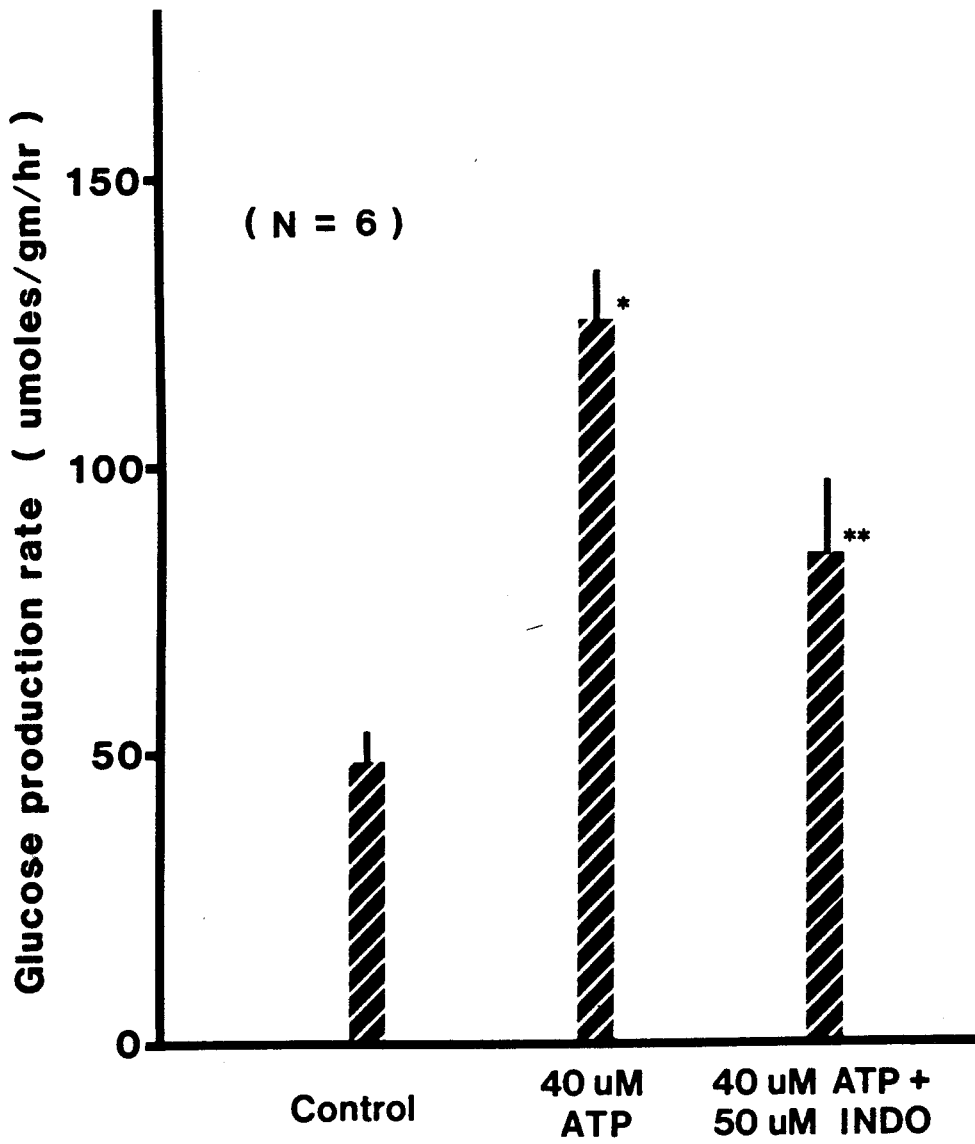
CHAPTER V

DISCUSSION

The major findings of this dissertation research deal with the effects of exogenous ATP on hepatic oxygen consumption, K^+ movements, glucose production, and hemodynamics. Each of the above effects will now be discussed in reference to underlying mechanisms of ATP action. However, since these effects were obtained in the context of the use of exogenous ATP in the isolated perfused rat liver, the key characteristics of the liver perfusion model and ATP will first be addressed.

A.   Isolated Liver Perfusion Model

There are several experimental models for the study of hepatic circulation and metabolism--including in vitro studies, isolated perfusion techniques, slice techniques, isolated hepatocytes and isolated subcellular preparations.   The isolated perfused liver preparation has many advantages: it preserves intact cells; it provides an environment similar to in vivo; the chemical composition, substrate and hormone concentration and temperature can be easily controlled; and it eliminates complicated interactions with other organs via neural and humoral effects (116). Liver perfusion as an analytical physiological method has a history of over one hundred years. In early studies, mainly large animal livers such as dog or cat were used for perfusions. The perfusion model of the isolated rat liver originated

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from Trowell (333). Subsequently Miller and his associates (248) developed an extra-corporeal apparatus and an operative procedure for the perfusion of the isolated rat liver which has been used by many investigators up to the present. The rat liver has several advantages for perfusion. One, it requires less operative time (about 10 minutes from anesthesia to total removal of the liver) than larger animals such as rabbits, dogs or pigs; two, the rat liver is less susceptible to vasoactive factors than that of the dog and rabbit; and three, it requires small amounts of perfusion medium (249). There are two types of isolated liver perfusion--recirculating and non-recirculating systems. The non-recirculating system has several advantages; one, it maintains constant substrate or drug concentration of the perfusate and two, there is no accumulation of products during the perfusion period. On the other hand, a recirculating system has the advantage that it is possible to determine small changes in metabolites (for example, glucose and lactate production or ion movements), and it requires small amounts of perfusion medium. A recirculating system was employed for the present study.

Most rat liver perfusions are performed with either a red blood cell free-medium or with a semisynthetic medium (physiological buffer plus washed red blood cells). Red blood cells (from many species including the human, cow, dog, pig, sheep and rat) increase oxygen carrying capacity and permit perfusion flow rates sufficiently low as to maintain adequate oxygenation. Miller et al. (248) perfused livers at flow rates of 1.0 - 3.0 ml/gm/min for their definitive study of hepatic

protein synthesis. Schimassek (295) employed 1 ml/gm/min of perfusion flow for study of mitochondrial respiration. For the study of gluconeogenesis livers were perfused at a flow rate of 6.8 - 7.2 ml/min in 90 to 140 gm rats--approximately 1.3 ml/gm/min (119). In most studies of rat liver perfusion with buffer enriched with erythrocytes, a low perfusion flow was employed. Exceptionally, Brauer et al. (41) perfused rat livers at an extremely high perfusion flow of 93 ml/min (approximately 8.4 ml/gm of liver/min) in their study of bile secretion. A significant problem with the red blood cell medium is that erythrocytes consume glucose and produce lactate and also may affect ion movement (151). Therefore, in the present study which had an interest in the effects of ATP on glucose production, lactate production and K^+ movement, the red blood cells were omitted from the perfusion medium.

Since red blood cell free-medium (for instance, KRB) has a low oxygen-carrying capacity, a high perfusion flow is required to achieve adequate oxygenation. Friedman and Rasmussen (140) perfused rat livers with erythrocyte free-medium at a flow rate of 24 ml/min in 100 gm rat (5.4 ml/gm/min) in studies of gluconeogenesis. Glycogenolysis by glucagon and metabolic inhibitors was studied under KRB perfusion at a flow rate of 30 ml/min (3.3 ml/gm/min, 197) and Debeer et al. (101) perfused livers with Krebs-Henseleit bicarbonate buffer at flow rates of 7-8 ml/gm/min. Eisenstein et al. (113) perfused adrenalectomized rat livers with KRB at flow rates of 10-12 ml/gm/min in a study of adrenal cortical influences on carbohydrate metabolism.

As described above, perfusion flow rates with erythrocyte free-

medium are commonly much higher than those with erythrocyte containing medium. At a high perfusion flow the endothelial cell may be injured; on the contrary, at a low perfusion flow tissue may fall into hypoxic states (297). Therefore, it is important to maintain an adequate perfusion flow to prevent cell injury or hypoxia. Sugano et al. (323) reported that when rat livers were perfused with oxygenated erythrocyte free-medium at flow rates between 3-3.5 ml/gm/min, the rates of glucose, pyruvate and lactate production, the rate of oxygen consumption, and the tissue contents of adenine nucleotides were similar to those observed with perfusion system containing erythrocytes. However, in that study they maintained the perfused liver at $32^{o}C$ to reduced oxygen demand. If they carried out their experiment at normal temperature (for instance at $37^{o}C$), a higher perfusion flow might have been required.

In the present study rat livers were perfused with KRB at flow rates of 60 to 64 ml/min (5.1 ml/gm liver/min) at $36^{o}C$. This flow rate is higher than that used by Kimura et al. (214, 3.1 ml/gm/min) and Jakob and Diem (197, 3.3 ml/gm/min) but less than that used by Brauer et al. (41, 8.4 ml/gm/min), Eisenstein et al. (113, 10-12 ml/gm/min), Bloxam (37, 9.9 ml/gm/min) and almost the same as that used by Friedman and Rasmussen (140, 5.3 ml/gm/min). One reason this perfusion flow was chosen was that oxygen consumption showed saturation phenomenon at this range in the relationship between oxygen consumption and perfusion flow (Fig. 11).

B. Characteristics of ATP

Adenosine triphosphate (ATP) is the universal free energy source in biological systems. It is formed mainly via oxidative-phosphorylation in the inner compartment of mitochondria. ATP contains two high energy bonds. Free energy is liberated when ATP is hydrolyzed to adenosine diphosphate (ADP) and orthophosphate (Pi) or when ATP is hydrolyzed to adenosine monophosphate (AMP) and pyrophosphate (PPi). Thus ATP provides free energy for the performance of mechanical work in muscle contraction, active transport of molecules and ions across the membrane, and the synthesis of cellular components such as protein, lipid and carbohydrate.

The ATP formed in the mitochondria is transported across the mitochondrial membrane into the cytosol by an ATP-ADP carrier (341). The intracellular concentration of ATP varies among different cell types--namely, 4-5 mM in muscle cells (160, 262), 1.8-4.5 mM in liver cells (197, 208, 334, 337), 1.3 mM in fibroblasts, 2.2 mM in PCM3 hybrid cells and 0.6 mM in P-388 lymphoma cells (13).   In addition intracellular ATP concentration is decreased under various cell conditions--in hepatic ischemia (180, 266), by burns (352), after administration of endotoxin, in hypoxia and in hemorrhagic shock (269).

In contrast to high intracellular concentrations, only 1 uM of ATP is found in the human plasma (135). There is, however, an arterio-venous difference of plasma ATP concentration during sustained exercise (137). The existance of extracellular ATP indicates that ATP can be released out of cells. The sources of plasma ATP were reported as active skeletal muscle (134, 136), erythrocytes (20, 133), blood

platelets (137), vascular endothelial cells (276), and nervous cells (182). In addition, the release of ATP from purinergic nerve endings as a neurotransmitter or co-transmitter has been well-recognized (52, 55, 58, 320).

As described above, the major function of intracellular ATP is as a free energy source for most active process in the body. In addition, extracellular ATP may have important physiological functions including changes in vascular resistance, regulation of tissue metabolism and as a neurotransmitter.

C.   Mechanisms of ATP Action on Oxygen Consumption

The liver is a unique organ which has two afferent blood supplies--hepatic arterial and portal venous, both of which participate in the supply of oxygen. In the fasting state, about 50% of hepatic oxygen is supplied by the portal vein and 50% by the hepatic artery (65). Many authors have reported an oxygen consumption rate in the perfused rat liver between 50 and 70 ml/gm liver/min (17, 101, 151, 232, 295, 298). Although values as low as 16.7 (331) 26.7 (159) and 36.7 ml/gm/min (323) have been reported, these results might be due to low temperature ($32^{\circ}C$) or extremely low perfusion flow (0.2 ml/gm/min). The oxygen consumption rate of 53 ml/gm/min in the basal state in present study is therefore consistant with other published studies.

The oxygen extraction or consumption by the liver is dependent upon the conditions of perfusion flow, substrate level, hormone concentration and various drugs. At low oxygen supply or low perfusion

flow, the oxygen extraction rate increased (17, 233). Exogenous substrates for gluconeogenesis also increased oxygen consumption (101, 322). Catecholamines or glucagon also increased oxygen consumption (83, 322). In the current study ATP decreased not only perfusion flow but also oxygen extraction in early phase after ATP (40 and 160 uM) addition; consequently it decreased oxygen consumption at these concentrations. Among possible mechanisms for the decrease in hepatic oxygen extraction due to ATP is that ATP may decrease oxygen consumption of the hepatocyte directly. According to Maxild (239), externally added ATP (0.1 - 10 mM) caused an elevation of ATP content in renal cortical tissue accompanied by a reduction of renal glucose utilization and oxygen consumption. This effect may be due to allosteric inhibition of glycolytic enzymes by ATP. Another mechanism of ATP action on oxygen use may involve redistribution of perfusion flow within the liver. The liver has heterogeneity in glycogen content (100), mitochondrial number and size (231, 343) and enzyme distribution (304) in different hepatic zones. The peripheral hepatocytes (periportal cell) contains fewer mitochondria than central hepatocytes; however peripheral cells have larger total mitochondrial volumes, membrane area of cristae, and larger matrix per cell (231). Therefore, the peripheral region is associated with high oxygen consumption. Daniel and Prichard (95, 96) demonstrated uneven distribution of perfusion flow by certain stimuli using serial angiography. Stimulation of the hepatic nerves or administration of adrenaline shifted the portal perfusion flow through central, deeper parts due to constriction of small portal vessels. Daniel and Prichard (95) also observed that when the intrahepatic blood flow is shifted in

this manner, the portal blood reached the inferior vena cava more rapidly than when the liver is perfused throughout. If ATP constricted small branches of the portal vein, the perfusate would flow readily through the central zone. The perfusion flow passing through this area would have less chance for contact with parenchymal cells. Conversely, if ATP led to much more flow through the less oxygen-consuming region (central zone), then the perfusate would retain a high oxygen content. Although anatomical intrahepatic shunts (portal-hepatic venous shunts) have not been proved in the rat liver (96), the shift of hepatic portal perfusion flow by ATP may serve as a kind of physiological shunt. During the period of ATP action, the hepatocytes in the less-perfused region (peripheral zone) might undergo a oxygen-debt which caused compensated reactive oxygen uptake in the late phase; that is the oxygen-debt was paid back by a greater extraction. A similar reactive oxygen uptake was reported in the cat liver following partial occlusion of the hepatic artery (233). In addition, since ATP increased hepatic lactate production (Fig. 6), it is possible that the oxygen consumption might be increased as the lactate was used for gluconeogenesis as a substrate by the liver.

However, at low concentration of ATP (10 uM), there was no biphasic phenomenon of oxygen extraction. ATP only increased oxygen extraction. Two possibilities can be considered for the latter effect. First, at low concentrations ATP has a characteristics of increasing oxygen consumption by unknown mechanisms. Second, a reduction perfusion flow by ATP in certain region may increase oxygen

extraction because low perfusion flow or low oxygen supply increases oxygen extraction rate by a compensation mechanism (17, 323). Although we did not measure any acute low oxygen extraction in the early phase after ATP addition, it might be masked by following reactive oxygen extraction. Even though perfusion was decreased, oxygen consumption rate was increased by 10 uM of ATP. This may indicate that the reactive greater oxygen extraction overcame the reduction of flow itself.

D. Mechanism of ATP Action on K^+ Movements

D'Silva (109) first observed that the transient elevation of plasma K^+ which follows the intravenous administration of adrenaline is a consequence of the hepatic release of K^+. Many other drugs and procedures also elevate plasma K^+; the K^+ release is promoted by α-adrenoceptor agonists (46, 169, 171, 294), glucagon (198), cyclic AMP (316), ionophore 23187 (46, 287), hemorrhage (109), hypoxia (273) and carbon dioxide inhalation (123).

The present study indicated that ATP also increases K^+ release from the isolated perfused rat liver. Similar effects were observed in the isolated hepatocytes from guinea-pigs (46). The mechanism(s) by which ATP causes K^+ efflux in the liver is not well understood. However, the following evidence suggests that calcium is involved in K^+ movements. An elevation in intracellular Ca^{++} as induced by intracellular Ca^{++} injection, cation ionophore or α-receptor agonists specifically increased the passive K^+ permeability (P_K) of the plasma membrane in various tissues--rat and human erythrocytes (9, 287, 342),

cat aspersa neurons (244, 245), barnacle photoreceptor (163), rat parotid and sublingual glands (280, 281, 282), taenia coli (45) and liver cells (46, 169, 294, 316). Although there is no direct evidence that ATP increases intracellular Ca^{++} level in the liver cell, exogenous ATP increases Ca^{++} uptake by rat mast cells (91, 92), Ehrlich ascites-tumor cells (223), chicken intestinal epithelial cells (213) and erythroleukemia cells (69). Thus ATP may increase intracellular Ca^{++} levels in the hepatocyte and consequently increase K^+ efflux just as elevations in intracellular Ca^{++} by catecholamine affects K^+ efflux in other tissues. It is of interest that catecholamines and the adenine nucleotides--ATP, ADP and cyclic AMP--hyperpolarize liver cells (170, 200, 316). This is further evidence of Ca^{++}-dependent, K^+-conductance change by ATP in the hepatocytes. However, it is not clear how intracellular Ca^{++} alters K^+ permeability in the plasma membrane.

Another possibility for the K^+ efflux is that ATP alters the Na-K^+ transport system in the plasma membrane. In rat liver intracellular Na^+ and Cl^- concentrations are low, and intracellular K^+ concentration is high with respect to extracellular fluid. The maintenance of the intracellular cation concentration is dependent upon transport mechanisms which are linked to metabolic activity of the cells (111, 312). Therefore, when the metabolism of liver cell is depressed by low temperature or by metabolic inhibitors such as cyanide and 2,4-dinitrophenol, the liver cell loses K^+ and gains Na^+ (115, 172, 173, 224, 335, 336). Anoxia also caused release of K^+ in the perfused rat liver (273). In the present study, ATP decreased perfusion flow (Fig.

15) and decreased oxygen consumption (Fig. 13). If ATP produced an extreme decrease in perfusion flow in certain local regions--particularly in the peripheral zones--oxygen delivery into this area and ATP availability in the liver cell should be low. The limitation of ATP in the liver could inhibit the Na^+-K^+ pump activity and then cause K^+ efflux to occur.

E. Glucose Production by ATP

The liver is an especially key organ in glucose regulation. Blood glucose levels, critical for life, are maintained by glycogenolysis in the fed state and by hepatic gluconeogenesis in the fasted state. Glycogenolysis and gluconeogenesis are determined by many factors--hormones, hepatic blood flow, oxygen supply and intracellular substrate levels as well as ATP, AMP and glucose-6-phosphate. In addition extracellular ATP also affects glucose metabolism. Levine (228) demonstrated that intravenous administration of ATP caused hyperglycemia in the rat. In the present study exogenous ATP increased glucose output in the perfused fed rat liver (Fig. 4). Clemens et al. (84) observed a similar result.

Two possible mechanisms for glucose production by ATP will now be discussed. First, vasoconstriction of the portal vein by ATP may indirectly produce glucose and lactate release. Since ATP decreases portal vein perfusion flow, and oxygen extraction by the liver (Fig. 13, 15), it may lead to a regional hypoxic condition. Since hepatic glycogenolysis is very sensitive to the oxygen tension in the liver,

glucose efflux occurs. As oxygen delivery to the liver is further reduced, glycogenolysis is also accelerated (62, 174, 197, 351). Hepatic glycogenolysis is catalyzed by glycogen phosphorylase which exists in two forms—phosphorylase a and b. In normal states, phosphorylase exists mainly as phosphorylase b (261) which is the dephosphorylated form. Phosphorylase b is subjected to allosteric regulators. For example, phosphorylase b is inhibited by ATP or glucose-6-phosphate and it is activated by AMP. Therefore, in the normal state, glycogenolysis is inhibited by high levels of intracellular ATP or glucose-6-phosphate and by low concentration of 5′-AMP. However, in hypoxic conditions, tissue ATP levels decline and AMP and Pi accumulate rapidly (122, 174, 344). Therefore, phosphorylase may be activated by low ATP levels (via deinhibition) and by high AMP levels in the liver cell. Lactate production is also one of the responses to hypoxic condition in the liver (16, 17, 174, 301, 351). In the present study, ATP increased lactate production in the perfused liver (Fig. 6). This may also be due to hypoxic stimuli by decreasing oxygen delivery to the liver by the same mechanism as described above.

To eliminate the hypoxic effect by low perfusion flow, the effect of ATP on glycogenolysis was observed under constant perfusion flow. Although glucose production rate with constant perfusion flow was lower than with constant perfusion pressure, ATP still increased glucose output (Fig. 5). This indicates that low perfusion flow and consequently local hypoxia is not the sole mechanism for glycogenolysis by ATP.

The question arises whether ATP has direct effects on glycogenolysis in the hepatic parenchymal cell. In the present study using isolated hepatocytes, ATP actually decreased glucose production rather than increased it (Fig. 8). However, Clemens et al (85) reported different results; in their study 10^{-4}M of ATP increased glucose output 23% in the isolated hepatocyte. Although they used ATP-MgCl$_2$ instead of ATP-Na as in present study, this may not be the critical cause of discrepancy between two results. To interpret the inhibitory effect of ATP on glucose production in the isolated hepatocyte, ATP-entering the cell and its intracellular role will now be considered. Generally, it is assumed that ATP does not cross the plasma membrane (150). However, there are many direct and indirect evidences that ATP does cross the plasma membrane in some tissues including kidney (239), skeletal muscle (74, 76), lymphomas cells (13), nervous cell (270) and liver (80, 152, 181). As described above, intracellular ATP can control the rate of glycogenolysis by its action on phosphorylase b without interconversion of enzyme forms. Low glucose output by ATP may be due to inhibition of phosphorylase via allosteric effects. However, it is uncertain whether 200 micromolar concentration of exogenous ATP can change the millimolar range of intracellular ATP enough to suppress the phosphorylase activity. But it may be possible that the entering ATP is accumulated in cell and thus breaks the steady state of intracellular ATP concentration. Thus entering ATP may act as a "priming" effector on the resynthesis of ATP (266).

On the other hand, in the present study glucagon increased

glucose output in the isolated hepatocyte (Fig. 8). It has been well known that a variety of hormones including catecholamines and glucagon have a stimulatory effect on hepatic glycogenolysis which is mediated by cyclic AMP mechanism and a cascade of reactions (178, 183, 186, 197, 263). Therefore, the stimulatory effect of glucagon on glycogenolysis in the present study agree with numerous investigations. ATP inhibited glucagon-stimulated glucose output (Fig. 8). Under hormone stimulation, phosphorylase b converts to phosphorylase a which is not subjected to any allosteric regulators even under high levels of ATP or glucose-6-phosphate. Thus the mechanism of the ATP action on glucagon-stimulated glucose output in the hepatocyte is not clear.

Phosphofructokinase (PFK) is one of the regulatory enzymes of glycolysis in the liver and its activity is suppressed by high levels of intracellular allosteric inhibitors including ATP, creatine phosphate and low levels of activators such as AMP, ADP and fructose-1,6-diphosphate (145, 225). Glucagon also inhibits PFK and pyruvate kinase of the liver cell (82, 202). In the present study ATP and glucagon significantly inhibited lactate production in the hepatocyte (Fig. 10) and similar results were reported by other investigators (152, 218, 219) in the normal and shocked hepatocytes. The effects of ATP and glucagon on lactate production may be due to inhibition of PFK by allosteric modulation.

A second mechanism of ATP action on glucose production may be related to alteration in prostaglandins in the perfused liver preparation. Prostaglandins or prostaglandin-like substance (PLS) are

released from many tissues by various stimuli. One of the stimuli for prostaglandin release is adenine nucleotide levels. ATP and adenosine diphosphate (ADP) acts as potent releasers of prostaglandins from a wide variety of isolated perfused organs including liver, kidney, spleen, fat pad, heart and lung (252, 260). There are many kind of prostaglandins (PG) in biological systems for example, the PGE-series, PGF-series, PGA-series and PGB-series. Each tissue releases different kinds of prostaglandins and the response to each prostaglandin depends on the respective tissues. Glycogen breakdown by PGE, was demonstrated in the isolated perfused rat liver (227, 347) and in the rat liver slice (38). This finding implicates that glycogenolysis by PGE, is related to PG receptors and adenylate cyclase activation in the liver plasma membrane. Purified prostaglandin E_1 receptors are isolated from rat liver plasma membrane (288, 289, 314, 315). Although some controversy exists (229), many investigators have demonstrated that prostaglandin F_1 stimulated adenylate cyclase and consequently increased cyclic AMP content in the hepatocyte (267, 288, 289, 325, 347, 353). In addition, indomethacin, an inhibitor of prostaglandin synthesis, depressed glucagon- or epinephrine-stimulated hepatic glucose output in vivo (146, 250). In the present study indomethacin attenuated the ATP-induced glucose production (Fig. 28). Therefore, glucose production by ATP in the perfused liver may be mediated by prostaglandin E_1. In summary, 1) ATP may release prostaglandin E_1 from liver cells including vascular endothelium, Kupffer cells and vascular smooth muscle cells, 2) PGE_1 may increase adenylate cyclase in the plasma membrane and increase c-AMP level in the hepatocyte and 3) c-AMP may increase phosphorylase

activity and finally lead to glycogenolysis.

F.   Role of ATP in Hepatic Hemodynamics

ATP, ADP, AMP and adenosine are potent vasodilators in most vascular beds including skeletal muscle (110, 132, 216), skin (132), ear (23), intestinal (90, 132, 161) and coronary system (23, 28, 97, 107, 349). However, vasoconstrictor responses to ATP occur in various tissue preparations. ATP increased the basal tension of dog's saphenous strip (338), aortic strip (143) and human umbilical artery strip (127). Contractile responses to ATP were also demonstrated in other smooth muscle such as rat uterine (94), guinea-pig taenia coli (87, 236), guinea-pig vas deferens (207), urinary bladder (42, 58) and stomach (44). ATP increased renal vascular resistance in the isolated perfused rat kidney (185, 339) and adenosine caused vasoconstriction in the perfused rat liver (193). Thus the hemodynamic response to ATP depends upon the tissue under study.

In the present study, effects of ATP on hepatic hemodynamics were studied in the isolated perfused rat liver. ATP decreased portal vein perfusion flow under constant perfusion pressure (Fig. 15). Although the vasoconstrictor effect of adenosine has been already reported in the perfused rat liver (193), vasoconstrictor response to ATP in the perfused liver have received little attention. The vasoconstrictor effects of ATP were also proved under constant perfusion flow by demonstration of portal vein pressure development. The increase in vascular resistance occurred rapidly and continued for several minutes

and then returned to normal. This decrease may be due to decay of ATP after initiation of vasoconstriction. There are three ectoenzymes for metabolism of external adenine nucleotides--nucleoside triphosphatase, nucleoside diphosphatase, and 5'-nucleotidase (274). These ectonucleotidase system are found in various tissue including red blood cell (272), vascular endothelial cell (88, 230, 274, 275), fibroblasts (102, 104), and plasma (138, 188, 251). In the perfused liver the ectoenzyme of the vascular endothelial cell and smooth muscle cell may degrade ATP. The ectonucleotidase system degrades ATP to ADP, then to AMP and finally to adenosine (275).

Since hepatic vessels have a rich innervation, the hepatic nerves may have significant effects on the hepatic circulation (158). Hormones and some vasoactive drugs including catecholamines, histamine, serotonin, dopamine, angiotensin and vasopressin also affect the hepatic circulation (6, 7, 19, 158, 303). However, the exact sites of action of the hepatic vasopressors are not known. Two major sphincteric sites have been found in the hepatic circulation--inlet sphincters at the presinusoidal region and outlet sphincters at the postsinusoidal region (36, 168, 189, 241). Hepatic arterioles, portal venules and hepatic venules also contribute to the regulation of hepatic circulation (156). Therefore, the hepatic inflow and outflow are determined by net effects between presinusoidal and postsinusoidal resistance change. To clarify the site of ATP action, liver weight changes were studied. ATP decreased liver capacitance (Fig. 21). This result suggests that either 1) ATP increases resistance only in presinusoidal region or 2)

ATP increases vascular resistance both in presinusoidal regions and postsinusoidal regions but the effect of ATP in the presinusoidal region exceeds that in postsinusoidal. However, it does not mean that the original response of presinusoidal region to ATP is greater than postsinusoidal. Because most of ATP might be degraded during passing through sinusoids, the concentration reaching the postsinusoidal region might be very low.

Not only ATP but also other adenine nucleotides--ADP, AMP, and adenosine--increased portal vein resistance in the perfused liver (Fig. 23). Although their potency was lower than that of ATP, ADP, AMP and adenosine also increased glucose output. This similar character indicates that purine compounds (ATP, ADP, AMP and adenosine) may be possessed of similar action site in the perfused liver. Burnstock (51) has proposed the existance of two purinergic receptors on vascular smooth muscle: the P_1 purino-receptors are most sensitive to adenosine and P_2 purino-receptors are most sensitive to ATP. According to his hypothesis the vasodilation effect of purine compounds on vascular beds of skeletal muscle or the coronary system is mediated by purino-receptor mechanism. However, the exact mechanism for vasodilation after activation of such receptor is not clear.

The question still remains as to how ATP increases hepatic vascular resistance. Prostaglandins may also be involved in ATP-induced vasoconstriction of hepatic vessels. It is clear that prostaglandins are released from most animal tissues into the circulation spontaneously and following drug, nerve and hormonal stimulation (283). As referred

to previously, ATP and ADP are potent stimulators of prostaglandin release from various isolated perfused organ including liver, kidney, spleen, heart and lung (252, 260). Endothelial cell, vascular smooth muscle cell, fibroblast and Kupffer cell have been recognized as prostaglandin releasing cells (1, 2, 15, 264, 341). With respect to prostaglandin responses, a single prostaglandin compound can exert opposite actions in different tissue preparations. For example PGI_2 increased blood flow through kidney, adrenal gland, small intestine, brain and pituitary gland; whereas pancreatic and skeletal muscle perfusion were diminished by PGI_2 (299). PGE_1 decreased the resistance in the coronary vessel, femoral vein, and renal artery; whereas PGE_1 caused a biphasic change in the portal venous pressure, an initial increase followed by a decrease (255, 257), and it also caused contraction in several isolated smooth muscle preparation (191, 253). However, generally, prostaglandins of the F-series increase vascular resistance and increase contractility of isolated smooth muscle including aortic strip, stomach muscle, collateral arteries, colon and bladder (3, 5, 190, 253, 345). Smooth muscle contraction by prostaglandins is accomplished by Ca^{++} ion mobilization from calcium storages. PGA_2, PGE_1, PGE_2 and $PGF_{2\alpha}$ increase Ca^{++} influx from extracellular fluid and increases intracellular release of Ca^{++} from sarcoplasmic reticulum, mitochondria or plasma membrane itself (5, 66, 67, 153, 154). In a preliminary study the vasoconstrictor effect of ATP could not be demonstrated in Ca^{++}-free perfusion medium. This may indicate that Ca^{++} influx from extracellular fluid is important to the ATP-prostaglandin-vasoconstriction axis.

Needleman et al (260) did not identify the prostaglandins which are released from the perfused liver by ATP stimulation, nor were prostaglandins measured in the present study. However, if prostaglandins are released from the perfused liver, as previous reports indicate for PGE and $PGF_{2\alpha}$, then vasoconstriction by $PGF_{2\alpha}$ in the present study can be an interpretation of ATP's effects on hepatic resistance change. One point of the evidence for the involvement of prostaglandins in ATP-induced vasoconstriction in the perfused liver is that indomethacin, a prostaglandin synthesis inhibitor, attenuated the ATP-induced portal vein pressure (Fig. 27). Several investigators also reported that indomethacin depressed ATP-induced contraction in guinea-pig digestive tract (205), guinea-pig myometrium (106), rabbit detrusor (99, 105), guinea-pig taenia coli (57) and human umbilical artery (127). This suggests that the vasoconstrictor effect of ATP in the perfused liver is mediated by prostaglandin(s).

Fig. 29 represents a summary of possible mechanisms of ATP action on hepatic hemodynamics and glucoregulation in the perfused rat liver. ATP may release prostaglandin(s) from liver cells such as endothelial cells, vascular smooth muscle cells, and Kupffer cells. Two lines of prostaglandin action--glucoregulation and hemodynamics--are considered. First, prostaglandin(s) act on parenchymal cells and lead to increase in adenylate cyclase activity, increase in phosphorylase activity, and finally increase in glucose and lactate production. Second, prostaglandin may act on vascular smooth muscle cells and lead to an increase in Ca^{++} availability in the cell, cause smooth muscle

FIGURE 29

SUGGESTED MECHANISM OF ATP ACTION ON HEPATIC CIRCULATION

AND GLUCOSE PRODUCTION IN THE ISOLATED PERFUSED RAT
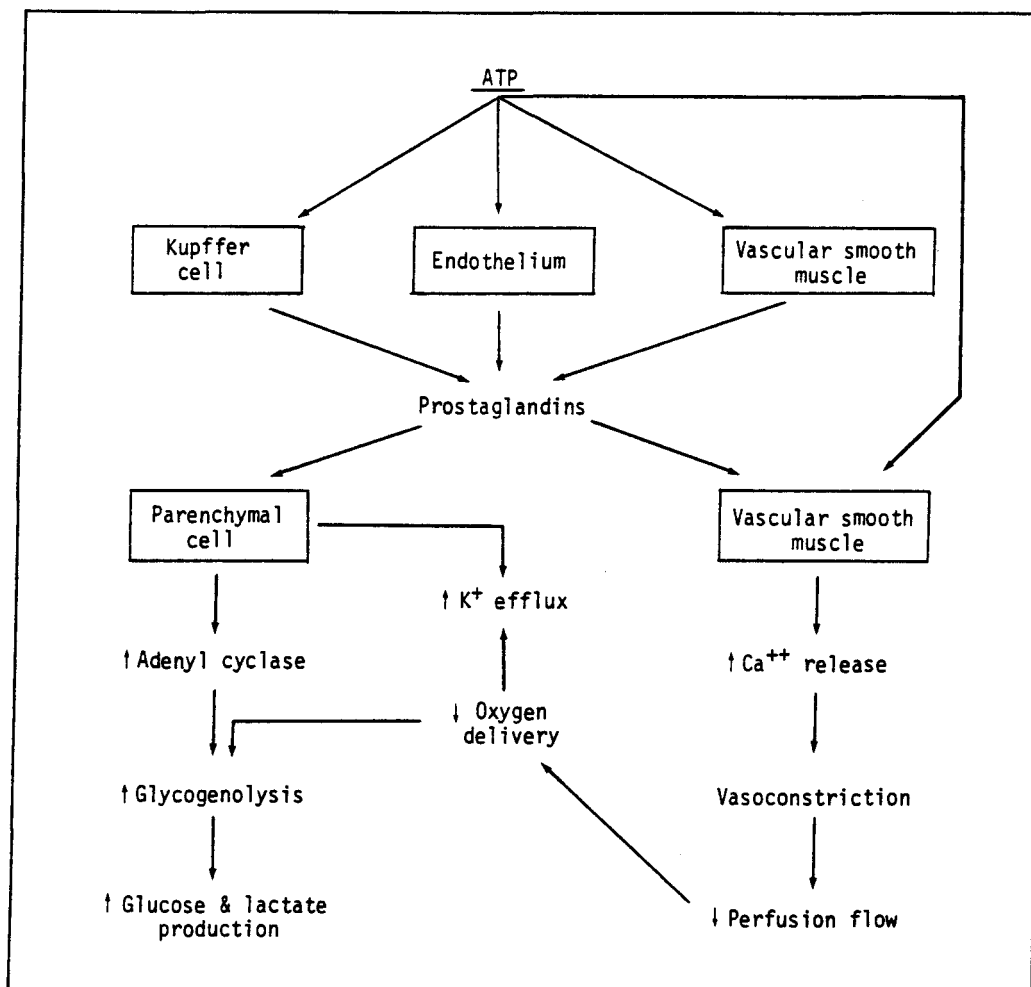
LIVER

contraction, and finally increase resistance of the hepatic vessel. The resultant low perfusion flow may decrease oxygen delivery into the liver which then stimulates glycogenolysis.

SUMMARY AND CONCLUSIONS

The present study was designed to investigate the action of ATP on liver carbohydrate metabolism and on hepatic hemodynamics. Two experimental models—the isolated perfused rat liver preparation and the isolated rat hepatocyte preparation—were employed. In the first series of experiments several metabolic changes including glucose production, lactate production, oxygen consumption and K^+ ion movement were measured. In the second series of experiments the effects of ATP on hepatic circulatory resistance, changes in portal vein perfusion flow, portal vein perfusion pressure, hepatic arterial perfusion pressure and hepatic capacitance were measured.

The main results of the experiments were:

1. ATP increased glucose production in the isolated perfused rat liver under either constant perfusion pressure or constant perfusion flow. Lactate production was also increased by ATP with constant perfusion pressure.

2. ATP did not increase glucose production in the isolated hepatocytes. However, it inhibited glucagon-stimulated glucose output. ATP also inhibited lactate production in the isolated hepatocytes.

3. ATP increased oxygen consumption at low concentration (10 uM) and

inhibited it at high concentration (40 and 160 uM) in the perfused liver.

4. ATP increased K^+ efflux in the isolated perfused liver.

5. Indomethacin attenuated ATP-induced glucose production in the isolated perfused liver.

6. ATP decreased perfusion flow and increased portal vein perfusion pressure. ATP also increased hepatic arterial perfusion pressure.

7. ATP reduced hepatic capacitance by increasing the resistance of the presinusoidal region in the liver.

8. The adenine derivatives including adenosine, AMP, ADP and ATP also decreased perfusion flow and increased glucose output. ADP decreased oxygen consumption, however, adenosine and AMP did not. The order of activity of the adenine compounds were ATP, ADP, AMP = adenosine.

9. Prostaglandin $F_{2\alpha}$ increased portal vein pressure. Indomethacin attenuated ATP-induced portal vein pressure.

In conclusion, the increments of glucose production and hepatic circulatory resistance with ATP in the isolated perfused rat liver may be mediated by prostaglandins which are released from hepatic cells by exogenous ATP stimulation.

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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