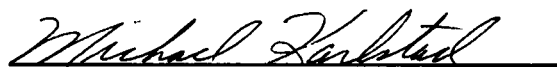


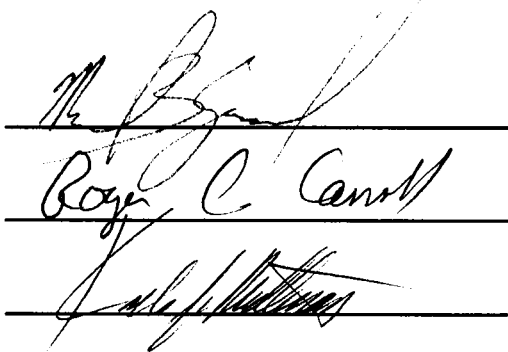
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I am submitting herewith a dissertation written by Darunee Buripakdi entitled "The Role of Platelet-Activating Factor in Alterations of Amino Acid Transport and Protein Metabolism During Shock." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Life Sciences.



Michael D. Karlstad, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor
and Dean of the Graduate School

**THE ROLE OF PLATELET-ACTIVATING FACTOR IN
ALTERATIONS OF AMINO ACID TRANSPORT AND PROTEIN
METABOLISM DURING SHOCK**

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Darunee Buripakdi

December, 1997

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DEDICATION

This dissertation is dedicated to my family:

คุณเฉลียว และ คุณจุฑา บุรีภักดิ์

my parents, Mr. Chalio and Mrs. Chuta Buripakdi,
for their love and never-ending support and encouragement;

คุณทิพพา บุญยประสาธ

my grandmother, Mrs. Thippa Boonyaprasart, who patiently awaits my return;

คุณเทพ บุญยประสาธ

and in memoriam of my grandfather, Mr. Thep Boonyaprasart,
for his living inspiration and wisdoms.

Without their love, this dissertation would never have been completed.

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ABSTRACT

Platelet-activating factor (PAF), a biologically active phospholipid, has been implicated as an important mediator of endotoxin shock and sepsis. An attenuation of amino acid transport and acceleration of net protein loss from skeletal muscle are major characteristics of the protein catabolic response to septic shock. This dissertation is composed of two parts and is devoted to understanding the role of PAF as a potential mediator of protein catabolism during shock and sepsis. The first part demonstrates that a) PAF attenuates system A amino acid transport in skeletal muscle during endotoxic shock by producing conditions and/or factors in plasma that might be similar to those existing during endotoxemia and, b) the direct attenuation of amino acid transport depends upon previous exposure of skeletal muscle to those conditions or factors generated by PAF *in vivo*. The second part demonstrates that PAF induces net protein catabolism as a result of a) elevated leucine oxidation at the expense of protein synthesis, b) attenuated rate of fractional protein synthesis in skeletal muscle, c) reduced efficiency of muscle protein synthesis, and d) decreased myosin relative abundance in skeletal muscle. PAF attenuates amino acid uptake and reduces protein synthesis in skeletal muscle and thus is a potential mediator of protein catabolism during shock. The first part of this dissertation was previously published (*SHOCK* 2(1):53-59, 1994) and the second part is in preparation for publication.

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LIST OF ABBREVIATIONS

AIB	α -aminoisobutyric acid
B	breakdown
BCAA	branched-chain amino acid
CLP	cecal-ligation and perforation
EAA	essential amino acid
<i>E. coli</i>	<i>Escherichia coli</i>
EDL	extensor digitorum longus muscle
FSR	fractional synthesis rate
G-protein	guanine nucleotide binding protein
HDL	high-density lipoprotein
HPLC	high-pressure liquid chromatography
I	dietary intake
Ig-E	immunoglobulin-E
IL	interleukine
i.v.	intravenous
i.p.	intraperitoneal
LDL	low-density lipoprotein
LPS	lipopolysacharide
MABP	mean arterial blood pressure
MDF	myocardial depressant factor

NEAA	non-essential amino acid
NOLD	non-oxidative leucine disappearance
OX	oxidation
PAF	platelet-activating factor
PAF-AH	PAF-acetylhydrolase
PLA ₂	phospholipase A ₂
cPLA ₂	cytosolic phospholipase A ₂
RNA	ribonucleic acid
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	total RNA
S	synthesis
SE	standard error of means
SSA	sulfosalicylic acid
TNF	tumor necrosis factor

I. INTRODUCTION

Platelet activating factor (PAF) is now known to be a potent mediator with diverse actions on various target cells. It has been implicated in a number of clinical states including inflammation, ischemia (circulatory insufficiency), and shock. During shock and trauma, a variety of protein metabolic abnormalities occur such as accelerated skeletal muscle proteolysis, increased amino acid flux, loss of skeletal muscle mass, and an overall loss of body protein termed a negative nitrogen balance. A preliminary result and previous study from this laboratory indicated that PAF might be involved in protein catabolic response to injury and endotoxemia. This laboratory reported that a) an administration of a PAF antagonist (WEB 2086) to injured rats decreased urinary nitrogen excretion and improved nitrogen balance and, b) an iv bolus injection of PAF attenuated basal and insulin-stimulated system A amino acid transport in isolated rat skeletal muscle. Endotoxic shock induced by infusion of endotoxin causes the reduction of both protein synthesis and the active transport of neutral amino acids in rat skeletal muscle. Thus, stimulation of PAF production *in vivo* by bacterial endotoxin suggests that PAF may be one of the key mediators that cause alterations of amino acid and protein metabolism during endotoxemia and septic shock.

This dissertation was divided into two parts to study the effect of PAF on skeletal muscle amino acid transport and whole-body and tissue protein

metabolism in the rats during shock. The first part was designed to investigate the effect of PAF on amino acid transport via system A of skeletal muscle during endotoxic shock in the rat. The second part was designed to explore the effect of PAF on whole-body and tissue leucine kinetics, plasma and tissue amino acid profiles, muscle RNA activity for protein synthesis, and muscle myofibrillar protein content in the rat. Both parts of this dissertation have been respectively published (1994, 2(1):53-59) or in preparation for publication in a peer-reviewed scientific journal, **SHOCK: *Molecular, Cellular and Systemic Pathological Aspects and Therapeutic Approaches***.

II. LITERATURE REVIEW

PLATELET-ACTIVATING FACTOR

Historical Background

The discovery of platelet activating factor, a phospholipid compound commonly known as PAF, was reported in the late 1960s by Benveniste (Benveniste, 1990), a biochemist who studied the hypersensitivity immunologic mechanism of leukocyte-dependent histamine release from rabbit platelets (Henson, 1969; Benveniste et al., 1972). During the first few years of his investigation of PAF, Benveniste had referred to PAF as a lipid-like compound released from basophils and stimulated platelets (Benveniste et al., 1972; Benveniste, 1974). PAF was found to trigger the release of histamine from rabbit platelets in the presence of sensitized-leukocytes and antigen in a calcium- and temperature-dependent reaction (Henson, 1969; Siraganian and Osler, 1971; Benveniste et al., 1972). Following a compilation of reports from Benveniste's group (Henson, 1969; Benveniste et al., 1972; Benveniste, 1974; Chignard et al., 1979) and others (Barbaro and Zvaifler, 1966; Siraganian and Osler, 1971; Pinckard et al., 1979), PAF was later known as a mediator released from rabbit and human basophils during anaphylaxis and immune complex deposition by an IgE-dependent process which stimulated the aggregation of platelets.

Coincidentally, another research group that investigated antihypertensive functions of kidney reported the antihypertensive activity of an alkyl ether analog of phosphatidylcholine (Blank et al., 1979; Muirhead, 1980; Muirhead et al., 1981). This group, which was led by Muirhead, had studied an antihypertensive polar lipid from renomedullary interstitial cells during the approximate time PAF was discovered. It turned out that this antihypertensive polar renomedullary lipid shared significant similarities with the molecular structure of PAF (Muirhead et al., 1981).

The merger of these two different fields of study of the same compound, in 1979, resulted in an increasing interest in other biochemical and physiological effects of PAF than just platelet activation and antihypertension (Hanahan, 1986; Koltai et al., 1991b; Koltai et al., 1991c; Braquet et al., 1989). The following decades have provided wider interest in PAF research activity for its role in shock and ischemia (Feuerstein and Siren, 1988; Lefer and Stahl, 1989; Anderson et al., 1991; Sanchez Crespo and Fernandez-Gallardo, 1991), sepsis and multi-organ failure (Braquet et al., 1989; Anderson et al., 1991; Ayala and Chaudry, 1996), immune responses (Hanahan, 1986; Mencia-Huerta and Braquet, 1989; Peplow and Mikhailidis, 1990; Koltai et al., 1991b; Koltai et al., 1991a), hemodynamics and cardiovascular pathology (Lefer and Stahl, 1989; Mathias and Behrens, 1990; Koltai et al., 1991a; Soloviev and Braquet, 1992), and alterations of metabolism in various organs (Evans et al., 1991; Koltai et al., 1991b; Koltai et al., 1991c).

Nonetheless, because of its platelet activation ability (Benveniste, 1990), PAF became a common name for the compound that was later chemically identified as alkyl-acetyl-glycero-phosphocholine (occasionally referred to as PAF-acether) (Demopoulos et al., 1979; Hanahan et al., 1980; Godfroid et al., 1980; Benveniste, 1990) and characterized as a biologically active phospholipid product of Ca^{2+} -sensitive cytosolic phospholipase A_2 (PLA_2) enzyme (Demopoulos et al., 1979; Chap et al., 1981; Kramer et al., 1988; Benveniste, 1990; Peplow and Mikhailidis, 1990; Triggiani et al., 1990; Kramer, 1994).

Biochemical Characteristics of PAF

During the 1970s, several studies on PAF focused on extraction and purification procedures for further investigations of its chemical structure and biochemical function, especially its role in platelet aggregation (Benveniste, 1974; Benveniste et al., 1977; Chignard et al., 1979). A soluble PAF was prepared from the antigen-induced *in vitro* degranulation of basophils from rabbits or hogs (for large amount of PAF production) that were immunized with peroxidase (Benveniste, 1974; Benveniste et al., 1977). A highly active purified PAF was obtained in a chloroform-soluble fraction. Since the PAF molecule is highly positively charged (Benveniste, 1974; Benveniste et al., 1977; Benveniste, 1990), the purification of PAF involved several steps

including centrifugal separations and chromatographic purifications by ion-exchange and high pressure liquid chromatography.

In vitro identification of a soluble PAF produced by antigen-stimulated leukocytes was achieved between the late 1970s and early 1980s. By using a variety of lipase enzymes to analyze purified PAF, Benveniste et al. (Benveniste et al., 1977) revealed that PAF's unique biological activity was at the site of an alkyl group attached to the glycerol backbone via an ether linkage. In 1979, Demopoulos et al. (Demopoulos et al., 1979) provided evidence that PAF had the structure of 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (AGEPC). They identified the similarity in chemical structure between the synthesized PAF analogue, AGEPC, and the native PAF which was produced under *in vitro* conditions, from antigen-stimulated basophils that were obtained from IgE-sensitized rabbits (Demopoulos et al., 1979; Pinckard et al., 1979; Hanahan et al., 1980). The total synthesis of PAF, in a form of 1-O-octadecyl-2-O-acetyl-*sn*-glycero-3-phosphoryl-choline, was later accomplished by Benveniste's research group (Godfroid et al., 1980; Polonsky et al., 1980).

The structural formula of PAF is shown in Figure 1 (Blank et al., 1979; Godfroid et al., 1980; Hanahan, 1986). Although a variation in the alkyl group for the side chain of AGEPC causes multiple molecular species of PAF, generally, PAF has a molecular weight of approximately 530 daltons (Blank et al., 1979; Hanahan et al., 1980; Godfroid et al., 1980; Hanahan, 1986;

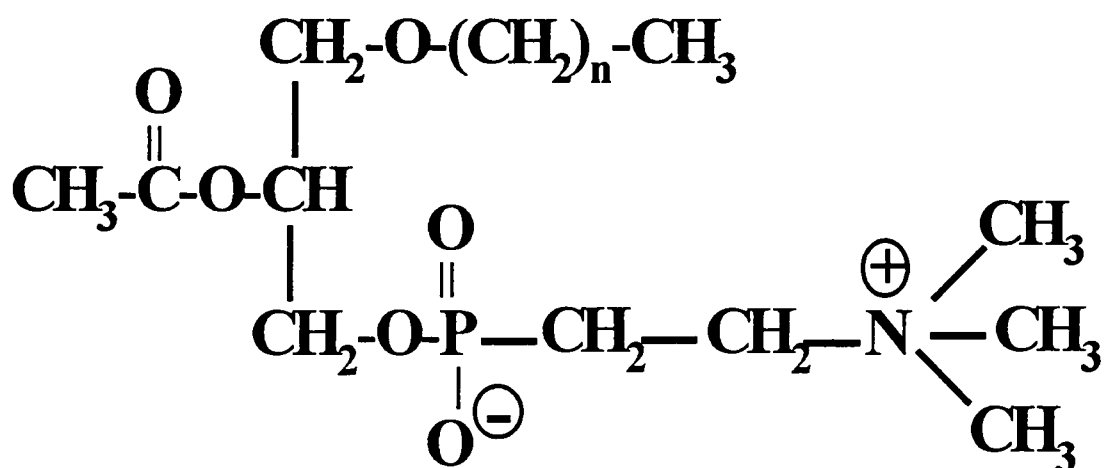


Figure 1. Structural formula of PAF, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. $n = 13:0$ to $17:1$, represents the existence of multiple molecular species of PAF that vary in the nature of alkyl group, $(\text{CH}_2)_n\text{-CH}_3$; including C14:0, C15:0, C16:0, C17:0, C18:0, and C18:1 alkyl chain homologs of PAF.

Sanchez Crespo and Fernandez-Gallardo, 1991). The most abundant O-alkyl side chain for the biologically active phospholipid AGEPC are hexadecyl and octadecyl (Hanahan et al., 1980; Hanahan, 1986; Lefer and Stahl, 1989), which are designated as C16 and C18 in Figure 1. It was reported that hexadecyl- and octadecyl-AGEPC composed of <10% and >90%, respectively, in PAF extracted from IgE-sensitized rabbit basophils (Hanahan et al., 1980).

Biosynthesis and Metabolism of PAF

Unlike other circulating mediators, PAF is not stored in cells but exists in a form of inactive precursors associated with a cellular membrane, and can be synthesized rapidly upon cellular stimulation (Hanahan, 1986; Peplow and Mikhailidis, 1990). PAF can be produced by two independent enzymatic pathways from two different precursors (Hanahan, 1986; Peplow and Mikhailidis, 1990; Blank et al., 1990; Snyder et al., 1991), and inactivated by hydrolysis of the acetyl group with PAF-acetylhydrolase (PAF-AH) (Hanahan, 1986; Touqui et al., 1987; Peplow and Mikhailidis, 1990; Snyder et al., 1991; Kirschbaum, 1991).

One enzymatic pathway for PAF synthesis is via PLA_2 which catalyses a cleavage of a long chain acyl moiety from a membrane phospholipid precursor, especially 1-O-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (1-O-alkyl-2-acyl-GEPC) (Snyder et al., 1991). This pathway is commonly referred to as a remodeling PAF synthesis (Peplow and Mikhailidis, 1990; Snyder et al., 1991).

Another pathway is via acetylhydrolase that is specific for an immediate precursor of PAF, 1-alkyl-2-acetyl-*sn*-glycerol (Blank et al., 1990; Snyder et al., 1991). This particular pathway is referred to as a *de novo* PAF synthesis (Hanahan, 1986; Peplow and Mikhailidis, 1990; Blank et al., 1990; Snyder et al., 1991).

The synthesis and metabolism of PAF is generally similar in most organs and cells that are capable of PAF production (Denizot et al., 1989; Peplow and Mikhailidis, 1990; Snyder et al., 1991; Sugiura et al., 1993). Interestingly, PAF synthesis from exogenous precursors by the prokaryote, *Escherichia coli*, was also reported (Denizot et al., 1989), in addition to evidences of PAF production in invertebrates such as earthworm, slug, and protozoa (Sugiura et al., 1993).

Sources

PAF can be produced and released from a variety of cells such as renomedullary interstitial cells (Muirhead, 1980; Muirhead et al., 1981), endothelial cells (Camussi et al., 1983; Zimmerman et al., 1985; Whatley et al., 1990), peritoneal cells (Inarrea et al., 1985), platelets (Siraganian and Osler, 1971; Benveniste et al., 1972; Chap et al., 1981; Ban et al., 1986), and leukocytes (Siraganian and Osler, 1971; Benveniste et al., 1977; Vargaftig et al., 1980) including basophils (Demopoulos et al., 1979; Pinckard et al., 1979; Hanahan et al., 1980), neutrophils (Betz and Henson, 1980; Chilton et al.,

1984; Denichilo et al., 1991; Glaser et al., 1991), mononuclear leukocytes (Henson, 1969), and macrophages (Zhang et al., 1991). Many other tissues have also been reported to produce PAF. Those tissues include amnion tissue in fetal membrane (Ban et al., 1986), brain (Kumar et al., 1988), bowel (Hsueh et al., 1987), jejunum (Whittle et al., 1987), spleen (Gomez-Cambronero et al., 1985; Inarrea et al., 1985), and lung (Snyder et al., 1991; Davidson and Drafta, 1992).

The production and release of PAF has been reported in the presence of bacterial endotoxin (lipopolysaccharide, LPS) (Inarrea et al., 1985; Hsueh et al., 1987; Whittle et al., 1987; Mozes et al., 1991), or calcium influx (Ban et al., 1986; Kramer et al., 1988; Kumar et al., 1988) and/or calcium ionophore (A23187) (Camussi et al., 1983; Zimmerman et al., 1985; Whatley et al., 1990; Denichilo et al., 1991; Glaser et al., 1991). This wide array of cell types that have the ability to synthesize PAF suggests a biological significance of PAF (Anderson et al., 1991).

Key Enzymes for PAF Synthesis: Phospholipase A₂ and Acetylhydrolase

PAF is one of the biologically active products (among arachidonic acid and eicosanoids) that PLA₂ generates (Chap et al., 1981; Vigo, 1987; Kramer et al., 1988; Benveniste, 1990; Peplow and Mikhailidis, 1990; Sanchez-Crespo et al., 1993; Kramer, 1994; Roberts, 1996) following cellular stimulation and an increase in cytosolic Ca²⁺ (100-1000 nM) (Kramer et al., 1988; Lapetina and

Crouch, 1989; Peplow and Mikhailidis, 1990; Roberts, 1996). Although currently there are various types of PLA₂ identified, the PLA₂ that plays a major role in PAF production resides in the cytosol and is Ca²⁺-dependent, with a Ca²⁺ binding site that requires Ca²⁺ in the micro- rather than milli-molar levels (Kramer et al., 1988; Roberts, 1996). Ca²⁺ concentrations in the millimolar range, however, were reported to heighten the action of PLA₂ to maximal activity in platelets (Kramer et al., 1988).

This PLA₂ specifically hydrolyzes fatty acids which are esterified at the *sn*-2 position on the phospholipid molecule of phosphatidylinositol, phosphatidylethanolamine, and phosphatidylcholine (Kramer et al., 1988; Peplow and Mikhailidis, 1990; Roberts, 1996). The phosphocholine molecule in the cellular membrane is most reported as the precursor for PAF biosynthesis (Hanahan, 1986; Touqui et al., 1987; Snyder et al., 1991). Therefore, the primary product of the initial step of PAF biosynthesis via the hydrolytic activity of PLA₂ on 1-O-alkyl-2-acyl-GEPC is 1-O-alkyl-2-(lyso)-glycero-3-phosphocholine (lyso-AGEPC or lyso-PAF) (Hanahan, 1986; Peplow and Mikhailidis, 1990; Snyder et al., 1991; Sanchez-Crespo et al., 1993).

The arachidonate-containing phospholipid, 1-O-alkyl-2-arachidonoyl-GEPC, was reported by many investigators (Chilton et al., 1984; Kramer et al., 1988; Peplow and Mikhailidis, 1990; Sanchez-Crespo et al., 1993) to be a common precursor of PAF via PLA₂ activity in the initial step of PAF biosynthesis which generates lyso-PAF. It has been reported also that PLA₂

can utilize eicosapentaenoate (EPA)- as well as arachidonate-containing phospholipids as precursors for PAF production (Triggiani et al., 1990). Although it has been conceptualized that EPA has an inhibitory effect on PAF biosynthesis, Triggiani et al. (Triggiani et al., 1990) found that EPA was incorporated into a PAF precursor pool.

PAF is also a product of another cytosolic enzyme, acetylhydrolase which catalyzes the hydrolysis of acetate from 1-alkyl-2-acetyl-*sn*-glycerol (Godfroid and Braquet, 1986; Hanahan, 1986; Blank et al., 1990; Peplow and Mikhailidis, 1990; Vallari et al., 1990; Snyder et al., 1991). This enzyme is present both in the cytosol of various cells as well as in plasma (Godfroid and Braquet, 1986; Vallari et al., 1990; Snyder et al., 1991), and is not affected by Ca^{2+} (Godfroid and Braquet, 1986; Blank et al., 1990). It has been suggested to play an important role in regulating the level of PAF synthesized by the *de novo* pathway via hydrolysis of the immediate precursor of PAF (Blank et al., 1990; Vallari et al., 1990; Snyder et al., 1991; Lee et al., 1986). However, the majority of PAF that is produced through the *de novo* pathway was reported to be present in the intracellular organelles, associated with the internal membranes of the endoplasmic reticulum and Golgi-apparatus, and not released from the intact cells (Vallari et al., 1990; Lee et al., 1986). Since the remodeling pathway is relatively inactive without cellular stimulation that result in an increase of calcium influx, thus, the *de novo* pathway may be important

for maintaining the physiologic level of PAF for the intracellular function of resting cells (Vallari et al., 1990; Lee et al., 1986; Anderson et al., 1991).

PAF Metabolism

The initial reaction that produces lyso-PAF in the remodeling pathway is also depending upon the regulation of a CoA-independent transacylase (Hanahan, 1986; Peplow and Mikhailidis, 1990; Snyder et al., 1991; Sanchez-Crespo et al., 1993). This enzyme is capable of catalyzing both the hydrolysis of the acyl moiety of the 1-O-alkyl-2-acyl-GEPC precursor and the acyl-transfer to another lyso-phospholipid (Snyder et al., 1991).

Once formed, lyso-PAF can serve as a substrate for two opposing pathways (Hanahan, 1986; Peplow and Mikhailidis, 1990; Snyder et al., 1991). As an immediate precursor to PAF, lyso-PAF can be acetylated by an acetyltransferase and acetyl CoA (acetyl-CoA : 1-O-alkyl-2-lyso-glycero-3-phosphocholine acetyltransferase) into PAF, or acylated by an acyltransferase back to 1-O-alkyl-2-acyl-GEPC (Gomez-Cambronero et al., 1985; Hanahan, 1986; Peplow and Mikhailidis, 1990; Snyder et al., 1991; Sanchez-Crespo et al., 1993). The acetyltransferase is the rate limiting enzyme in PAF production (Hanahan, 1986; Peplow and Mikhailidis, 1990; Sanchez-Crespo et al., 1993). The activation of this enzyme is regulated by calcium influx and phosphorylation via protein kinase (Gomez-Cambronero et al., 1985). The

acyltransferase enzyme, however, is inhibited by an elevated intracellular Ca^{2+} (Touqui et al., 1987; Peplow and Mikhailidis, 1990).

Calcium has a regulatory effect on PAF metabolism. During PAF production, calcium influx activates the acetylation of lyso-PAF (Gomez-Cambronero et al., 1985) and inhibits lyso-PAF reacylation (Touqui et al., 1987). However, calcium ions do not affect acetylhydrolase enzyme that is responsible for PAF biosynthesis via the *de novo* pathway (Blank et al., 1990). The PAF-acetylhydrolase, or PAF-AH, which initially inactivates PAF by catalyzing the hydrolysis of the acetyl group, in a manner that was earlier thought to be calcium-independent (Ban et al., 1986; Hanahan, 1986), was however reported to be blocked by an increase of intracellular Ca^{2+} (Touqui et al., 1987). Therefore, it is apparent that calcium ions when elevated intracellularly, promote PAF biosynthesis via PLA_2 activity and suppress PAF degradation via the deacetylation-reacylation process (Gomez-Cambronero et al., 1985; Hanahan, 1986; Touqui et al., 1987; Peplow and Mikhailidis, 1990; Snyder et al., 1991).

Bacterial endotoxin has been indicated in cellular stimulation that results in increased calcium influx and thus represents a strong stimulus for PAF production via the remodeling process which involves activation of phospholipase A_2 and acetyltransferase enzymes (Vallari et al., 1990; Feuerstein and Siren, 1988). PAF has also been shown to stimulate its own synthesis *in vivo* through a positive feedback loop, which could be blocked by

intravascular volume expansion with saline (Zhang et al., 1991). The diagrammatic summary of PAF metabolism is illustrated in Figure 2.

Biological Effects of PAF

The known biological effects of PAF in the early phases of PAF research included the activation and aggregation of platelets (Henson, 1969; Siraganian and Osler, 1971; Benveniste et al., 1972; Benveniste, 1974; Benveniste, 1990) and antihypertensive effects (Blank et al., 1979; Muirhead, 1980; Muirhead et al., 1981). It was not until 1980 that Benveniste's research group documented a bronchoconstrictive effect of PAF (Vargaftig et al., 1980). The implication from that study was that PAF also played a pathological role in bronchoconstriction during anaphylaxis in addition to its earlier known effect of platelet stimulation (Vargaftig et al., 1980). The findings from that investigation stimulated interest in PAF research for other biological effects of PAF.

PAF has been investigated for its role in many other biological and clinical states for more than three decades (Hanahan, 1986; Feuerstein and Siren, 1988; Lefer and Stahl, 1989; Anderson et al., 1991; Evans et al., 1991; Ayala and Chaudry, 1996). The findings from these studies indicate that PAF has diverse biological effects on a variety of cellular targets and organ systems. Examples of PAF's effects are as follows: activation of platelets and leukocytes in the peripheral circulation (Korth et al., 1989; Mathias and Behrens, 1990), bronchoconstriction (Vargaftig et al., 1980; Casals-Stenzel,

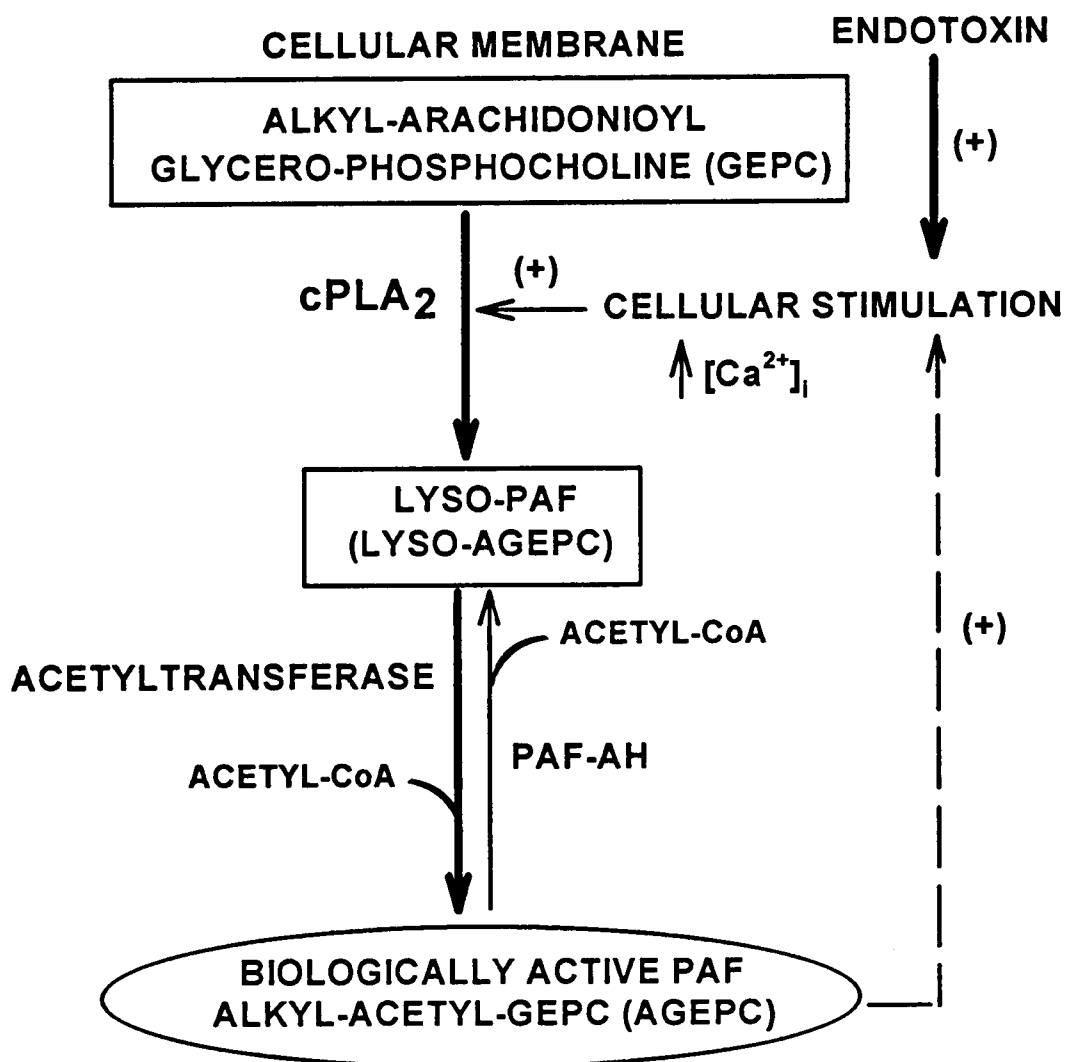


Figure 2. Biosynthesis of PAF via the Ca^{2+} -sensitive cytosolic phospholipase A_2 (cPLA₂) following endotoxin-induced cellular stimulation (coupled with an increased level of intracellular Ca^{2+} , $\uparrow [Ca^{2+}]_i$) and the positive feedback of PAF production (represented by a broken line). PAF-AH is the PAF-acetylhydrolase, a key enzyme in PAF inactivation.

1987a; Lohman and Halonen, 1990), hypotension (Casals-Stenzel, 1987b; Hsueh et al., 1987; Lang et al., 1987; Lang et al., 1988; Lang and Dobrescu, 1988; Mathias and Behrens, 1990; Qi and Jones, 1990; Karasawa et al., 1990), increased vascular permeability (Anderson et al., 1991), hyperglycemia (Lang et al., 1987; Lang et al., 1988; Lang and Bagby, 1990) induction of bowel and intestinal ischemia (Casals-Stenzel, 1987a; Hsueh et al., 1987), lung injury (Chang et al., 1987; Chang et al., 1990), cardiac contractility depression (Karasawa et al., 1990; Qi and Jones, 1990; Soloviev and Braquet, 1992), and lethality (Casals-Stenzel, 1987b; Terashita et al., 1989; Karasawa et al., 1990).

It is apparent that PAF plays an important role in many pathological conditions including anaphylaxis, multi-organ (gastrointestinal tract, heart, kidney, and lung) failure, ischemia, sepsis, and shock states (Hanahan, 1986; Hsueh et al., 1987; Feuerstein and Siren, 1988; Lang et al., 1988; Lefer and Stahl, 1989; Mathias and Behrens, 1990; Anderson et al., 1991; Evans et al., 1991; Rabinovici et al., 1991; Ayala and Chaudry, 1996).

Effects of Intravenous PAF Infusion

Lang et al. (Lang et al., 1988) studied the effects of PAF infusion, bolus or continuous, of different doses on hemodynamics and glucose metabolism in rats. A bolus i.v. infusion of PAF at the dose of 300 ng/kg caused a transient hypotension, a 30% decrease in blood pressure for 5 min that returned to

basal level by 30 min. Transient increases in plasma glucose and lactate were also observed. A constant infusion of 20 ng/min/kg (or 1.2 $\mu\text{g/kg/h}$) caused only slight changes in blood pressure (11% decrease for 2 h) and plasma glucose level (28% increase). On the other hand, prominent and prolonged changes in hemodynamics (34% decrease in blood pressure for 4 h) and plasma glucose level (150% increase) were observed in rats that received a constant infusion of a much higher dose of PAF, 12 $\mu\text{g/kg/h}$.

Since the constant infusion of PAF stimulated many of the hemodynamic and metabolic alterations (Lang et al., 1988) that were similar to the changes induced by endotoxin (Lang et al., 1987), burn (Lang and Dobrescu, 1988), and sepsis (Lang and Bagby, 1990), PAF was suggested to be an important mediator of the early hemodynamic and metabolic disorders of endotoxic and septic shock.

The cardiovascular effects of PAF infusion (even at nanomolar concentrations), such as peripheral vasodilation with hypotension, decrease in cardiac output, increase in vascular permeability, coronary vasoconstriction and arrhythmias involve PAF receptors which are expressed in cardiovascular tissues (Sugimoto et al., 1992).

PAF Receptors

All of PAF biological effects are a result of the binding of PAF to its specific cellular receptor (Godfroid and Braquet, 1986; Feuerstein and Siren,

1988). The stimulation of PAF responses by only the naturally occurring stereoisomer (*R*) but not the *s*-isomer (synthetic configuration) strongly supports the involvement of specific receptor(s) in mediating the biological effects of PAF (Godfroid and Braquet, 1986; Anderson et al., 1991). The study of PAF receptor in human platelets by Valone et al. (Valone et al., 1982) revealed that the PAF binding site was very specific with high affinity in a nanomolar level of concentrations, $K_D \approx 40$ nM. They showed that an ether linkage in position 1, a short-chain fatty acid in position 2, and a choline moiety in the polar head group (see Figure 1) were essential for the ligand (AGEPC molecule) binding to PAF receptor and the initiation of platelet aggregation (Valone et al., 1982).

Further studies on the molecular structure of PAF receptor suggested a homology of the PAF receptor and the superfamily of guanine nucleotide binding protein (G-protein)-coupled receptors (Shukla, 1992; Chau et al., 1993; Sugimoto et al., 1992). By using a hydrophobicity analysis of the deduced amino acid sequence, Sugimoto et al. (Sugimoto et al., 1992) revealed that the cloned PAF receptor from cardiac tissue had seven transmembrane-hydrophobic-domains which represent a common feature to the superfamily of G-protein-coupled receptors. They showed that human cardiac PAF receptor protein was similar to the PAF receptor expressed in human leukocytes, but also indicated that the regulatory mechanisms in the expression of this protein might be tissue specific (Sugimoto et al., 1992).

It has also been suggested that PAF receptors exist in various subtypes because several PAF antagonists elicited differences in the potency ranking order in various cells (Chau et al., 1993). Since there are different G-protein-coupled receptors in various cell types, together with possible PAF receptor subtypes, PAF responses may be mediated by a variety of signal transduction pathways (Chau et al., 1993; Sugimoto et al., 1992; Shukla, 1992). Thus, the binding of PAF ligand to the receptor has been suggested to activate GTPase and lead to the activation of the effector enzymes, such as phospholipase A₂, phospholipase C, phospholipase D, protein kinase C, and tyrosine kinase (Shukla, 1992). Further, PAF can stimulate the opening of ion channels, such as calcium, potassium, chloride, and proton (Chau et al., 1993; Sugimoto et al., 1992; Shukla, 1992).

PAF-Antagonists

The use of specific PAF-receptor antagonists became the most useful method of investigating the role of PAF in various biological states, including ischemia, shock, and injury (Lang et al., 1987; Lefer and Stahl, 1989; Anderson et al., 1991; Houlihan, 1991; Rabinovici et al., 1991). PAF antagonists can be categorized into two basic groups, PAF analogs such as CV- and SRI-compounds, and non-PAF analogs such as BN- and WEB-compounds (Lefer and Stahl, 1989; Houlihan, 1991). For example, CV-3988, the first phospholipid analog of PAF (Houlihan, 1991), has been shown to

attenuate systemic hypotension, preserve hypoxic pulmonary vasoconstriction, and prolong survival rate of endotoxin-treated rats (Chang et al., 1987). CV-6209, another derivative of a CV-compound, has been shown to improve survival rate, decrease the plasma accumulation of lysosomal hydrolase, cathepsin D, and a cardiotoxic peptide (myocardial depressant factor, or MDF) in traumatized rats (Terashita et al., 1988).

SRI 63-441, another PAF analog, was reported to prevent early intestinal necrosis in hypoxic rats (Caplan et al., 1990), and depressed hypoxic pulmonary vasoconstriction in endotoxin-treated rats (Chang et al., 1987). In addition, this compound was also shown to lower the plasma catecholamine levels and attenuate increased glucose metabolism during endotoxic shock (Lang et al., 1987).

BN 5039, a ginkgolide PAF-receptor antagonist which is a terpenoid isolated from an extract of the Ginkgo tree (*Ginkgo biloba*) (Houlihan, 1991), was shown to prolong survival time and attenuate the pathogenesis of traumatic shock (Karasawa et al., 1990). This compound lessened hypotension and reduced the elevated plasma levels of cathepsin D (lysosomal protease enzyme), free amino-nitrogen, and the cardiotoxic peptide MDF in traumatized rats (Karasawa et al., 1990).

WEB 2086, a derivative of triazolobenzodiazepines, was shown to inhibit hypotension and the lethal effects of PAF and bacterial endotoxin (Casals-Stenzel, 1987b; Qi and Jones, 1990), ameliorate endotoxin-induced

lung injury (Chang et al., 1990), and protect the gut mucosa during shock (Casals-Stenzel, 1987a). WEB 2086 is of particular interest for this dissertation because it was previously shown by this laboratory to improve nitrogen balance in injured rats (McKeen et al., 1990). This compound was previously indicated to have the PAF antagonistic effect at the PAF receptor level in target cells (Korth et al., 1989). The triazolo-thieno-benzodiazepine WEB 2086 inhibited platelet aggregation induced by PAF and the binding of [^{3} H]-PAF to endothelial cells. Korth et al. (Korth et al., 1989) demonstrated that partial displacement of platelet-bound [^{3} H]-PAF by WEB 2086 was in a concentration-dependent manner reaching a plateau at 400 nM. Qi and Jones (Qi and Jones, 1990) showed that WEB 2086, at a dose of 5 mg/kg, prevented hypotension, maintained heart rate, and prevented increases in plasma lactate and catecholamine levels during endotoxic shock in rats.

Blockers of PAF activity have been found to attenuate the severity of anaphylactic (Casals-Stenzel, 1987a; Lohman and Halonen, 1990), endotoxic (Casals-Stenzel, 1987b; Chang et al., 1987; Lang et al., 1987; Chang et al., 1990; Qi and Jones, 1990; Anderson et al., 1991), and traumatic (Lang et al., 1988; Lang and Dobrescu, 1988; Terashita et al., 1988) shock, myocardial ischemia (Karasawa et al., 1990; Soloviev and Braquet, 1992), and hypoxia (Caplan et al., 1990), thus, implicating an important role of PAF in the pathophysiology of these conditions. The early administration of specific PAF receptor antagonists, therefore, has been suggested for therapeutic benefits in

a variety of life-threatening disorders, such as severe hypotension, cardiac dysfunction, lung injury, bowel ischemia, and multi-organ failure (Casals-Stenzel, 1987b; Casals-Stenzel, 1987a; Terashita et al., 1988; Feuerstein and Siren, 1988; Karasawa et al., 1990; Caplan et al., 1990; Chang et al., 1990; Dhainault et al., 1994).

Recently, Dhainaut et al. (Dhainault et al., 1994) evaluated a clinical use of PAF receptor antagonist (BN 52021) for its safety and efficacy in the treatment of patients with sepsis syndrome in a randomized, double-blind, placebo-controlled, multicenter (21 medical facilities in France) clinical trial. The study provided the promising results that this PAF receptor antagonist was safe and able to reduce the mortality rate among patients with Gram-negative septic shock, dramatically from ~70% to ~30%. The administration of PAF receptor antagonist BN 52021 also significantly improved the outcome of organ failures by 64% in the survivals of Gram-negative sepsis with three or more organ dysfunction at entry. A larger clinical trial was encouraged to confirm the potential therapeutic use of a PAF receptor antagonist in Gram-negative sepsis (Dhainault et al., 1994).

PAF as a Shock Mediator

The role of PAF as a shock mediator in the pathogenesis of sepsis and trauma has been revealed through documentation of a) biosynthesis and release of PAF during shock in septic patients (Lopez Diez et al., 1989;

Graham et al., 1994) and injured (Stahl et al., 1988), endotoxemic (Hsueh et al., 1987; Whittle et al., 1987; Mozes et al., 1991), or septic animals (Inarrea et al., 1985), and b) pathophysiologic studies of PAF by using synthetic PAF (Lagente et al., 1988; Davidson and Drafta, 1992; King et al., 1995) and numerous analogs in experimental animal models for endotoxemia (Chang et al., 1987; Lagente et al., 1988), sepsis (Inarrea et al., 1985), and injury (Terashita et al., 1988; Ikeuchi et al., 1992).

Production of PAF endogenously has been shown to increase PAF under several circumstances including endotoxemia (Mozes et al., 1991), sepsis (Inarrea et al., 1985; Lopez Diez et al., 1989), and injury (Lang and Dobrescu, 1988; Stahl et al., 1988). A temporal increase in the level of PAF in plasma was related to the hypotension of endotoxic shock (Doebber et al., 1985; Mathias and Behrens, 1990). Since PAF has been shown to be responsible for early hemodynamic changes and hemoconcentration (Doebber et al., 1985; Lang et al., 1987; Lang and Dobrescu, 1988; Mathias and Behrens, 1990), the release of PAF in response to endotoxemia and trauma has been connected with the pathogenesis of shock and ischemia.

PAF Production in Trauma, Sepsis, and Endotoxemia

Stahl et al. (Stahl et al., 1988) detected an elevation of PAF in the peritoneal fluid of rats following traumatic shock that was developed by subjecting anesthetized rats to Noble-Collip drum trauma. The developed

traumatic shock was characterized by 80-min survival time and a severe hypotension (less than 50% of normal mean arterial blood pressure). PAF was detected by high performance liquid chromatography (HPLC) and confirmed for its biological activity by a platelet aggregation bioassay using washed rabbit platelets. They found that the activity of PAF in aggregating platelets was increased 4 fold in traumatized rats as compared with controls. A PAF receptor antagonist, CV-6209, specifically blocked the aggregation of washed rabbit platelets induced by aliquots of a chromatographic peak containing PAF activity. The ameliorating effect of PAF receptor antagonist and the formation of PAF in traumatic shock support a role of PAF in the pathogenesis of traumatic shock (Stahl et al., 1988).

Inarrea et al. (Inarrea et al., 1985) measured the formation of PAF in rat peritoneal cells and spleen at different times and in response to various doses of i.p. gram-negative bacteria, *Escherichia coli* (*E. coli*). They showed that a substantial amount of PAF had risen preceding development of blood volume depletion and increased mortality. This development of the depletion of the circulating blood volume was a result of pathological changes of hemodynamics and permeability which also induced the accumulation of peritoneal exudate. They, therefore, suggested that PAF that was formed in peritoneal cells and spleen during sepsis which might be involved in the pathogenesis of the hemodynamic alterations seen during gram-negative sepsis in rats.

It has been shown also that endotoxin was a potent stimulator of PAF production *in vivo* and the temporal increase of PAF level in plasma was related to the hypotension of endotoxic shock (Doebber et al., 1985; Terashita et al., 1988; Terashita et al., 1989). In addition, PAF was suggested to be produced in the lung during endotoxemia and play an important role in systemic and pulmonary hemodynamic changes as well as acute lung vascular injury after endotoxemia (Chang et al., 1987). This is due to the fact that the key enzyme of PAF synthesis, PLA₂, is activated in endotoxic shock and its activation catalyzes the release of PAF from tissues which increases the level of PAF in the circulation (Vallari et al., 1990). Thus, the presence of endotoxin in the circulation can stimulate PAF production (Inarrea et al., 1985; Feuerstein and Siren, 1988; Mozes et al., 1991), as shown in Figure 2.

PAF Level and Activity of PAF-Acetylhydrolase (PAF-AH) in Septic Patients

An accurate quantitative measurement of PAF concentration in blood has been difficult to achieve in the clinical setting due to a rapid degradation of the biologically active PAF to biologically inactive lyso-PAF by PAF-AH enzyme that is present in plasma. Therefore, the elevation of PAF concentration in blood was implicated in septic patients by the increased amount of platelet-associated PAF and the occupancy of PAF receptors (Lopez Diez et al., 1989).

The activity of PAF-AH which is the key enzyme in PAF deactivation has been studied to determine the regulation of PAF level in a number of clinical settings (Kirschbaum, 1991; Graham et al., 1994; Van Lenten et al., 1996). The lack of degradation of PAF in plasma because of decreased PAF-AH activity may contribute to the pathophysiology of sepsis (Graham et al., 1994). Plasma PAF-AH regulates the PAF level in the circulation and prevents PAF from reaching toxic levels (Kirschbaum, 1991; Graham et al., 1994). The PAF-AH enzyme in plasma is highly associated with high density (HDL) and low density (LDL) lipoproteins (Van Lenten et al., 1996). During the acute phase response, a significant depression in PAF-AH activity of HDL was found in patients who were 2-3 d recovering from cardiac surgery (Van Lenten et al., 1996). In addition, an increase in the plasma half-life of PAF as a result of decreased PAF-AH activity was reported in the severely ill patients with clinical sepsis (Graham et al., 1994).

The activity of PAF-AH in urine, however, was elevated in patients with diabetic nephropathy, glomerulonephritis, hypertension with renal damages, polycystic kidney disease, and obstructive uropathy (Kirschbaum, 1991). This elevation of urine PAF-AH activity was reportedly essential to signal the response by the kidney to PAF stimulation. In this case, the urinary PAF-AH activity was suggested to reflect the stimulation level of PAF-dependent responses and was suggested to provide a measure of PAF involvement in the pathophysiology of renal diseases (Kirschbaum, 1991).

Relationship Between PAF and Other Shock Mediators

The intravenous infusion of PAF upregulates the release of glucagon, norepinephrine, and epinephrine, and results in hemodynamic and glucose metabolic alterations similar to those seen during endotoxemia and septic shock (Lang et al., 1988; Bonavida et al., 1990). It has also been suggested that the hemodynamic and metabolic effects of PAF are mediated via an elevation of catecholamine (epinephrine and norepinephrine) levels in plasma (Lang et al., 1987; Lang et al., 1988; Qi and Jones, 1990). Lang et al. (Lang et al., 1987) showed that an endotoxemia-induced elevation of plasma catecholamine levels was lessened (30-70%) by anaphylactic PAF infusion. In another study (Lang et al., 1988), they showed that PAF infusion (bolus or continuous) induced dose-related increases in plasma glucagon and catecholamine levels. Adrenergic blockade was also found to prevent both the PAF-induced hyperglycemia and glycogenolysis (Lang et al., 1988).

PAF has been shown to regulate the production of cytokines such as tumor necrosis factor (TNF) (Bonavida et al., 1990) and interleukin-1 (IL-1) (Pignol et al., 1990b) by monocytes (Braquet et al., 1989; Mencia-Huerta and Braquet, 1989). PAF potentiates the production of IL-1 and TNF induced by endotoxin through the production of lipxygenase metabolites, such as leukotriene B₄ and C₄, in macrophages (Braquet et al., 1989; Mencia-Huerta and Braquet, 1989; Bonavida et al., 1990; Pignol et al., 1990a; Pignol et al., 1990b). In addition, an intimate relationship between PAF and the eicosanoids

was studied by Moore et al. (Moore et al., 1991) where they suggested that activation of PAF release and eicosanoid synthesis might occur simultaneously. An amplification role of PAF in eicosanoid synthesis during endotoxemia was also implicated (Moore et al., 1991). Therefore, PAF may contribute to the inflammatory process by mediating the biological activity of the peripheral blood monocyte (Braquet et al., 1989; Mencia-Huerta and Braquet, 1989; Bonavida et al., 1990; Pignol et al., 1990a; Pignol et al., 1990b; Angeras et al., 1991). Furthermore, TNF and IL-1 are in turn capable of stimulating PAF production as previously shown by Evan et al. (Evans et al., 1991) and others (Braquet et al., 1989; Mencia-Huerta and Braquet, 1989).

SHOCK

Shock Syndrome

Shock is a symptom of insufficient circulating blood volume that leads to inadequate tissue perfusion in various disease conditions (von Euler, 1962; Clowes, Jr., 1988; Canadian Multiple Organ Failure Study Group et al., 1991; Smith et al., 1991; Geller, 1993; Dhainault et al., 1994; Kinney and Tucker, 1994). The major causes of shock are trauma and infection which result in a decrease in blood volume, failure of the heart, and an increase in peripheral vasodilation (von Euler, 1962; Canadian Multiple Organ Failure Study Group et al., 1991; Smith et al., 1991; Parrillo, 1993; Dhainault et al., 1994). Due to any cause, shock represents circulatory failure with all of its ramifications.

Due to the lack of understanding of the complexities of the pathophysiologic mechanisms of shock, the mortality rate of shock is still unacceptably high, especially in septic patients (patients with a systemic inflammatory response to an overwhelming infection) (Canadian Multiple Organ Failure Study Group et al., 1991; Smith et al., 1991; Dhainault et al., 1994). Shock has a mortality rate in the range of 40-50% (Canadian Multiple Organ Failure Study Group et al., 1991; Smith et al., 1991; Parrillo, 1993; Dhainault et al., 1994) and as high as 70% in patients with Gram-negative sepsis (Dhainault et al., 1994). Thus, sepsis has been presented as an important factor in the high mortality rate of shock (Fink and Heard, 1990).

History and Definition of Shock

Throughout history, recognition of the clinical signs and symptoms of acute circulatory failure following severe trauma and/or infection in man had been made through observations by those who dressed the wounds and cared for the sick and injured (Clowes, Jr., 1988; Baue, 1993; Geller, 1993; Kinney and Tucker, 1994). An introduction of non-invasive "Riva-Rocci" apparatus for estimating the blood pressure in man by a U.S. neurosurgeon, Dr. Harvey Cushing (1869-1939), had enabled the hemodynamic studies of conditions affecting the blood pressure such as after trauma, and during and after surgery (Clowes, Jr., 1988). Subsequently, it was revealed that circulation failure

following trauma and/or infection resulted in hypotension, weak thready pulse, and anuria (Clowes, Jr., 1988; Geller, 1993; Kinney and Tucker, 1994).

Historical Origin of "Shock"

It has been disclosed that the use of the word "shock" to indicate the clinical condition of the circulation failure, was introduced in error back in the 18th century (Clowes, Jr., 1988; Baue, 1993; Geller, 1993). An unknown translator of Le Dran's treatise on gunshot wounds used the word "shock" to translate the French "choc" which was used in the treatise to state an impact, a jolt, or a sudden deceleration of the severely wounded person (Clowes, Jr., 1988; Baue, 1993). However the use of the word "shock" to denote a failure of blood circulation after trauma has continued to the present day.

Concept and Definition of Shock

Until the early 20th century after intense study and observation during World War I, American and English members of the committee for medical research came up with the characteristics of "clinical shock" (Baue, 1993). The committee characterized "clinical shock" as a) a reduction of the circulating blood volume and the rate of capillary blood flow, and b) a concentrated blood in the capillary bed that was a result of capillary stagnation (Clowes, Jr., 1988). During the same period of time, a U.S. physiologist Dr. Walter B. Cannon (1871-1945), one of the committee members, carefully

emphasized in his 1923 classic monograph *Traumatic Shock* that hemoconcentration was found in stagnant capillary blood and not in circulating venous blood (Clowes, Jr., 1988; Geller, 1993).

As early as in the middle of World War II, the concepts of hypovolemia, volume replacement, and blood transfusion were understood and accepted (Baue, 1993). Resuscitation with blood or plasma became a primary treatment for the injured. Thus, the morbidity and mortality of traumatic shock was improved significantly by resuscitation (von Euler, 1962; Clowes, Jr., 1988; Baue, 1993). Though shock is no longer the limiting morbidity following injury, it has still remained a problem in many hospitalized and injured patients when it persists or is not treated adequately (Baue, 1993). Especially, when infection becomes a factor, shock (occasionally referred to as irreversible shock) can cause multiple organ failure even in resuscitated patients (von Euler, 1962; Baue, 1993).

By contemporary clinical definition, shock is an abnormal physiological state characterized by a disproportion between the circulating blood volume and the size of the vascular bed, resulting in circulatory failure, hypotension, and tissue hypoxia (von Euler, 1962; Clowes, Jr., 1988; Baue, 1993; Geller, 1993).

Physiology of Shock

Shock is a product of abnormalities to one of the four physiologic components (preload, afterload, contractility, and heart rate) that determine cardiac output or blood flow in the circulatory system.

Classification of Shock

The etiology of shock is classified into four general categories (Baue, 1993): hypovolemic, cardiogenic, septic, and neurogenic shock. Hypovolemic shock is represented by inadequate blood volume for ventricular filling (low preload). Cardiogenic shock is a result of inadequate cardiac output due to failure of inotropic contractility function in myocardium (pump failure). Septic shock is characterized by decreased peripheral vascular resistance (low afterload) following the inability of the circulation to meet an increase in blood flow demand which is mediated by various mediators during infection. Finally, neurogenic shock is the nervous system's inability to regulate the peripheral circulation (irregular contractility and heart rate) in order to maintain normal vascular resistance and blood pressure.

Shock as an Early Stage in the Physiologic Response to Trauma and Sepsis

Shock is considered by Clowes (Clowes, Jr. et al., 1983; Clowes, Jr. et al., 1985; Clowes, Jr., 1988) to be the early stage in the physiological response to trauma and sepsis. During shock or ebb phase, the loss of

circulating fluid is due to hemorrhage or increased permeability of capillaries. By lack of perfusion or by toxic agents from either patho-microorganisms (mainly bacteria) or endogenous sources, whole-body and tissue metabolism can be severely impaired. The countershock treatment by immediate resuscitation or spontaneous compensatory responses are directed toward the preservation of vital physiological and biochemical processes.

The ebb phase is followed by the acute stage, convalescent phase, and rehabilitation stage (Clowes, Jr., 1988). The acute or flow phase, then, initiates the mobilization of body resources (fat and protein) to assure adequate energy production and sufficient metabolic requirements for defense and healing mechanisms. The complete wound healing, elimination of infectious and toxic agents, and restoration of lean tissue protein occur in the convalescent phase. The patient can resume normal activities following physical and psychological adjustment in the rehabilitation stage, during which the muscular strength and depleted body fat are restored with sufficient protein and caloric intake. The failure in providing optimal responses to the imposed stresses of trauma or sepsis can cause a reversal to the earlier phase. The perpetuating response by revolving between the acute and shock stages, can lead to depleting and wasting of the body resources. Thus, the overwhelming infection (sepsis) and chronic shock eventually result in multiple organ-system failure and death (Clowes, Jr. et al., 1985; Clowes, Jr., 1988).

Pathophysiologic Mechanisms of Shock

Shock alters the homeostasis of every cell, tissue, organ, and system through a) the disproportion of fluid and electrolytes between intracellular, interstitial, and extracellular compartments (von Euler, 1962; Illner and Shires, 1981; Chaudry, 1985; Goodman, 1987; Hasselgren et al., 1988; Jennings et al., 1990; Murphy et al., 1991; Karlstad and Sayeed, 1992; Baue, 1993; Shoemaker et al., 1996), and b) the elevation of hormone and mediator productions and releases to the circulation as a compensatory response to the insults that induce shock and/or to shock itself (Chaudry, 1985; Filkins, 1985; Clowes, Jr., 1988; Lang et al., 1988; Hasselgren et al., 1990; Cerami, 1992; Baue, 1993). Thus, cellular metabolism is decreased during shock due to the lack of tissue perfusion which leads to a decreased supply of oxygen, nutrients, water, electrolytes, and hormones to cells (Harkema et al., 1990; Baue, 1993). In addition, it has also been suggested that cellular metabolism and function can be impaired due to a direct contact between cells and the effectors such as bacterial endotoxin, stress hormones, and shock mediators, prior to the occurrence of severe hypotension (Illner and Shires, 1981; Filkins, 1985). The adverse changes on cellular functions in early sepsis may lead to organ failure if it persists and is not reversed (Chaudry, 1985; Harkema et al., 1990; Jennings et al., 1990).

The manifestations of septic shock include those related to the systemic inflammatory response (tachycardia, tachypnea, thrombocytopenia,

leukocytosis, and alterations in body temperature), metabolic alterations (hyperglycemia, lactic acidosis, and negative nitrogen balance), and multiple organ-system dysfunction (cardiovascular, respiratory, renal, hepatic and hematologic abnormalities) (Clowes, Jr., 1988; Harkema et al., 1990; Jennings et al., 1990; Canadian Multiple Organ Failure Study Group et al., 1991; Smith et al., 1991; Fourrier et al., 1992; Baue, 1993; Parrillo, 1993).

Several experimental animal models have been developed to study the pathophysiologic mechanism of shock (Fink and Heard, 1990). One of the shock models that involves iv injection(s) of endotoxin (10 to 20 mg/kg) in the rat has been shown to produce typical signs of severe endotoxic shock such as hemorrhagic lesions of the bowel, alterations of intestinal mucosal permeability, acute lung injury, hyperlacticacidemia, hyperglycemia, and alterations of skeletal muscle system A amino acid transport (Karlstad and Sayeed, 1986; Hsueh et al., 1987; Lang et al., 1987; Chang et al., 1990). Other models including cecal-ligation and perforation (CLP), administration of viable bacteria, implantation of fecal pellet, and infusion of a single or combination of shock mediators (e.g. TNF, IL-1, or PAF) and stress hormones (corticosterone, glucagon, or catecholamine) have also been shown to cause similar changes to those seen during septic shock (Lang et al., 1988; Flores et al., 1989; Fong et al., 1989; Harkema et al., 1990; Hasselgren et al., 1990; Hall-Angeras et al., 1991; Yoshida et al., 1991; Boruff and Karlstad, 1993; Sakurai et al., 1994).

Metabolic Effects of Shock

General characterization of the host response to injury and infection is readily evident from the many reports of hypermetabolism and hypercatabolism over the past 50 years (Cuthbertson, 1930; Clowes, Jr., 1988; Long and Lowry, 1990). In most cases, the responses to injury, including elevated resting energy expenditure, disturbed carbohydrate metabolism, and increased protein wasting, exhibit variability that is related to the intensity of the insult (Cuthbertson, 1930; Cerra, 1987; Clowes, Jr., 1988; Long and Lowry, 1990).

It has been shown (Lang et al., 1987) that during endotoxemia, plasma levels of glucose and lactate, and the rate of glucose appearance (possibly from glycogenolysis) was increased. Thus, rats that were infused intravenously with *E. coli* endotoxin at a non-lethal dose of 1 ng/kg, developed hyperglycemia (Lang et al., 1987). However, Wolfe et al. (Wolfe et al., 1977) found that at the lethal dose of 1 mg/kg *E. coli* endotoxin, dogs developed hypoglycemia. In addition, they found that lactate became an important precursor for gluconeogenesis and an important metabolic substrate after endotoxemia. They suggested that the observed changes in carbohydrate metabolism after endotoxemia were due to an increase in peripheral uptake of glucose and a failure of the liver to maintain an increased rate of glucose appearance in the circulation (Wolfe et al., 1977).

Whole-body and skeletal muscle protein synthesis have been shown to be reduced following burn, sepsis, and endotoxic shock (Karlstad et al., 1988;

Hasselgren et al., 1990). Active transport of amino acids across the cellular membrane into skeletal muscle has also been shown to be inhibited during endotoxemia (Karlstad and Sayeed, 1985; Hasselgren et al., 1986; Karlstad and Sayeed, 1986; Hasselgren et al., 1988). Thus, it is possible that the reduction of amino acid transport during shock can limit the availability of amino acids for cellular metabolism and contributes to the loss of protein by limiting protein synthesis in skeletal muscle, as previously suggested (Karlstad and Sayeed, 1986; Hasselgren et al., 1988; Biolo et al., 1992).

Involvement of Stress Hormones and Shock Mediators

The pathophysiology of shock is mediated by a number of circulating factors including stress hormones (corticosterone, glucagon, and catecholamine), cytokines (TNF and IL-1), eicosanoids (thromboxane, prostaglandin, and leukotriene), and phospholipid mediators such as PAF (Clowes, Jr. et al., 1985; Filkins, 1985; Billiau and Vandekerckhove, 1991; Cerami, 1992; Baue, 1993; Parrillo, 1993). Levels of these mediators are significantly increased in the circulation during injury, shock, and systemic inflammation (Angeras et al., 1991; Byerley et al., 1992). These hormones and mediators play an important regulatory role in shock by contributing to the inflammatory and metabolic responses. Administration of these hormones to normal subjects results in metabolic alterations commonly observed in stressed and injured patients, such as hypermetabolism, negative nitrogen

balance, hyperglycemia, and peripheral insulin resistance (Bessey et al., 1984).

Recent observations suggest a metabolic regulatory role for other shock mediators (Flores et al., 1989; Hasselgren et al., 1990; Angeras et al., 1991; Byerley et al., 1992; Cerami, 1992). It has been shown that the metabolic alterations observed in the septic rat are associated with a complex cascade of several cytokines including TNF, IL-1, and interferon (Filkins, 1985; Mozes et al., 1991; Byerley et al., 1992; Cerami, 1992). The increase in TNF- α mRNA and serum peptide levels correlated with the early fall in serum glucose and rise in plasma glucagon (Byerley et al., 1992). TNF has been shown to be markedly increased in non-survival sepsis (Mozes et al., 1991).

Many of these shock mediators, such as IL-1, TNF, and PAF, have been shown to have both local and systemic effects (Feuerstein and Siren, 1988; Hummel, III et al., 1988; Lang et al., 1988; Terashita et al., 1988; Terashita et al., 1989; Tanaka et al., 1991). TNF and IL-1 are classified as cytokines, which are protein compounds of approximately 17,000 daltons. These cytokines are produced from the activated leukocytes, primarily macrophages, as part of the cellular immune response following exposure to endotoxin (Hummel, III et al., 1988; Byerley et al., 1992). These compounds are capable of activating other immune cells. The commonly known functions of IL-1 are to induce fever and regulate immune responses (Byerley et al., 1992).

The infusion of these cytokine mediators to rats reproduced many metabolic alterations found during sepsis and injury (Hummel, III et al., 1988; Sakurai et al., 1993; Sakurai et al., 1994; Ling et al., 1995). TNF has been shown to induce an increased utilization of carbohydrate (Sakurai et al., 1993), elevated oxidation of amino acid (Sakurai et al., 1994), and impaired protein synthesis (Sakurai et al., 1994). TNF was further suggested to cause an increase in net protein breakdown even though whole-body protein breakdown was not affected (Sakurai et al., 1994). In addition, IL-1 was reported (Ling et al., 1995) to have a greater effect on glucose metabolism in fasted rats, rather than TNF.

The interaction between different types of mediators may also be responsible for the manifestation of metabolic responses to severe injury and sepsis (Hasselgren et al., 1988). This is because recent observations have suggested that injury-induced catabolism of proteins and amino acids is associated with secretion of multiple stress hormones, production and release of lipid mediators that are products of the cyclo-oxygenase pathway, and elevation of plasma level of mediators from activated reticuloendothelial cells (Angeras et al., 1991; Cerami, 1992).

The alterations in carbohydrate metabolism during shock that were induced by endotoxemia or shock mediator (TNF or PAF), were suggested to be controlled by increased levels of glucose-counterregulatory hormones, glucagon and catecholamine (Lang et al., 1987; Lang et al., 1988; Sakurai et

al., 1993). The elevated concentration of catecholamines were suggested to represent the compensatory mechanism for stabilization of the cardiovascular system in response to hypotension (Lang et al., 1987; Lang et al., 1988). Thus, the hyperglucagonemia can be a result of catecholamine-enhanced stimulation of pancreatic alpha-cells (Lang et al., 1988).

Protein and Amino Acid Metabolism During Shock and Sepsis

In critical illness, a variety of metabolic abnormalities take place such as hypermetabolism, accelerated skeletal muscle proteolysis, increased amino acid flux, loss of skeletal muscle protein mass, and negative nitrogen balance (Cuthbertson, 1930; Clowes, Jr. et al., 1985; Cerra, 1987; Clowes, Jr., 1988). Whole-body protein catabolism has been shown to precede protein wasting of lean tissue such as skeletal muscle in sepsis (Hasselgren et al., 1990), burn (DeMichele et al., 1988; Karlstad et al., 1988), trauma (Shaw and Wolfe, 1989), and endotoxemia (Ling et al., 1995). In the fasted septic patient, amino acids were reportedly mobilized by degradation of muscle protein at rates which are three fold or more higher than those observed in normal men and women in a post-absorptive state (Jeevanandam et al., 1989; Jeevanandam et al., 1991).

In experimentally burned rats, the degradation of protein and first pass extraction of dietary leucine by the splanchnic bed were elevated during the hypermetabolic state (Karlstad et al., 1988). Though the supply of amino acids

to liver for gluconeogenesis and protein synthesis (of acute phase protein) are essential to survival, the prolonged mobilization of amino acid from skeletal muscle protein could lead to major loss of muscle mass and become life threatening (Clowes, Jr., 1988; Clowes, Jr. et al., 1985; Cerra, 1987).

Derangements of protein metabolism in sepsis and injury have been shown to lead to changes in the pattern of free amino acids in plasma and muscle (Roth et al., 1985; Jeevanandam et al., 1989; Petersson et al., 1992). Aminoaciduria and marked depletion of nonessential amino acids were observed in plasma of trauma victims (Jeevanandam et al., 1989). In addition, changes in the pattern of free amino acids and decreases in total free amino acids and glutamine levels, in skeletal muscle were observed on the third day after abdominal surgery (Petersson et al., 1992).

The demand for branched-chain amino acids (BCAA) in severe injury has been suggested to be increased relative to the demand for the other amino acids (Birkhahn et al., 1986). This suggestion was later supported by the study of Yoshida et al. (Yoshida et al., 1991) which showed an increase in the conversion of leucine to glutamine during sepsis. High utilization of BCAAs by skeletal muscle was also reported in trauma patients (Jeevanandam et al., 1989). However, a supplement of branched-chain amino acids did not improve nitrogen balance or reduce protein breakdown in postoperative patients, where a decrease in the plasma level of albumin was reported (Sandstedt et al., 1992).

Alterations in the amino acid pattern in liver were also expected in the protein catabolic stage during severe injury (Jepson et al., 1986; Hasselgren et al., 1988). A slight decrease in BCAA levels in liver were suggested to be contributed by plasma in early sepsis where the pattern of free amino acids in liver was not disturbed (Roth et al., 1985). Although liver amino acid pattern reflects the amino acid pattern in plasma, the pattern of free amino acids in liver was not disturbed in early sepsis. However, there was an increase in the flux of gluconeogenic amino acids because of a lack of accumulation of the free gluconeogenic amino acids in liver (Roth et al., 1985).

Hepatic protein synthesis was shown to be increase during endotoxemia in order to compensate for the significant reduction of protein synthesis rate in skeletal muscle, so that the sum total in the two tissues was maintained in rats (Jepson et al., 1986). These rats were fasted after daily endotoxin injection for over 3-d period, and their liver protein mass was increase instead of decrease as it was in the fasted control rats. Therefore, protein and amino acid metabolism in liver has been indicated to be altered during injury, sepsis, and shock (Roth et al., 1985; Jepson et al., 1986; Hasselgren et al., 1988).

III. RATIONALE AND SPECIFIC AIMS

RATIONALE

Metabolic complications have always been a serious problem in recovery from severe injury as patients undergo shock. Protein catabolism is a hallmark feature of injury. Several currently proposed mechanisms for protein catabolism during shock focus on released cytokines and other mediators. While the previous studies of cytokines (TNF and IL-1) have failed to demonstrate their roles in protein metabolism, PAF remains of interest. This dissertation considers the role of PAF in protein catabolism, including the effect of PAF on amino acid transport and protein metabolism during endotoxic shock in the rat. The mechanism of PAF in attenuating of amino acid transport during endotoxic shock was delineated by using a pharmacologically available PAF antagonist, WEB 2086, and a commercially available form of PAF, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. Amino acid transport via system A of skeletal muscle was assessed by determining *in vitro* cellular uptake of a radioisotope-labeled amino acid analog, 1-¹⁴C- α -aminoisobutyric acid (non-metabolizable). The *in vivo* effect of PAF on whole-body and tissue protein metabolism was explored by using a 4-h continuous intravenous infusion of L-(1-¹⁴C)-leucine to determine whole-body and tissue leucine kinetics. In addition, plasma and tissue amino acid profiles, muscle RNA activity for protein synthesis, and muscle myofibrillar protein contents were

also determined. Therapeutic strategies for treating metabolic complications related to alterations of amino acid and protein metabolism due to endotoxic shock can benefit from the findings of this doctoral dissertation research.

SPECIFIC AIMS

In addition to establishing an animal model to study the effect of PAF on amino acid transport of skeletal muscle, and protein metabolism of whole-body, liver, and skeletal muscle in the rat, the specific aims of this dissertation are divided into two parts as follows.

Part 1: The Effect of PAF on Amino Acid Transport

The main objective of this part is to investigate the effect of PAF on amino acid transport via system A of skeletal muscle during endotoxic shock in rats. Specific aims are as follows:

1. to determine if a prophylactic i.v. administration of a PAF antagonist, WEB 2086, would ameliorate endotoxin-induced attenuation of system A amino acid transport in rat skeletal muscle.
2. to determine if an i.v. administration of PAF at a sublethal dose would attenuate system A amino acid transport in skeletal muscle.

3. to determine if addition of PAF to incubation medium would affect system A amino acid transport in rat skeletal muscle *in vitro*.
4. to determine if addition of plasma from PAF-treated rats to incubation medium would affect system A amino acid transport in rat skeletal muscle *in vitro*.

Part 2: The Effect of PAF on Protein Metabolism

The main objective of this part is to explore the effect of PAF on whole-body and tissue leucine kinetics, plasma and tissue amino acid profiles, muscle RNA activity for protein synthesis, and muscle myofibrillar protein contents in rats. Specific aims are as follows:

1. to determine if the whole-body leucine kinetics would be affected by iv PAF infusion.
2. to determine if tissue fractional protein synthesis rates would be affected in liver and skeletal muscle of PAF-treated rats.
3. to determine if plasma and tissue amino acid profiles would be affected in PAF-treated rats.
4. to determine if the efficiency of protein synthesis or the RNA activity would be affected in skeletal muscle of PAF-treated rats.
5. to determine if the relative abundance of myofibrillar proteins, actin and myosin, would be affected in skeletal muscle of PAF-treated rats.

IV. MATERIALS AND METHODS

The general animal protocols for Part 1 and 2 are as follows. Male Sprague-Dawley rats weighing 51-75 g and 250-300 g were purchased from Taconic Farms (Germantown, NY) to be used respectively in Parts 1 and 2 of the experimental studies of this dissertation. Rats were housed in a temperature (23-24°C) and light (12 h light/12 h dark cycle) controlled animal facility. Rodent chow (Purina, no. 5001) and water *ad libitum* were provided during a 3-5 day acclimation period. Animals were maintained in accordance with the recommendations of the National Institutes of Health Guide for the Care, Use of Laboratory Animals, and the experiments were approved by the Institutional Animal Care and Use Committee of the University of Tennessee Medical Center.

PART 1: THE EFFECT OF PAF ON AMINO ACID TRANSPORT

Animal Protocols

Following the acclimation period, rats were used for the experimental study of Part 1 of this dissertation if body weight was between 80 and 100 g. Soleus and extensor digitorum longus (EDL) muscles weighed 40-50 mg. Rats were fasted overnight for 16 h prior to the experimental procedures.

Experimental Procedures

To determine if PAF is a mediator of attenuated amino acid transport during endotoxin shock, rats were given a bolus intravenous (i.v.) injection (0.2 mL/100 g) of 10 mg/kg *Salmonella enteritidis* endotoxin (Lipopolysaccharide B *S. enteritidis*, Difco Laboratories, Detroit, MI) or 30 mg/kg WEB 2086 immediately prior to endotoxin injection (WEB 2086/endotoxin). Control rats were treated similarly but received an equal volume of vehicle that used to solubilize endotoxin, PAF and WEB 2086. The vehicle was composed of 0.9 % saline, 0.25 % bovine serum albumin and 10 U/mL heparin sodium. The injections were given via the sublingual vein under metofane (Methoxyflurane; Pitman-Moore, Inc., Mundelein, IL) anesthesia. Rats were returned to their cages and allowed to recover from anesthesia. Rats were killed 5 h later by decapitation. WEB 2086 (Kilobetriebe or 3-(4-(2-Chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]-diazepin-2-yl)-1-(4-morpholinyl)-1-propanone, provided with courtesy by Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT) was solubilized in the vehicle to make a 30 mg/mL WEB 2086 solution. Solutions were freshly prepared prior to i.v. injections.

Rats were given a bolus i.v. injection of 4 µg/kg PAF and killed 1 hr later to determine the effect of PAF on cellular AIB transport. Control rats were treated similarly but received an equal volume of the vehicle (0.2 mL/100 g). PAF (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; Sigma Chemical Co., St. Louis, MO) in chloroform was evaporated to dryness and then resuspended

in the vehicle to make a 100 µg/mL PAF stock solution. The PAF stock solution was diluted to 2 µg/mL with the vehicle prior to i.v. injection. To determine the direct effect of PAF on cellular AIB uptake, transport was measured in paired muscles from control and PAF-treated rats during a 40 min incubation period in the absence (basal media) or presence of PAF (1 µg/mL PAF). In a separate series of experiments, cellular AIB uptake was also assessed in muscles of control rats incubated for 40 min in basal media or media containing PAF at 0.1, 1.0 and 10 µg/mL. PAF in chloroform was evaporated to dryness and then resuspended in Krebs-Ringer bicarbonate buffer (KRB) media to a concentration of 0.1, 1.0 or 10 µg/mL.

To study the effect of plasma on cellular AIB uptake *in vitro*, muscles from control rats were incubated in media containing plasma from PAF- or endotoxin-treated rats and control rats. Rats were anesthetized with metofane and blood was drawn from the abdominal aorta 1 h after the i.v. injection of either 4 µg/kg PAF, 10 mg/kg endotoxin or vehicle. Plasma from either PAF-treated, endotoxin-treated or control rats was obtained following centrifugation (2100 x g, 10 min at 4° C) and 0.2 mL was added to 2.8 mL KRB media.

Determination of Amino Acid Transport in Soleus and EDL

Rats were killed by decapitation and soleus and EDL muscles were removed from both hindlimbs with care to avoid any damage to the muscles. The tendons were attached to adjustable stainless holders to maintain isolated

muscles at resting length to facilitate perfusion of nutrients and oxygen to the muscle core as previously described (Karlstad and Sayeed, 1985; Karlstad and Sayeed, 1986). Soleus and EDL muscles were incubated separately in 25 mL Erlenmeyer flasks containing 3 mL KRB media (pH 7.4) at 37 °C under a 95%O₂-5%CO₂ atmosphere in a Dubnoff metabolic shaker incubator for 40, 60 or 80 min. KRB media contained 119 mM NaCl, 4.7 mM KCl, 1.1 mM KH₂PO₄, 1.1 mM MgSO₄, 2.5 mM CaCl₂, 24 mM NaHCO₃, 5.6 mM glucose, 0.25% bovine serum albumin, and 0.25 µCi 1-¹⁴C-α-aminoisobutyric acid (AIB; Amersham, Arlington Heights, IL) to assess cellular AIB uptake. Muscles were removed from the incubation media at the end of the incubation period and lightly blotted on Whatman (no. 42) filter paper to remove adherent surface radioactivity. The wet weight was obtained and the tissues dried in a vacuum oven for 16 h at 100°C to determine their dry weight. Muscles were rehydrated with 150 µL deionized water at 40°C in a water bath for 2 h and then digested in 1 mL TS-2 tissue solubilizer (Amersham) for 10 h at 40°C. We added 10 mL of 3a20 scintillation fluor (RPI, Mount Prospect, IL) to the dissolved muscles and 20 mL of Monofluor (National Diagnostics, Manville, NJ) to 100 µL aliquots of media. The radioactivity of dissolved muscles and media was counted in a Packard Tri-Carb 1600CA liquid scintillation analyzer (Packard Instrument Company, Downers Grove, IL).

Determination of Extracellular Space and Total Tissue Water

Total tissue water (mL/g) was calculated from the wet weight-to-dry weight ratio. The concentration of inulin in media was assumed to be equal to the concentration in muscle interstitial fluid. Extracellular (inulin) space (mL/g) was calculated by dividing total muscle inulin content (dpm/g muscle) by medium inulin concentration (dpm/mL). Intracellular volume (mL/g) was calculated as the difference between total tissue water and extracellular space (mL/g). Extracellular (inulin) space was measured in soleus and EDL at 60 min of incubation in separate experiments as previously described (Karlstad and Sayeed, 1985; Karlstad and Sayeed, 1986). Since there were no significant differences in extracellular space measurements at 60 min in soleus and EDL, the values used for extracellular space at 40 and 80 min of incubation were obtained from the previous studies of this laboratory (Karlstad and Sayeed, 1985; Karlstad and Sayeed, 1986; Boruff and Karlstad, 1993).

Determination of Cellular AIB Uptake

Total muscle radioactivity (dpm) was converted into AIB contents (nmol) by dividing it by medium AIB specific activity (dpm/nmol AIB). The intracellular concentration of AIB (nmol/mL) was calculated by dividing the difference between the amount present in the total muscle and that in the extracellular space by the intracellular volume. Cellular AIB uptake (nmol/g dry tissue) was

calculated as the amount present in the intracellular fluid divided by the dry weight of the muscle. The distribution ratio of AIB was equal to the intracellular [AIB]/extracellular [AIB].

Statistical Analysis for Part 1

Data from the Part 1 experimental study of this dissertation were analyzed using a one-way analysis of variance and differences between means were determined using the Ryan-Einot-Gabriel Welsch multiple range test. A paired Student's t-test was used for paired experiments. Data are expressed as means $\pm$ SE. $P \leq 0.05$ was considered significant.

PART 2: THE EFFECT OF PAF ON PROTEIN METABOLISM

Surgical Procedures

Rats weighing 250-300 g were fasted overnight for 16 h prior to surgery. Rats were anesthetized with isoflurane and a silastic catheter (0.025 in. ID, 0.047 in. OD) was aseptically placed in the jugular vein. The catheter was tunneled subcutaneously and exteriorized in the midscapular region, passed through a stainless steel spring and attached to a flow-through infusion swivel (Instech, Horsham, PA) to allow free movement by the rat. The rats were returned to their cages and allowed to recover from surgery until the next day. The jugular vein catheter was maintained patent by a heparinized saline (20 U/mL) infusion (0.5 mL/h) during the recovery period.

PAF Infusion and Leucine Kinetics

Rats were randomized to control ($n = 8$) and PAF-treated ($n = 8$) groups and fasted overnight for 16 h prior to being placed in metabolic chambers for the determination of whole-body leucine kinetics. PAF (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, Sigma Chemical Co., St. Louis, MO) in chloroform was evaporated to dryness under an airstream and resuspended in vehicle to a concentration of 1 $\mu\text{g/mL}$. The vehicle was composed of 0.9% saline, 0.25% bovine serum albumin and 10 U/mL heparin sodium. PAF-treated rats were given a priming iv bolus infusion of 2 $\mu\text{g/kg}$ PAF, followed 1 h later by a continuous iv infusion of 2 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ PAF for 4 h at a rate of 2.1 mL/h using an infusion pump (Harvard Apparatus, South Natick, MA). Control rats were treated similarly but were given the vehicle. In a preliminary group of rats ($n = 4$), we found that an iv bolus injection of 2 $\mu\text{g/kg}$ PAF produced an immediate reduction in mean arterial blood pressure to (MABP) 60 mmHg. Within 60 min after the bolus injection, MABP was maintained at 75 mmHg for another 60 min and returned to control level by 60 min. However, a continuous iv infusion of 2 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ PAF 1 h following the bolus injection was able and necessary to sustain the MABP reduction at 75 mmHg during the infusion period as previously reported (Lang et al., 1988). The hemodynamic effect of PAF was completely abolished by a prophylactic iv injection of 30 mg/kg WEB 2086 (PAF antagonist). There were no deaths in the control or PAF-treated rats during the experimental period.

L-(1- ^{14}C)-Leucine (50 mCi/mmol, ICN Radiochemicals, Irvine, CA) was added to the PAF solution and the vehicle. Since the plasma leucine pool has been shown to be at near isotopic equilibrium by 120 min of isotope infusion in rats (Laurent et al., 1984) and because we did not actually measure the rise to isotopic steady-state of plasma leucine specific activity in this study, we infused L-(1- ^{14}C)-leucine at a rate of 1.6 $\mu\text{Ci/h}$ for 4 h to determine leucine kinetics as previously reported (DeMichele et al., 1988; Karlstad et al., 1988). Consumption of O_2 and production of CO_2 were determined at 30-min intervals by measuring the airflow rate through the metabolic chambers and by analyzing O_2 and CO_2 gas concentrations in room air and in a mixture of room air and expired breath (room air/expired breath mixture) entering and exiting the metabolic chambers, respectively. Gas concentrations were measured by using S-3A oxygen and CD-3A carbon dioxide analyzers (Ametek Thermox Instrument, Pittsburgh, PA) and corrected for standard conditions of pressure and temperature (STPD). To determine the rate of $^{14}\text{CO}_2$ appearance in the breath, the room air/expired breath mixture was bubbled into 5 mL 40 mM hyamine hydroxide (National Diagnostics, Manville, NJ) in ethanol containing 0.004% phenolphthalein (Mallickrodt, Paris, KY).

Tissue Collection and Analytical Procedures

Rats were rapidly anesthetized with halothane near the end of the isotope infusion period, a midline laparotomy was performed, and blood was

drawn from the abdominal aorta into chilled heparinized (100 U) syringes.

Plasma was separated by centrifugation ($2100 \times g$, 10 min at 4°C), and stored at -70°C . Whole liver and skeletal muscles with different compositions of three fiber types (soleus and extensor digitorum longus (EDL), and a section of rectus abdominus muscles that are composed primarily of red, white, and a combination of red and white, respectively) were rapidly removed, weighed and frozen immediately in liquid nitrogen and stored at -70°C . Plasma protein was precipitated by 10% sulfosalicylic acid (SSA). Muscle and liver were homogenized in 10% SSA to precipitate tissue protein. The precipitated protein pellets were solubilized in 2 N NaOH and protein contents of plasma and tissues were determined by the biuret reaction. The specific activity of leucine (dpm/ μmol) in the acid-soluble fractions of plasma, muscle, and liver were determined after α -keto-isocaproic acid (KIC) was removed by cationic ion-exchange chromatography using Poly Prep columns filled with AG 50W-X8 (H^{+}) resins (Bio-Rad Laboratories, Richmond, CA). To determine the specific activity of leucine (dpm/ μmol) in the protein-bound fractions of muscle and liver, the tissue protein was corrected for its leucine content using the assumption that the leucine content was 8% of total tissue protein and the molar ratio of leucine to total amino acid in protein was 0.053 (Waterlow et al., 1978). Monofluor (National Diagnostics, Manville, NJ) was added to hyamine hydroxide saturated with expired-breath CO_2 and KIC-free acid-soluble fractions of plasma and tissues, and EcoLite (+) (ICN, Costa Mesa, CA) was

added to protein-bound fractions of tissues for counting of ^{14}C -radioactivity (dpm/mL). Radioactivity was counted in a Packard Tri-Carb 1600CA liquid scintillation analyzer (Packard Instrument Company, Downers Grove, IL) and was corrected for quenching by an external standard technique.

Amino Acid Determination

Amino acids were analyzed by high-pressure liquid chromatography (HPLC) using the PICO•TAG System (Waters Chromatography Division, Millipore Corporation, Milford, MA). This system involved pre-column derivatization of the sample with phenylisothiocyanate to produce phenylthiocarbamyl amino acids followed by reverse phase HPLC (Heinrikson and Meredith, 1984; Ebert, 1986). The concentrations of leucine ($\mu\text{mol/mL}$, for leucine specific activity determination) in KIC-free acid-soluble fractions of plasma, muscle, and liver were determined using norleucine (Sigma Chemical Co., St. Louis, MO) as an internal standard. The concentrations of the free amino acids in acid-soluble fractions of plasma (nmol/mL) and tissues (nmol/mg wet wt) were determined using norleucine and methionine sulfone (Sigma Chemical Co., St. Louis, MO) as internal standards.

Calculations of Leucine Kinetics

The rate of appearance and disappearance of free leucine in plasma (Flux, $\mu\text{mol}\cdot\text{h}^{-1}\cdot 100\text{ g}^{-1}$) was estimated at the end of the 4-h infusion as follows:

$$Flux = I/S_p$$

where I is the isotope infusion rate (disintegration per minute (dpm)·h⁻¹·100 g⁻¹) and S_p is the specific activity of leucine in plasma (dpm/μmol) at isotopic steady state (Laurent et al., 1984). The following equation was used to calculate whole-body leucine kinetics:

$$Ox + NOLD = Flux = B + I$$

where, the disappearance rate of leucine in plasma is due to leucine oxidation (Ox) and leucine incorporation into protein (or nonoxidative leucine disappearance, NOLD, used as an indicator of whole-body protein synthesis) and the rate of leucine appearance in plasma was assumed to represent leucine release from protein breakdown (B) since rats were post-absorptive and given no dietary intake (I). The rate of leucine oxidation (μmol·h⁻¹·100 g⁻¹) was determined by dividing the rate of ¹⁴CO₂ appearance in the breath (dpm·h⁻¹·100 g⁻¹) by the specific activity of leucine in plasma (dpm/μmol). Leucine oxidation was divided by 0.8 to correct for the radioactivity that did not appear in the expired breath because approximately 20% of ¹⁴CO₂ has been estimated to be retained in the body or lost through other pathways (Laurent et al., 1984). The rate of whole-body leucine incorporation into protein represented by NOLD (μmol·h⁻¹·100 g⁻¹) was subsequently calculated as the difference between leucine flux and oxidation. The ratio of leucine oxidation to NOLD (Ox/NOLD) was calculated to determine the rate of leucine oxidation relative to the rate of NOLD. The ratio of leucine oxidation to leucine flux (Ox/Flux) was

calculated to determine the rate of leucine oxidation relative to the rate of leucine flux.

The fractional synthetic rate (FSR) in muscle and liver was calculated using the equation of Garlick et al. (Garlick et al., 1973) as previously reported (DeMichele et al., 1988; Karlstad et al., 1988).

$$\frac{S_b}{S_i} = \frac{\lambda_i}{\lambda_i - K_s} \cdot \frac{(1 - e^{-K_s \cdot T})}{(1 - e^{-\lambda_i \cdot T})} - \frac{K_s}{\lambda_i - K_s}$$

where S_b and S_i were the specific activities of leucine in the protein-bound and free acid-soluble pools, respectively, in dpm/ μ mol, λ_i was the rate (38/day) of rise in specific activity of ^{14} C-leucine in the acid-soluble amino acid pool, T was the length of the L-(1- ^{14} C)-leucine infusion in days, and K_s was the fraction of protein mass renewed each day (or FSR), in %/day.

RNA Extraction

RNA was isolated from rectus abdominus muscle using GlassMAX RNA Microisolation Spin Cartridge system (GIBCO BRL, Life Technologies, Inc., Gaithersburg, MD) which is suitable for isolation of RNA ranging in size from 0.16 to 9.5 Kb. By this method, mRNA and rRNA could be fully recovered. However, tRNA, whose size is smaller, was not recovered with this method. RNA contents in rectus abdominus muscle were reported as μ g/mg protein and presented in relation to FSR values of each individual muscle. RNA

activity in PAF-treated and control muscles were expressed as the slopes of the simple regression relationship between FSR values and RNA contents in muscle of both groups.

Myofibrillar Protein Determination

Determinations of myosin and actin contents by electrophoresis and western blot analysis were conducted. Both myofibrillar proteins were determined and were reported as ratio of relative abundance (% of total tissue protein) of myosin and actin obtained from the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot of total-tissue extracts of rectus abdominus muscle from control and PAF-treated rats. Equal quantities of protein were applied to each gel, therefore, a change of any protein relative to the total amount of protein would result in a change in the relative abundance of that protein.

We qualitatively determined myosin and actin (by PAGE and western blot) in rectus abdominus muscle soluble-cytosolic, detergent soluble-membrane, and insoluble-cytoskeletal fractions to explore the possible changes in myosin and actin distributions as a result of PAF-treatment. Due to sample limitation, the fractionated rectus abdominus muscle from each treatment were pooled prior to the determination of myosin and actin.

Muscle Fractionation

Approximately 35-40 mg of rectus abdominus muscle was homogenized in 1 mL of Tris buffer (20 mM Tris HCl, pH 7.4, 5 mM EGTA, 2 mM β -mercaptoethanol, 0.05 mM leupeptin and 0.01 mM PMSF) on ice. The muscle homogenate was fractionated by ultra-centrifugation (140,000 x g, 45 min, 4 °C) into fractions of 1) cytosolic-supernatant; 2) Triton-X-100-soluble membrane proteins; and 3) Triton-X-100-insoluble cytoskeletal proteins. The cytosolic-supernatant fractions were supernatants from the homogenized muscle in Tris buffer. The pellets were resuspended in 1 mL of Tris buffer containing 0.5% Triton X-100, and the Triton-X-100-soluble fractions were collected after centrifugation. The insoluble materials from Triton X-100 fractions were completely solubilized by the addition of 1 mL 2% SDS and 0.5% Triton X-100 in Tris buffer and sonicated in a Vibracell bath sonicator at 0 °C. The total-tissue extract was obtained by homogenizing muscle in 1 mL 2% SDS and 0.5% Triton X-100 in Tris buffer and solubilized in a Vibracell bath sonicator at 0 °C. Protein content for all fractions was determined by the biuret reaction.

Polyacrylamide Gel Electrophoresis (PAGE)

Tissue extracts were denatured with an adjusted volume of three fold concentrated denaturing buffer (450 mM Tris HCl (pH 6.8), 6% SDS, 30% glycerol, 6% 2-mercaptoethanol, 2 mM EDTA, 12 mM EGTA, and 0.03%

bromophenol blue) to make a final sample concentration of 1 mg/mL. All samples were incubated at 100 °C for 5 min and equal aliquots loaded onto a 6-17.5% polyacrylamide gradient SDS slab gel as described by Studier (Studier, 1973). The molecular weight markers (Sigma Chemical Co., St. Louis, MO) which include a myosin standard were used to identify molecular weights of the proteins in each sample. The Coomassie-blue-staining intensity of myosin and actin bands were detected by Zeineh soft-laser densitometer with slit width set to cover the entire band. Peak areas were quantitated on an interfaced Shimadzu recording integrator.

Western Blot

Following separation by 6% to 17.5% gradient PAGE, all protein was transferred overnight from the gel to nitrocellulose using an electrophoretic transblot apparatus (LKB-Produkter AB, Bromma, Sweden). The blots were blocked with 10 mM Tris-HCl (pH 8.0), 0.14 M NaCl, and 0.05% Tween 20 (TBST) containing 1% BSA, 1% goat serum, and 0.01% sodium azide (as a preservative) at 37 °C for 1 h in a shaking water bath. The protein-blocked membranes were incubated with the mouse-conjugated monoclonal anti pan-myosin or actin (Amersham Corp., Arlington Heights, IL) for 1 h at 37 °C followed by washing with TBST containing goat anti-mouse Ig-alkaline phosphatase conjugate. After washing, color was developed with the addition of 0.5 mg/mL 5-bromo-4-chloro-3-indolyl phosphate and 0.25 mg/mL nitro blue

tetrazolium in 0.1 M NaHCO₃ and 10 mM MgCl₂, pH 9.8. The density of the developed color was determined by an Applied Imaging-Lynx-4000 Workstation video-densitometer.

Statistical Analysis for Part 2

Student's t-test was used to analyze the data. Data were presented as means $\pm$ SE. A *p*-value of less than or equal to 0.05 was considered significant. The relationship between FSR and RNA content in rectus abdominus muscle was examined by the Pearson product moment correlation. The equation of the line and its significance was determined by a simple regression analysis. The difference in the equality of slopes from different regression lines was examined by *F*-test (Sokol and Rohlf, 1995).

V. RESULTS

PART 1: THE EFFECT OF PAF ON AMINO ACID TRANSPORT

Active Transport of System A Amino Acid in Skeletal Muscle

The effect of incubation time on cellular AIB uptake and the distribution ratio of AIB was determined in soleus and EDL of control rats. Cellular AIB uptake (Figure 3) and the distribution ratio of AIB (Table 1) increased in a linear fashion for 80 minutes of incubation time for soleus and EDL. Since cellular AIB uptake both muscles was linear for up to 80 minutes, subsequent experiments were performed at shorter time periods with incubations of 40 or 60 minutes. The distribution ratio of AIB was greater than unity after 60 minutes of incubation for soleus and EDL of control rats. There was no significant difference in the maximum AIB distribution ratio between soleus and EDL. These findings indicate there is a similar level of active transport of AIB across the cell membrane of skeletal muscles of different fiber types.

Prophylactic i.v. Administration of PAF Antagonist and Endotoxemia

The rate of cellular AIB uptake in soleus and EDL of control, endotoxin-treated, and WEB 2086/endotoxin-treated rats are shown in Figure 4. The i.v. injection of 10 mg/kg endotoxin significantly reduced the rate of cellular AIB uptake in soleus and EDL by 32% and 33%, respectively, as compared with control muscles. The rate of cellular AIB transport increased 17% (although

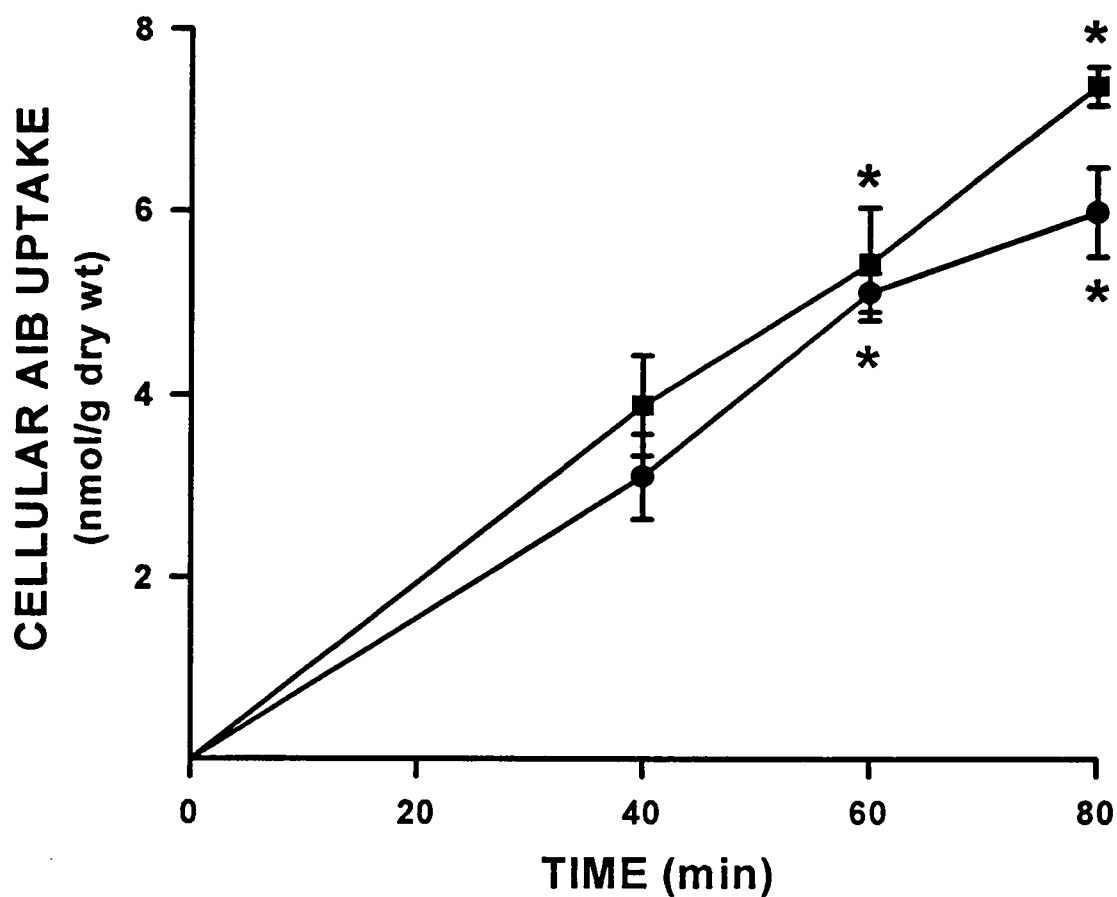


Figure 3. The effect of incubation time on cellular AIB uptake by soleus (solid circle) and EDL (solid square) of control rats incubated in basal media. Values are means $\pm$ SE; number of muscles = 5 for each time point; * $p \leq 0.05$ versus 40 min incubation of the same muscle type.

Table 1. Effect of incubation time on AIB distribution ratio in soleus and EDL of control rats incubated in basal media.

Muscle	AIB distribution ratio (Intracell [AIB]/Extracell [AIB])		
	40 min	60 min	80 min
Soleus	0.74 ± 0.12 (5)	1.27 ± 0.07* (5)	1.57 ± 0.15* (5)
EDL	0.84 ± 0.12 (5)	1.06 ± 0.21 (4)	1.68 ± 0.08* (5)

Values are means ± SE; () = number of muscles; * $p \leq 0.05$ versus 40 min incubation time of the same muscle type.

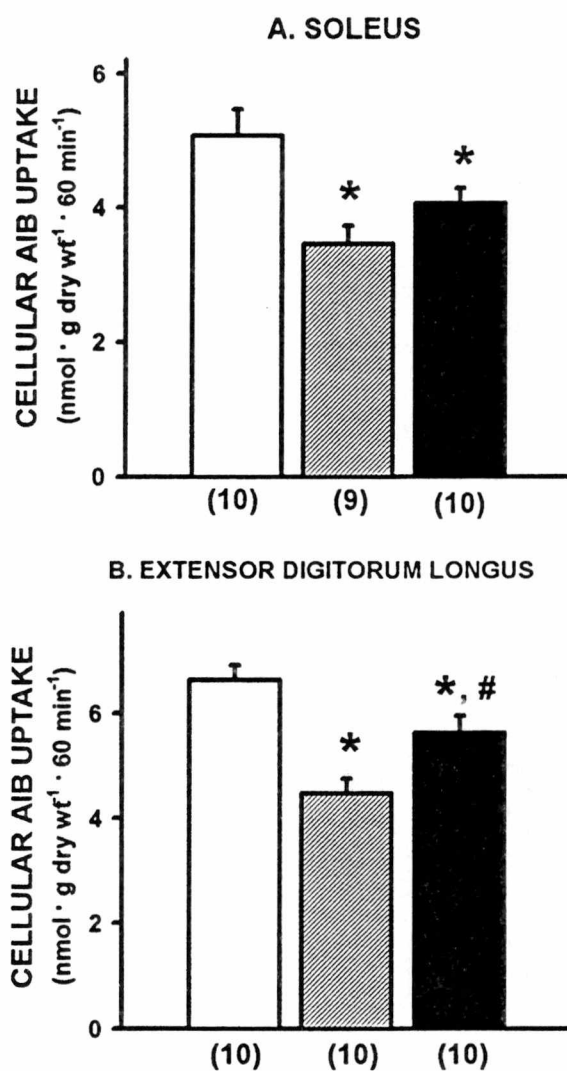


Figure 4. Cellular AIB uptake by soleus (A) and EDL (B) of rats given an i.v. injection of vehicle (open bars), endotoxin (10 mg/kg) (hatched bars), and WEB 2086 (30 mg/kg) prior to endotoxin (10 mg/kg) (solid bars).

Values are means $\pm$ SE; () = number of muscles; * $p \leq 0.05$ versus vehicle of the same muscle type; # $p \leq 0.05$ versus endotoxin of the same muscle type.

not significantly) in soleus and increased 26% in EDL in the WEB 2086/endotoxin-treated rats as compared with the endotoxin-treated rats. The distribution ratio of AIB in soleus and EDL of control, endotoxin-treated, and WEB 2086/endotoxin-treated rats are shown in Table 2. The distribution ratio of AIB in endotoxin-treated rats was significantly reduced in soleus and EDL by 35% and 33%, respectively, as compared with the controls. It should also be noted that the distribution ratio was less than one indicating a loss of active AIB transport by soleus and EDL from endotoxin-treated rats. The AIB distribution ratio increased 12% (although not significantly) in soleus and increased 32% in EDL in the WEB 2086/endotoxin-treated rats as compared with the endotoxin-treated rats. Thus, there was a return of active transport of AIB in EDL but not soleus in the WEB 2086/endotoxin-treated rats.

Addition of PAF to Incubation Media

The rate of cellular AIB uptake in soleus and EDL of control and PAF-treated rats incubated in the absence (basal) or presence of 1 $\mu\text{g/mL}$ PAF are shown in Table 3. The iv injection of 4 $\mu\text{g/kg}$ PAF significantly reduced the rate of cellular AIB uptake in soleus and EDL by 9% and 15%, respectively, as compared with the control rats in the basal state (PAF-free media). Incubation of soleus and EDL from control rats for 40 minutes in the presence of 1 $\mu\text{g/mL}$ PAF did not affect the rate of cellular AIB uptake. Additional experiments determined that incubation of soleus and EDL from control rats for 40 minutes

Table 2. AIB distribution ratio in soleus and EDL of rats that were injected i.v. with vehicle, 10 mg/kg endotoxin, and 30 mg/kg WEB 2086 prior to 10 mg/kg endotoxin (WEB 2086/Endotoxin) and incubated for 60 minutes in basal media.

Treatment	AIB distribution ratio (Intracell [AIB]/Extracell [AIB])	
	Soleus	EDL
Vehicle	1.31 ± 0.32 (10)	1.47 ± 0.17 (10)
Endotoxin	0.85 ± 0.15* (9)	0.98 ± 0.19* (10)
WEB 2086/ endotoxin	0.95 ± 0.17 (10)	1.30 ± 0.24* (10)

Values are means ± SE; () = number of muscles; * $p \leq 0.05$ versus vehicle of the same muscle type; * $p \leq 0.05$ versus endotoxin of the same muscle type.

Table 3. Cellular AIB uptake in soleus and EDL of control and PAF-treated rats incubated in PAF-free media (basal) and in the presence of PAF (1 µg/mL)*.

Group			AIB uptake (nmol/g dry wt/40 min)	
Treatment Muscle	N		Basal media	PAF (1 µg/mL)
Control Soleus	13		4.27 ± 0.29	4.10 ± 0.22
PAF-treated Soleus	22		3.87 ± 0.19 [#]	3.16 ± 0.17 ^{**}
Control EDL	14		4.37 ± 0.29	4.13 ± 0.34
PAF-treated EDL	22		3.73 ± 0.17 [*]	3.46 ± 0.19 [*]

*Control rats were injected with vehicle and PAF-treated rats were injected with 4 µg/kg PAF and killed 1 hour later and muscles removed for determination of cellular AIB uptake. Values are means ± SE; N = number of rats or pairs of muscle; ^{*} $p \leq 0.05$ versus paired muscle incubated in basal media; ^{*} $p \leq 0.05$ versus control group of the same muscle type.

in the presence of 0.1 and 10 $\mu\text{g/mL}$ PAF also did not affect the rate of cellular AIB uptake (Table 4). In addition, AIB transport rates in soleus and EDL from controls rats were not significantly affected by longer periods of incubation (60, 80 and 100 minutes) in the presence of 1 $\mu\text{g/mL}$ PAF (data not shown). However, the presence of 1 $\mu\text{g/mL}$ PAF in the incubation media for 40 minutes resulted in a further reduction in the rate of cellular AIB uptake in PAF-treated rat soleus by 18% ($p = 0.0004$) and in EDL by 7% ($p = 0.08$) as compared with paired-muscles in basal media.

Addition of Plasma from PAF-Treated Rats to Incubation Media

The rate of cellular AIB uptake in paired soleus and EDL of control rats incubated for 40 minutes in the presence of incubation media (2.8 mL) and 0.2 mL plasma from control or PAF-treated rats are shown in Figure 5. The rate of cellular AIB uptake in soleus and EDL was significantly reduced by 12% and 17%, respectively, in the presence of plasma obtained from PAF-treated rats. In addition, the rate of cellular uptake was attenuated significantly by 22% in EDL and not significantly by 8-9 % in soleus when incubated for 60 min in the presence of plasma obtained from endotoxin-treated rats as compared with PAF-treated rats (Figure 6).

Table 4. Cellular AIB uptake in soleus and EDL of control rats incubated in PAF-free media (basal) and in the presence of PAF at different concentrations.

Group			AIB uptake (nmol/g dry wt/40 min)	
PAF ($\mu\text{g/mL}$)	Muscle	N	Basal media	PAF-containing media
0.1	Soleus	10	3.43 ± 0.17	3.36 ± 0.24
1.0	Soleus	13	4.10 ± 0.29	4.23 ± 0.19
10.0	Soleus	12	3.95 ± 0.28	3.98 ± 0.26
0.1	EDL	10	3.69 ± 0.21	3.61 ± 0.25
1.0	EDL	13	4.36 ± 0.30	4.24 ± 0.28
10.0	EDL	12	4.07 ± 0.33	4.17 ± 0.28

Values are means $\pm$ SE; N = number of rats or pairs of muscle; $p > 0.05$ versus paired muscle incubated in basal media.

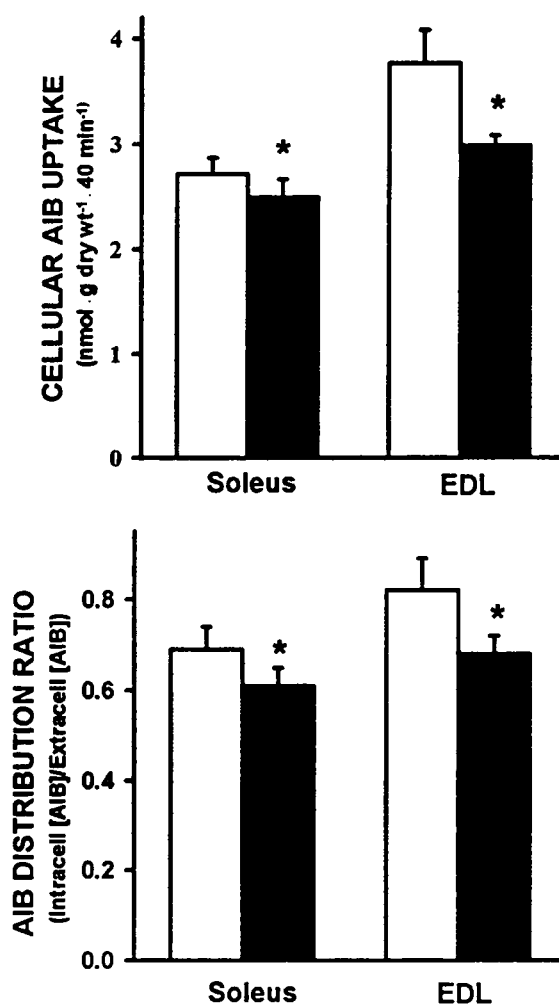


Figure 5. The effect of media containing plasma from control (open bars) or PAF-treated rats (solid bars) on cellular AIB uptake in soleus and EDL of control rats. Values are means $\pm$ SE; number of pairs of muscle = 4 and 8 for soleus and EDL, respectively; * $p \leq 0.05$ versus paired muscles incubated in the presence of plasma from control rats.

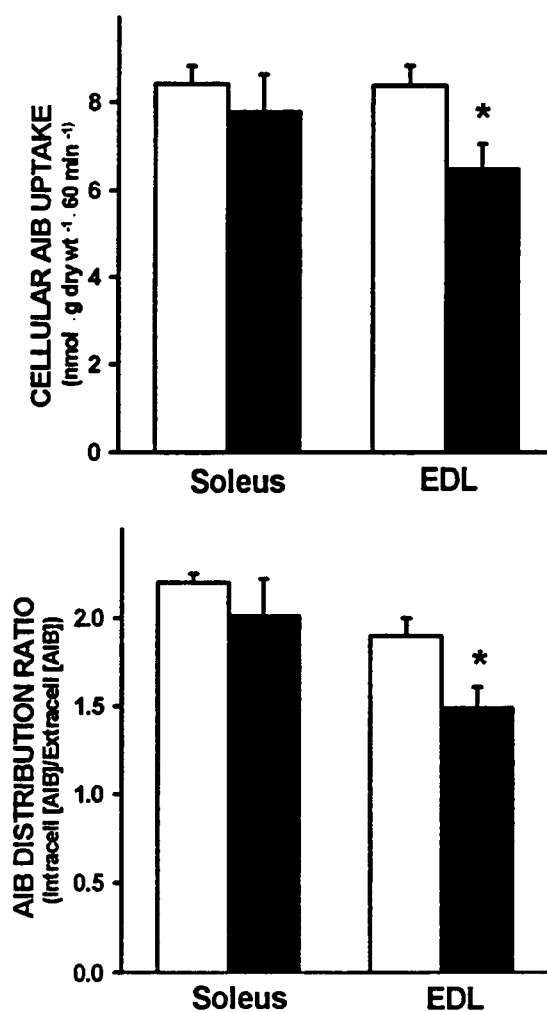


Figure 6. The effect of media containing plasma from PAF-treated (open bars) or endotoxin-treated rats (solid bars) on cellular AIB uptake in soleus and EDL of control rats. Values are means $\pm$ SE; number of muscles = 8 in each group; * $p \leq 0.05$ versus the same muscle type incubated in the presence of plasma from PAF-treated rats.

PART 2: THE EFFECT OF PAF ON PROTEIN METABOLISM

Whole-Body and Tissue Leucine Kinetics

Estimates of whole body leucine kinetics for control and PAF-treated rats are shown in Figure 7. Within 120 min of the start of the 4 h constant iv infusion of L-(1-¹⁴C)-leucine, the specific activity of ¹⁴CO₂ in expired breath was at near isotopic steady-state in both groups. Expired ¹⁴CO₂ specific activity during the last two h of the L-(1-¹⁴C)-leucine infusion was significantly greater in the PAF-treated rats (100.48 ± 1.37 dpm/ μ mol) as compared with the controls (74.90 ± 2.92 dpm/ μ mol). The rate of leucine flux was lower, although not significantly ($p = 0.07$, one-tailed t-test), in the PAF-treated rats as compared with controls. The rate of leucine oxidation was not different between the groups. The rate of protein synthesis represented as NOLD was significantly lower by 24% ($p \leq 0.05$, one-tailed t-test) in the PAF-treated rats as compared with controls. The iv infusion of PAF significantly increased the relative rate of leucine oxidation to protein synthesis (Ox/NOLD) by 36% (Figure 8). Leucine flux oxidized or the relative rate of leucine oxidation to leucine flux (Ox/Flux) was significantly increased by 23% in the PAF-treated rats as compared with the controls (Figure 8). There were no significant differences between groups for O₂ consumption (606 ± 16 and 647 ± 9 μ mol/min/kg in control and PAF-treated rats, respectively) and CO₂ production (587 ± 13 and 661 ± 5 μ mol/min/kg in control and PAF-treated rats, respectively).

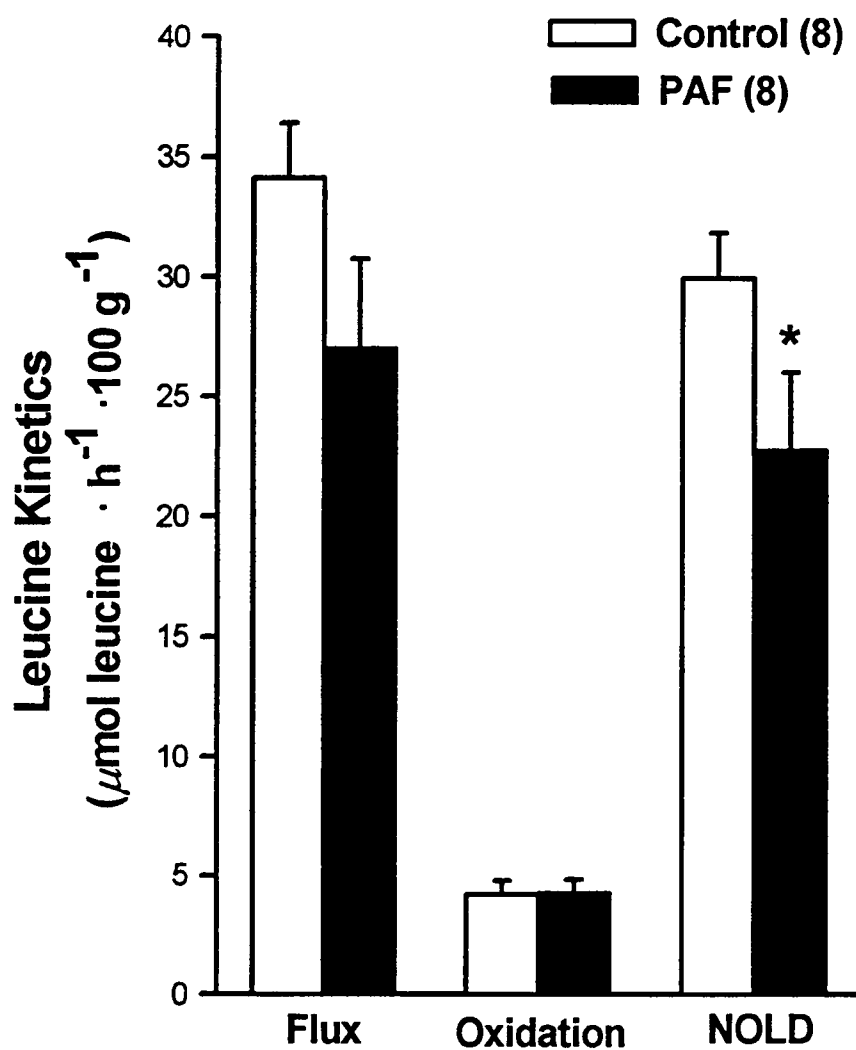


Figure 7. Whole-body leucine kinetics in control and PAF-treated rats.

Values are means $\pm$ SE; () = number of rats; * $p \leq 0.05$ versus control. NOLD = non-oxidative leucine disappearance.

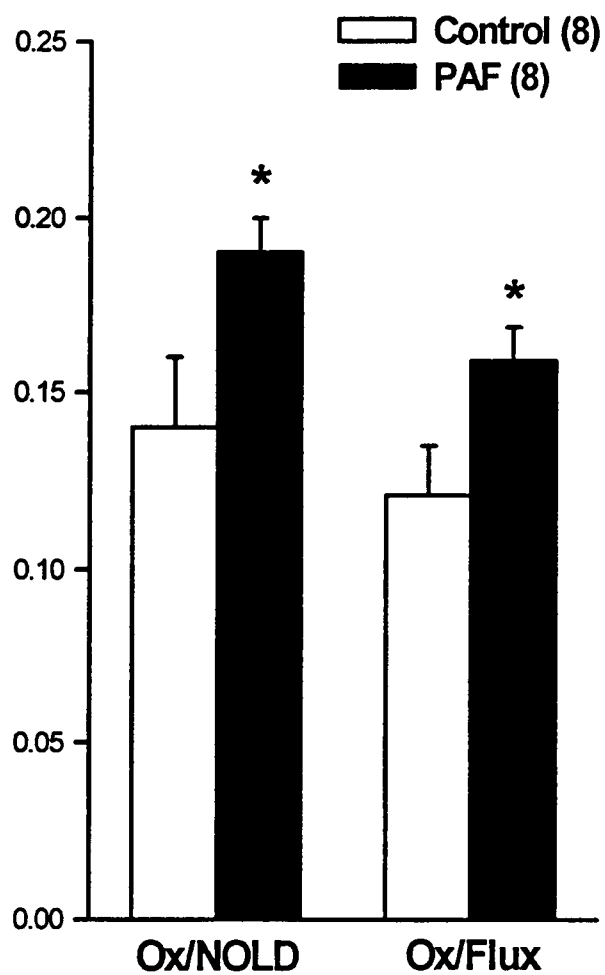


Figure 8. The effect of PAF on the ratio of leucine oxidation to non-oxidized leucine disappearance (OX/NOLD) and leucine oxidation to leucine flux (OX/Flux). Values are means $\pm$ SE; () = number of rats; * $p \leq 0.05$ versus control.

The rate of fractional protein synthesis (FSR) in rectus abdominus, soleus and EDL decreased significantly by 33%, 30% and 25%, respectively, in PAF-treated rats as compared with the controls (Figure 9). Liver FSR was not significantly different between the groups (Figure 9). The protein-to-wet weight ratios in rectus abdominus, soleus and EDL were not different between the groups (Table 5). The protein-to-wet weight ratio and total protein content in liver and plasma protein concentration were significantly lower in the PAF-treated rats as compared with controls (Table 5).

Plasma and Tissue Amino Acid Profiles

The effect of PAF on plasma amino acids is shown in Table 6. PAF significantly decreased the plasma concentrations of tryptophan (35%), isoleucine (36%), leucine (30%), valine (34%), glutamic acid (54%) and serine (29%), and increased histidine (44%) and glycine (45%) as compared with control rats. The sum of the branched-chain amino acids was reduced significantly by 33% in PAF-treated rats as compared with controls. The sum of the essential, nonessential, and total amino acids were not different between the groups. The concentration of taurine was significantly increased by approximately 4-fold in PAF-treated rats as compared with controls.

PAF caused several changes in free amino acid profiles in rectus abdominus, soleus, and EDL muscles (Table 7). In rectus abdominus muscle, free lysine was increased significantly by 55% in PAF-treated rats as compared

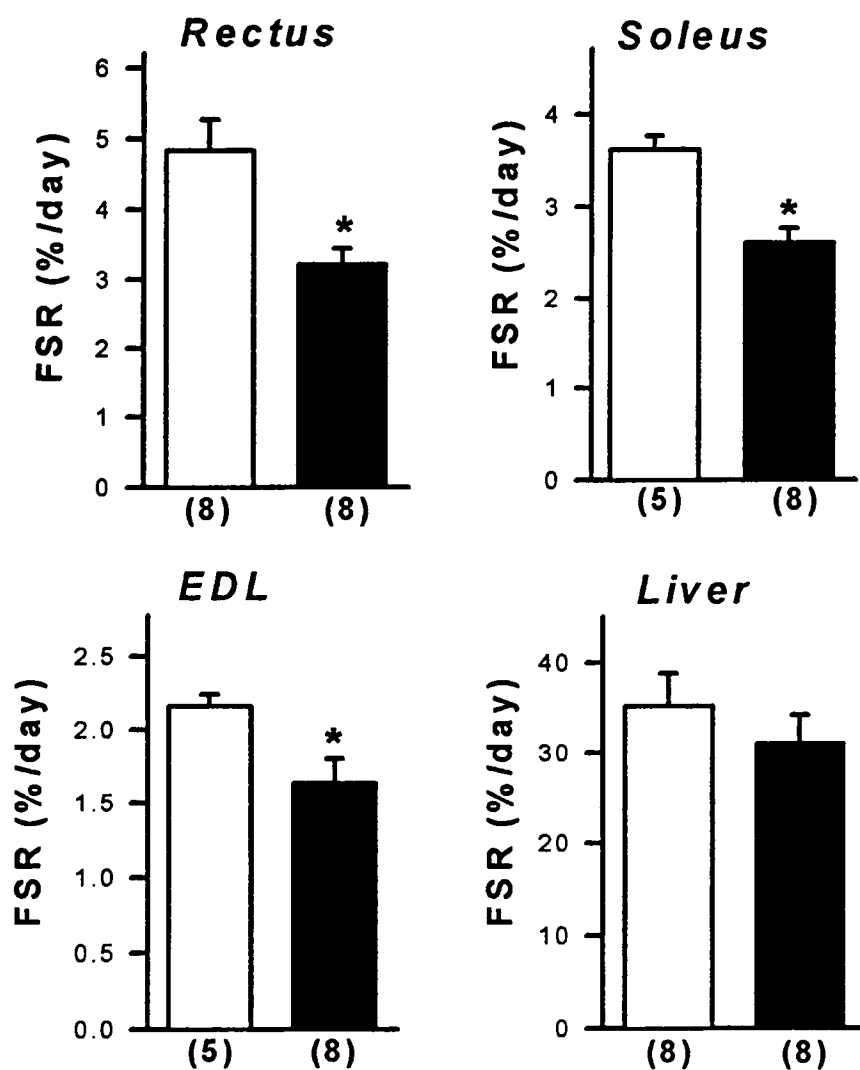


Figure 9. The effect of PAF on fractional protein synthesis rate (FSR) in rectus abdominous (top-left panel), soleus (top-right panel), EDL (bottom-left panel), and liver (bottom-right panel). Values are means $\pm$ SE; () = number of rats; * $p \leq 0.05$ versus control. The open- and solid-bars represent control and PAF-treated groups, respectively.

Table 5. Tissue protein-to-wet weight ratios and plasma and liver protein content in control and PAF-treated rats.

Parameter	Control	PAF-treated
<i>Tissue Protein (mg/100 mg tissue wet wt)</i>		
Rectus	12.4 ± 0.2 (8)	12.3 ± 0.2 (8)
Soleus	16.0 ± 0.6 (5)	16.4 ± 0.6 (8)
EDL	16.6 ± 0.2 (5)	16.1 ± 0.2 (8)
Liver	14.7 ± 0.2 (8)	13.7 ± 0.3* (8)
<i>Protein Content</i>		
Plasma (g/dL)	2.86 ± 0.07 (8)	2.58 ± 0.09* (8)
Liver (g/Liver)	1.33 ± 0.03 (8)	1.18 ± 0.04* (8)

Values are means ± SE; () = number of rats; * $p \leq 0.05$ versus control.

Table 6. Profiles of free L-amino acids in plasma from control and PAF-treated rats.

Amino acid (nmol/mL)	Control (8)	PAF-treated (8)
<i>Essential amino acids (EAA)</i>		
Histidine	62.0 ± 6.4	89.1 ± 6.7*
Lysine	376 ± 35	419 ± 18
Methionine	47.5 ± 1.2	40.5 ± 2.6*
Phenylalanine	61.2 ± 2.8	67.6 ± 3.4
Threonine	192 ± 26	198 ± 19
Tryptophan	65.8 ± 8.0	43.0 ± 2.9*
<i>Branched-chain amino acids (BCAA)</i>		
Isoleucine	74.2 ± 5.3	47.3 ± 3.6*
Leucine	186 ± 12	129 ± 9*
Valine	221 ± 15	146 ± 9*
<i>Nonessential amino acids (NEAA)</i>		
Alanine	302 ± 16	246 ± 21*
Asparagine	86.9 ± 4.2	80.8 ± 3.6
Aspartic acid	14.5 ± 1.5	16.2 ± 3.1
Cysteine	32.5 ± 4.4	39.8 ± 4.5
Glutamic acid	184 ± 13	85.3 ± 7.0*
Glutamine	721 ± 25	725 ± 24
Glycine	308 ± 14	447 ± 35*
Proline	131 ± 5	105 ± 6
Serine	238 ± 9	170 ± 8*
Tyrosine	60.4 ± 4.4	52.8 ± 3.5
Sum BCAA	481 ± 31	323 ± 21*
Sum EAA	1285 ± 53	1180 ± 59
Sum NEAA	2078 ± 66	1968 ± 83
Total amino acids	3363 ± 98	3148 ± 135
<i>Other amino acids and dipeptide</i>		
3-m-Histidine	6.9 ± 1.1	7.3 ± 1.3
Carnosine	ND	ND
Citrulline	30.7 ± 2.7	46.9 ± 9.2
Cystathionine	ND	ND
Ornithine	38.2 ± 4.1	46.9 ± 9.2
Taurine	125 ± 11	479 ± 86*

Values are means ± SE; () = number of rats; * $p \leq 0.05$ versus control; ND = not detectable.

Table 7. Profiles of free L-amino acids in rectus abdominous, soleus and EDL muscles from control and PAF-treated rats.

Amino Acid (nmol/mg wet wt)	Rectus		Soleus		EDL	
	Control (7)	PAF (8)	Control (5)	PAF (7)	Control (4)	PAF (8)
Essential amino acids (EAA)						
Histidine	0.20 ± 0.02	0.23 ± 0.02	0.20 ± 0.06	0.16 ± 0.03	0.20 ± 0.04	0.12 ± 0.03
Lysine	0.44 ± 0.05	0.68 ± 0.09*	0.25 ± 0.07	0.42 ± 0.06	0.24 ± 0.04	0.21 ± 0.03
Methionine	0.20 ± 0.05	0.18 ± 0.04	0.30 ± 0.10	0.68 ± 0.13*	0.65 ± 0.26	0.38 ± 0.10
Phenylalanine	0.09 ± 0.01	0.11 ± 0.02	1.72 ± 0.25	1.40 ± 0.11	1.32 ± 0.14	1.46 ± 0.08
Tryptophan	0.07 ± 0.01	0.06 ± 0.01	0.24 ± 0.08	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Branched-chain amino acids (BCAA)						
Isoleucine	0.36 ± 0.08	0.29 ± 0.04	0.15 ± 0.03	0.31 ± 0.02*	0.36 ± 0.02	0.36 ± 0.02
Leucine	0.17 ± 0.02	0.15 ± 0.01	0.12 ± 0.02	0.05 ± 0.01*	0.07 ± 0.01	0.07 ± 0.01
Valine	0.25 ± 0.03	0.20 ± 0.01	0.35 ± 0.04	0.23 ± 0.01*	0.46 ± 0.09	0.41 ± 0.05
Nonessential amino acids (NEAA)						
Alanine	1.00 ± 0.14	1.12 ± 0.07	0.62 ± 0.13	0.49 ± 0.10	0.77 ± 0.03	0.51 ± 0.10
Asparagine	0.28 ± 0.03	0.34 ± 0.02	0.31 ± 0.10	0.12 ± 0.03	0.14 ± 0.04	0.09 ± 0.02
Aspartic acid	0.27 ± 0.02	0.36 ± 0.05	0.67 ± 0.04	0.72 ± 0.13	0.78 ± 0.09	0.57 ± 0.07
Glutamic acid	4.80 ± 0.28	4.10 ± 0.45	1.89 ± 0.25	1.70 ± 0.07	2.21 ± 0.37	1.73 ± 0.14
Glutamine	1.71 ± 0.16	2.02 ± 0.14	1.99 ± 0.62	0.73 ± 0.17*	0.57 ± 0.16	0.44 ± 0.13
Glycine	3.03 ± 0.25	3.46 ± 0.32	1.46 ± 0.16	0.79 ± 0.08*	1.56 ± 0.45	1.15 ± 0.09
Proline	0.24 ± 0.02	0.25 ± 0.01	0.10 ± 0.01	0.06 ± 0.01*	0.05 ± 0.01	0.05 ± 0.01
Serine	0.53 ± 0.02	0.50 ± 0.03	0.63 ± 0.18	0.36 ± 0.06	0.36 ± 0.09	0.25 ± 0.04
Tyrosine	0.45 ± 0.12	0.29 ± 0.05	1.36 ± 0.26	0.71 ± 0.06*	0.75 ± 0.09	0.60 ± 0.06
Sum BCAA	0.79 ± 0.11	0.64 ± 0.06	0.63 ± 0.04	0.59 ± 0.03	0.89 ± 0.11	0.84 ± 0.06
Sum EAA	1.80 ± 0.20	1.90 ± 0.12	3.33 ± 0.37	3.36 ± 0.18	3.43 ± 0.22	3.12 ± 0.23
Sum NEAA	12.33 ± 0.61	12.43 ± 0.91	9.01 ± 1.27	5.68 ± 0.37*	7.20 ± 0.82	5.40 ± 0.40*
Total amino acids	14.12 ± 0.65	14.34 ± 0.98	12.34 ± 1.47	9.04 ± 0.50*	10.62 ± 0.93	8.52 ± 0.59
Other amino acids and dipeptide						
3-m-Histidine	3.28 ± 0.20	3.07 ± 0.19	2.38 ± 0.33	1.04 ± 0.17*	1.32 ± 0.28	1.14 ± 0.10
Carnosine	3.90 ± 0.34	3.70 ± 0.22	1.57 ± 0.17	0.63 ± 0.05*	1.13 ± 0.28	0.97 ± 0.09
Cystathionine	1.51 ± 0.15	1.72 ± 0.07	1.80 ± 0.24	2.66 ± 0.55	0.14 ± 0.05	0.09 ± 0.03
Ornithine	0.06 ± 0.02	0.03 ± 0.00	0.07 ± 0.01	0.11 ± 0.03	2.90 ± 0.53	2.38 ± 0.41
Taurine	14.79 ± 0.99	13.70 ± 0.71	24.44 ± 2.37	22.54 ± 0.88	19.35 ± 1.46	15.65 ± 1.04

Values are means ± SE; () = numbers of rats; * $p \leq 0.05$ versus control.

with controls. There was a significant change in the soleus free amino acid concentration profile characterized by a 58%↓ in leucine, 34%↓ in valine, 126%↓ in glutamine, 46%↓ in glycine, 40%↓ in proline, 48%↓ of tyrosine, 56%↓ in 3-m-histidine, 60%↓ in carnosine, 127%↑ in methionine, and 107%↑ in isoleucine in PAF-treated rats as compared with controls. Furthermore, the sum of soleus nonessential and total amino acids were reduced by 36% and 27%, respectively, in PAF-treated rats as compared with controls. There was no significant change in the EDL amino acid profile other than a 25% reduction in the sum of nonessential amino acids in PAF-treated rats as compared with controls. PAF decreased leucine, valine, and the sum of branched-chain amino acids in liver by 58%, 44%, and 46%, respectively (Table 8). All other amino acids and the sum of the essential, nonessential, and total amino acids were not significantly different between the groups (Table 8).

RNA Activity and Myofibrillar Protein in Rectus Abdominus Muscle

The relationship between the fractional rate of protein synthesis and RNA content in rectus abdominus muscle of PAF-treated and control groups is shown in Figure 10. The fall in muscle RNA content ([RNA]) in control and PAF-treated rats was correlated with the fall in FSR [$FSR = (-1.09 \pm 1.44) + (10.29 \pm 2.89) [RNA]$ for control ($r = 0.93$, $n = 4$, $p = 0.070$); and $FSR = (2.30 \pm 0.18) + (1.28 \pm 0.19) [RNA]$ for PAF-treated ($r = 0.97$, $n = 4$, $p = 0.007$)]. There was a highly significant difference ($p < 0.01$) in the slopes of regression

Table 8. Profiles of free L-amino acids in liver from control and PAF-treated rats.

Amino acid (nmol/mg wet wt)	Control (8)	PAF-treated (8)
<i>Essential amino acids (EAA)</i>		
Histidine	0.44 ± 0.04	0.43 ± 0.07
Lysine	0.57 ± 0.13	0.45 ± 0.05
Methionine	0.47 ± 0.06	0.58 ± 0.05
Phenylalanine	0.36 ± 0.04	0.30 ± 0.06
Tryptophan	0.15 ± 0.02	0.23 ± 0.06
<i>Branched-chain amino acids (BCAA)</i>		
Isoleucine	0.17 ± 0.03	0.14 ± 0.04
Leucine	0.53 ± 0.13	0.22 ± 0.03*
Valine	0.55 ± 0.08	0.31 ± 0.04*
<i>Nonessential amino acids (NEAA)</i>		
Alanine	0.85 ± 0.11	0.90 ± 0.08
Asparagine	0.27 ± 0.08	0.16 ± 0.02
Aspartic acid	0.61 ± 0.15	0.49 ± 0.08
Cysteine	2.22 ± 0.53	1.41 ± 0.31
Glutamic acid	4.43 ± 0.49	3.49 ± 0.26
Glutamine	3.76 ± 0.37	4.13 ± 0.24
Glycine	2.09 ± 0.20	2.71 ± 0.14
Proline	0.20 ± 0.07	0.09 ± 0.01
Serine	0.62 ± 0.16	0.31 ± 0.01
Tyrosine	1.08 ± 0.26	0.64 ± 0.11
Sum BCAA	1.25 ± 0.23	0.67 ± 0.09*
Sum EAA	3.23 ± 0.42	2.66 ± 0.23
Sum NEAA	16.13 ± 1.11	14.34 ± 0.76
Total amino acids	19.36 ± 1.32	17.00 ± 0.93
<i>Other amino acids and dipeptide</i>		
3-m-Histidine	0.73 ± 0.12	0.45 ± 0.09
Carnosine	0.04 ± 0.01	0.08 ± 0.03
Cystathionine	0.51 ± 0.12	0.82 ± 0.18
Ornithine	0.31 ± 0.06	0.20 ± 0.06
Taurine	6.72 ± 1.15	6.04 ± 0.48

Values are means ± SE; () = number of rats; * $p \leq 0.05$ versus control.

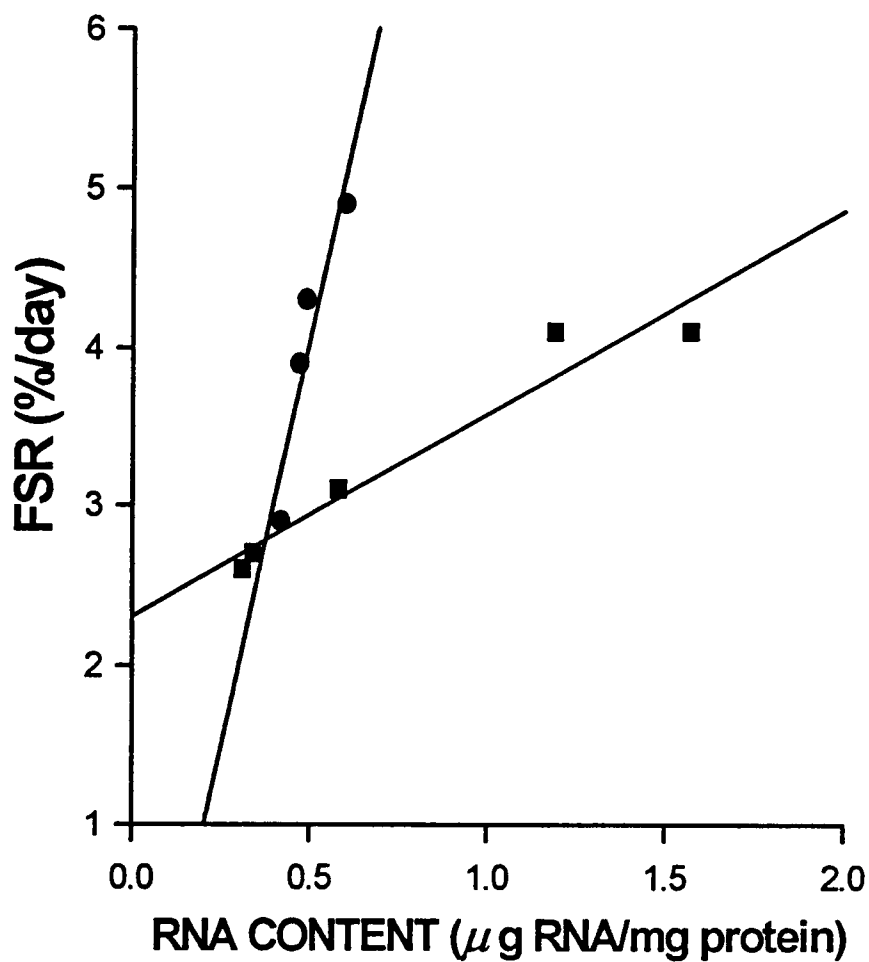


Figure 10. Relationship between FSR and RNA content in rectus abdominous muscle from control (solid circle) and PAF-treated (solid square) rats. Values are individual samples; number of rats = 4 in control and 5 in PAF-treated group; solid lines represent the regression lines in each group; slopes of both lines are significantly different ($p < 0.01$).

lines of control and PAF-treated rats when using the *F*-test for the comparison of slope equality (Sokol and Rohlf, 1995). This significant decline in the slope of PAF-treated rats (from 10.29 ± 2.89 to 1.28 ± 0.19) reflected the 32% decline in muscle RNA activity (from 8.07 ± 0.41 to 5.52 ± 1.15 μg protein synthesis/ μg RNA/day; $p = 0.05$, one-tailed *t*-test versus control).

Rectus abdominus muscle was fractionated into three different fractions based on the solubilities of tissue proteins. The cellular soluble fraction was prepared using Tris buffer and the protein content of this fraction was not different between the groups (0.041 ± 0.002 mg/mg tissue in control versus 0.039 ± 0.001 mg/mg tissue in PAF-treated rats). The protein content of Triton-X-100-soluble fraction, however, showed a significant 18% decrease in the PAF-treated rats (0.028 ± 0.002 mg/mg tissue) as compared with controls (0.037 ± 0.002 mg/mg tissue). In the Triton-X-100-insoluble fraction, there was no significant difference in the protein content of both groups (0.079 ± 0.004 and 0.088 ± 0.004 mg/mg tissue in control and PAF-treated rats, respectively). The sum of the protein contents in all three fractions (mentioned above) was not different between PAF-treated and control rats. There was also no difference in the protein content of total tissue extracts of rectus abdominus muscle from both groups (0.134 ± 0.004 and 140 ± 0.005 mg/mg tissue in control and PAF-treated rats, respectively).

There was a significant reduction (60%) of myosin-to-actin ratio in the total tissue extract of rectus abdominus muscle from PAF-treated rats as

compared with controls while there was no difference in the relative abundance of actin in both groups (Figure 11). Pooled samples of different fractions, including the total tissue extract, of rectus abdominus muscles from control (n=5) and PAF-treated (n=8) rats were separated on SDS-PAGE and blotted for the myosin and actin with specific monoclonal antibodies (Figures 12 and 13). Myosin was intensely stained in Triton-X-100-soluble, Triton-X-100-insoluble, and total tissue fractions while there was very little myosin in the cytosolic-supernatant fraction. Actin bands were prominent in all fractions though they were less intensely stained in the cytosolic-supernatant fraction. In agreement with the analysis of the relative abundance of myosin in total tissue extracts analyzed separately for each animal (Figure 11), the intensity of myosin bands in the pooled Triton-X-100-soluble, Triton-X-100-insoluble, and total tissue fractions of rectus abdominus muscle from PAF-treated rats were all decreased as compared with controls while the actin bands were not different between the groups.

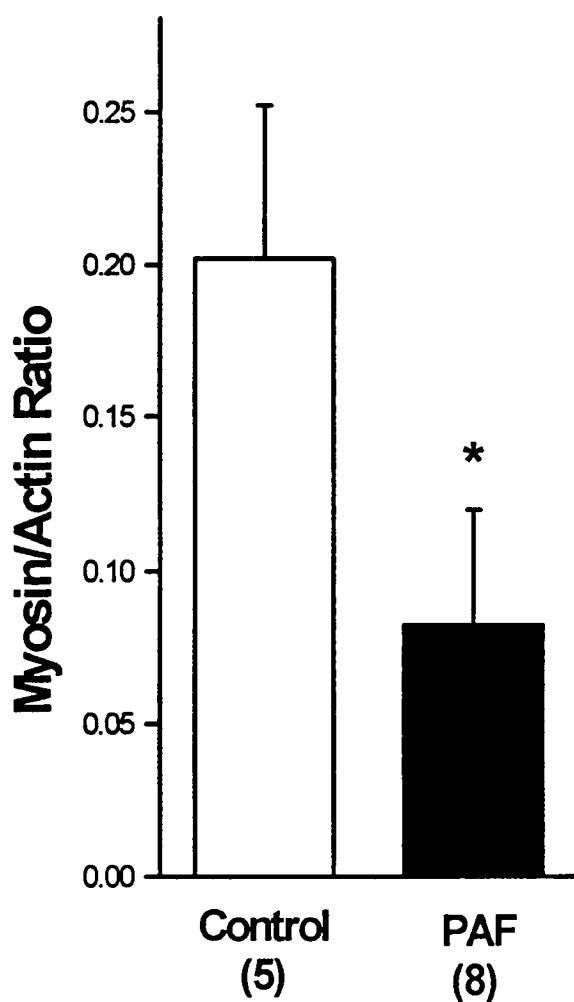


Figure 11. Ratio of relative abundance (% of total tissue protein) of myosin to actin obtained from the SDS-polyacrylamide gel electrophoresis of total tissue extracts of rectus abdominous muscle from control and PAF-treated rats. Values are means $\pm$ SE; () = number of rats; * $p \leq 0.05$ versus control.

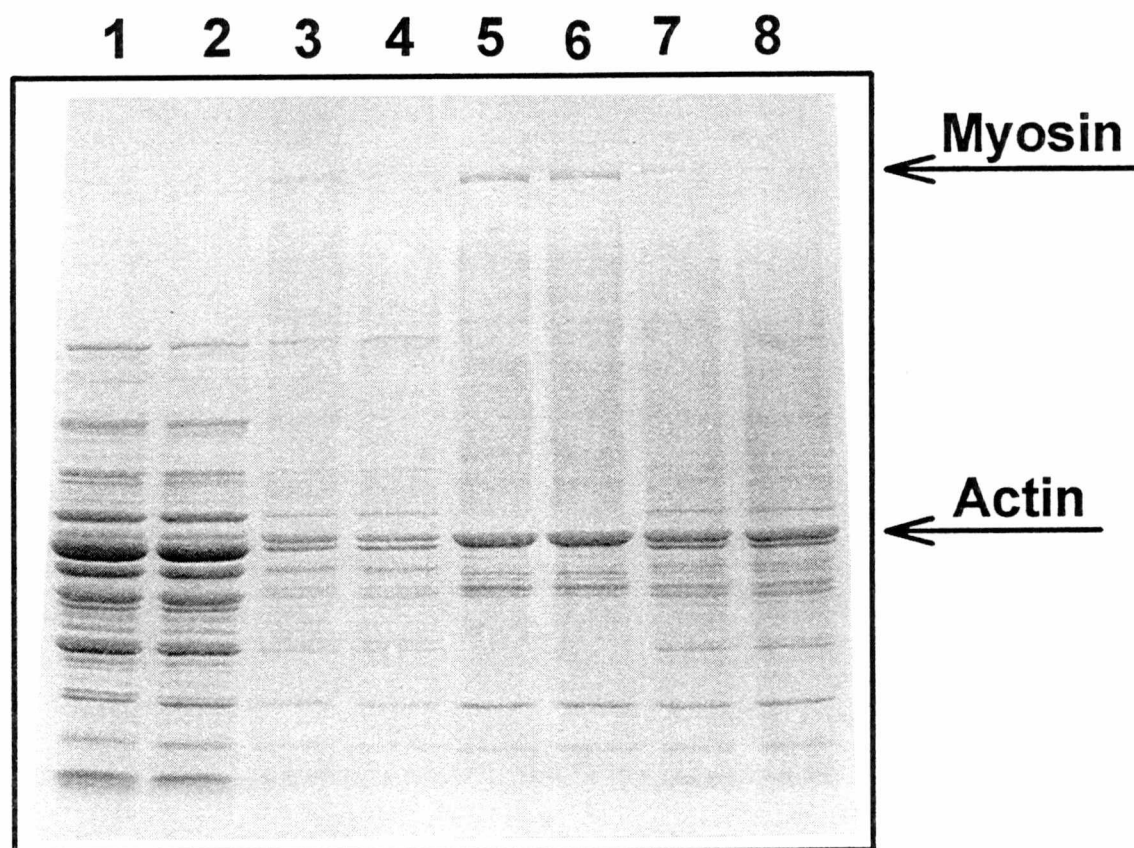


Figure 12. SDS-polyacrylamide gel electrophoresis of myosin and actin in pooled-sample of the cytosolic-supernatant (*lanes 1-2*), Triton-X-100-soluble (*lanes 3-4*), Triton-X-100-insoluble (*lanes 5-6*), and total tissue (*lanes 7-8*) fractions of rectus abdominous muscle from control (*lanes 1, 3, 5, and 7*) and PAF-treated (*lanes 2, 4, 6, and 8*) rats.

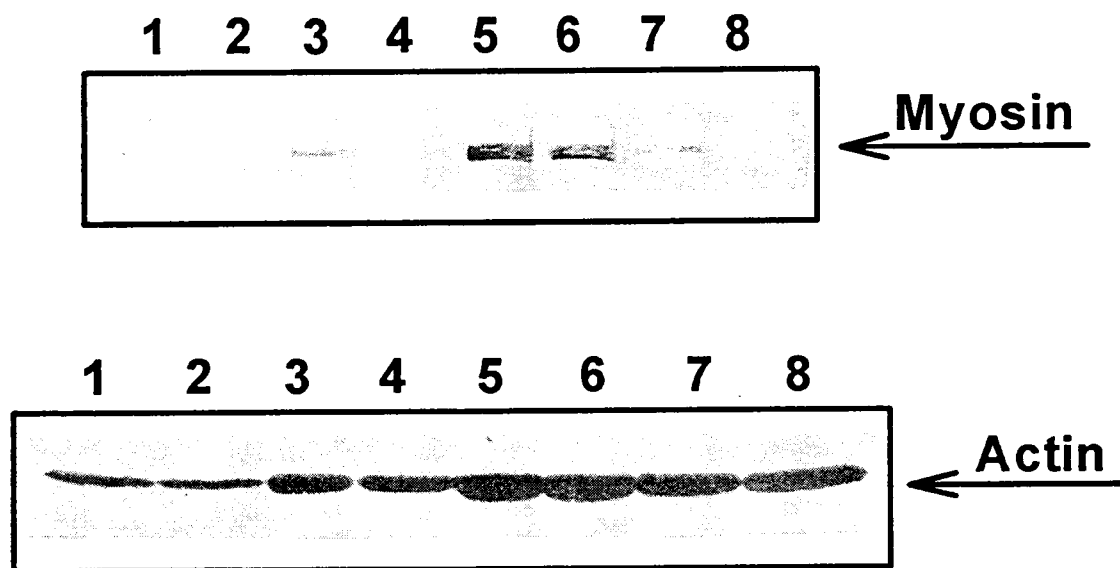


Figure 13. Western blot of myosin and actin in pooled-sample of the cytosolic-supernatant (*lanes 1-2*), Triton-X-100-soluble (*lanes 3-4*), Triton-X-100-insoluble (*lanes 5-6*), and total tissue (*lanes 7-8*) fractions of rectus abdominous muscle from control (*lanes 1, 3, 5, and 7*) and PAF-treated (*lanes 2, 4, 6, and 8*) rats.

VI. DISCUSSION

PART 1: THE EFFECT OF PAF ON AMINO ACID TRANSPORT

Involvement of PAF in the Attenuation of Skeletal Muscle Transport of Amino Acid During Endotoxic Shock

A recent study from this laboratory showed that PAF, when administered *in vivo*, significantly attenuated basal and insulin-mediated system A amino acid transport in rat soleus muscle (Boruff and Karlstad, 1993). These findings were similar to previous studies that also showed an inhibition of both basal and insulin-mediated system A amino acid transport in skeletal muscle during septic and endotoxic shock (Karlstad and Sayeed, 1985; Karlstad and Sayeed, 1986). System A transports amino acids such as alanine, glycine, proline and other neutral amino acids across the cell membrane in skeletal muscle (Guidotti et al., 1978; Shotwell et al., 1983). It is an important transport system because it is the only amino acid carrier in skeletal muscle that is hormone-sensitive and capable of active transport (Guidotti et al., 1978; Shotwell et al., 1983). The active transport of amino acids by system A requires the presence of extracellular Na^+ and that most if not all of the energy used to transport type A amino acids is supplied by the free energy of the Na^+ electrochemical gradient (Bombara and Bergamini, 1968; Karlstad and Sayeed, 1985). This laboratory showed that the mechanism of Na^+ -dependent amino acid transport alterations in rat soleus muscle during endotoxic shock was due to a decrease

in V_{MAX} with no change in the apparent affinity (K_M) of the amino acid carrier for system A amino acids (Karlstad and Sayeed, 1986). A reduction in amino acid transport would affect the availability of amino acids for protein synthesis by regulating the size and the composition of the intracellular amino acid pools in skeletal muscle (Biolo et al., 1992). It is possible that the reduction in protein synthesis in skeletal muscle following sepsis (Hasselgren et al., 1990; Hall-Angeras et al., 1991) and endotoxin shock (Jepson et al., 1986) may be due to alterations of amino acid transport.

Effect of PAF on Muscle Amino Acid Transport, *In Vivo*

The first aim of Part 1 of this dissertation was to determine the degree of involvement of PAF on the attenuation of system A amino acid transport in skeletal muscles of different fiber types during endotoxic shock. The uptake of AIB into muscle reflects its net influx into the muscle. Isolated rat soleus and EDL, which were used in this study for the assessment of amino acid transport, clearly were viable preparations as evidenced by the near linear uptake of AIB during the incubations. This study also showed that the uptake of AIB was an active process since the distribution ratio of AIB was greater than unity. The attenuation of system A amino acid transport by approximately 33% in soleus and EDL by endotoxic shock both supports and extends the previous findings from this laboratory (Karlstad and Sayeed, 1985; Karlstad and Sayeed, 1986).

The prophylactic administration of WEB 2086, a PAF receptor antagonist (Casals-Stenzel, 1987b; Chang et al., 1990), significantly attenuated the endotoxin-induced inhibition of system A amino acid transport by 26% in EDL. PAF receptor blockade also tended to attenuate the endotoxin-induced inhibition of amino acid transport in soleus (17%). These findings suggest that PAF is, in part, responsible for the inhibition of system A amino acid transport in skeletal muscle during endotoxic shock. The results also showed that the intravenous injection of PAF attenuated amino acid transport to a greater extent in EDL (15%↓) than in soleus (9%↓). In addition, there was a greater attenuation of amino acid transport in EDL (20%↓) than in soleus (7%↓) when incubated in the presence of plasma from PAF-treated rats. It was not surprising, therefore, that WEB 2086 provided a greater level of protection against the endotoxin-induced attenuation of amino acid transport in EDL than in soleus.

Ameliorating Effect of PAF Antagonist on Endotoxemia-Induced Attenuation of Muscle Amino Acid Transport

These results and a previous study from this laboratory (Boruff and Karlstad, 1993), taken together, have clearly shown that PAF, administered intravenously, reduces system A amino acid transport in both soleus and EDL in the rat. Previous studies from this laboratory (McKeen et al., 1990) and others (Qi and Jones, 1990; Zhang et al., 1991)

have shown severe and immediate hypotension with a dose of PAF similar to that used in these experimental studies given by bolus or constant iv infusion in the rat. A transient fall in mean arterial blood pressure to approximately 40 mmHg within minutes after an iv injection of 2 or 4 $\mu\text{g/kg}$ PAF and a return of blood pressure to near control levels within 1 to 2 hr has been measured. Since preliminary experiments in this laboratory demonstrated that the prophylactic administration of WEB 2086 at the dose used in this work completely blocked the hypotensive effects of PAF (2 or 4 $\mu\text{g/kg}$ iv) in the rat, this dose was used to assess the role of PAF as a mediator of system A amino acid transport alterations in skeletal muscle during endotoxic shock. Qi and Jones (Qi and Jones, 1990) showed that WEB 2086 at a dose (5 mg/kg) lower than that used in this study prevented hypotension, maintained heart rate, and prevented increases in plasma lactate and catecholamine levels in a rat model of endotoxic shock. In the current study, a control group of rats given WEB 2086 alone was not included because it has been shown that WEB 2086 has no effect on basal hemodynamics or metabolic variables in rats (Qi and Jones, 1990). Previous studies have shown that a bolus iv injection of endotoxin (10 to 20 mg/kg) in the rat produces typical signs of severe endotoxic shock such as hemorrhagic lesions of the bowel, alterations of intestinal mucosal permeability, acute lung injury, hyperlacticacidemia, hypoglycemia, and alterations of skeletal muscle system A amino acid transport (Karlstad and Sayeed, 1985; Karlstad and Sayeed, 1986; Hsueh et

al., 1987; Lang et al., 1987; Chang et al., 1990). The bolus iv injection of PAF or endotoxin did not cause death at 1 or 5 hr, respectively, but both groups of rats showed many of the typical signs of the shock response in this study. The PAF-treated rats and endotoxin-treated rats were studied at 1 and 5 h, respectively, because the typical signs of shock are present at this time in both groups.

Effect of PAF on Muscle Amino Acid Transport, *In Vitro*

The second aim of Part 1 was to determine if PAF added to the *in vitro* incubation media would directly attenuate system A amino acid transport in muscles obtained from control rats and/or further reduce amino acid transport in muscles from PAF-treated rats. Amino acid transport was not affected by the addition of PAF over a 100-fold concentration range to incubation media containing soleus and EDL of control rats. In contrast, PAF that was added to the incubation media caused a further reduction in amino acid transport in soleus and to a lesser extent, though not significantly ($p = 0.08$), reduced amino acid transport in EDL of PAF-treated rats. Further reduction of amino acid transport in muscles of PAF-treated rats after the addition of PAF to the incubation media, while the control muscles remained unaffected, implies that the attenuation of amino acid transport by PAF depends on previous exposure of soleus and EDL to conditions or factors generated following the i.v. injection of PAF. This implication is supported by the fact that an i.v. administration of

PAF to the rats induces similar metabolic changes seen during sepsis and endotoxemia, such as hyperglycemia, hyperlactacidemia, and increases of plasma catecholamines and glucagon concentrations in plasma (Lang et al., 1987; Lang et al., 1988). In addition, PAF has been shown to upregulate the production and release of other shock mediators including TNF and IL-1 (Braquet et al., 1989; Mencia-Huerta and Braquet, 1989; Bonavida et al., 1990; Pignol et al., 1990a; Pignol et al., 1990b).

Effect of Plasma from PAF-Treated Rats on Muscle Amino Acid

Transport, *In Vitro*

System A amino acid transport was significantly attenuated in EDL and tended to be lower in soleus from control rats that were incubated in media containing plasma from endotoxin-treated rats as compared with plasma from PAF-treated rats. It was noted that amino acid transport was reduced to a greater extent in EDL (22%↓) than in soleus (9%↓) by plasma from endotoxin-treated rats as compared with plasma from PAF-treated rats. These results were not compared with the amino acid transport measured in muscles from control rats incubated in media containing plasma obtained from control rats because previous experiments revealed that plasma of PAF-treated rats reduced amino acid transport in both soleus and EDL as compared with plasma of control rats. Since endotoxin administration increases PAF production (Inarrea et al., 1985; Feuerstein and Siren, 1988; Mozes et al.,

1991) it is reasonable to assume that PAF levels were increased following the i.v. injection of endotoxin. It is possible that the endogenous release of PAF (due to endotoxin) was greater or more potent than exogenously administered PAF to generate the conditions and/or factors that reduced amino acid transport following the i.v. injection of PAF in soleus and EDL. Thus, the conditions or factors responsible for the attenuation of system A amino acid transport may be similar to those present in plasma from endotoxin-treated rats and the i.v. injection of PAF. This is because the attenuation of amino acid transport due to the i.v. injection of PAF and plasma from PAF-treated rats as well as the improvement of amino acid transport by WEB 2086 during endotoxemia was greater in EDL than soleus.

Conclusion of the Effect of PAF on Amino Acid Transport

Part 1 of the study showed that system A amino acid transport was attenuated in soleus and EDL following the i.v. injection of PAF or endotoxin and that the prophylactic administration of WEB 2086, a PAF receptor antagonist, partially ameliorated the attenuation of amino acid transport induced by endotoxic shock. Furthermore, PAF receptor blockade improved the endotoxin-induced attenuation of amino acid transport to a greater extent in EDL than in soleus. This may be due to the differences in muscle metabolism since soleus and EDL are different in their fiber types and glycolytic and oxidative capacity. It has been shown that sepsis may affect energy

metabolism differently in different types of skeletal muscle and that might lead to other metabolic disturbances in muscle (Angeras et al., 1991). The Na^+ -electrochemical gradient which provides the free energy for the transport of amino acids via system A may be reduced to a greater extent in EDL than in soleus due to these metabolic differences. Results from this part of the dissertation suggest that *in vivo* administration of PAF presumably releases factors in plasma that are responsible for the attenuation of amino acid transport and that these factors may be similar to those released during endotoxic shock. Furthermore, the direct attenuation of amino acid transport by PAF depends upon previous exposure of soleus and EDL muscles to the conditions or factors generated following the i.v. injection of PAF. Since the composition of the intracellular amino acid pools may be important in the regulation of protein synthesis, the attenuation of amino acid transport via system A in skeletal muscle mediated by PAF may contribute to alterations of protein metabolism during injury and shock.

PART 2: EFFECT OF PAF ON PROTEIN METABOLISM

This study demonstrates that PAF may be an important mediator of protein catabolism in such protein wasting conditions as endotoxemia (Ling et al., 1995), burn (DeMichele et al., 1988; Karlstad et al., 1988), trauma (Shaw and Wolfe, 1989), and sepsis (Hasselgren et al., 1990), where it is known that the level of PAF is elevated in the circulation (Feuerstein and Siren, 1988;

Lopez Diez et al., 1989; Qi and Jones, 1990; Zhang et al., 1991). The iv infusion of PAF provided a 35% reduction of MABP (~75 mmHg) similar to that seen during hypotensive shock. The constant infusion of PAF following the bolus injection was necessary to sustain this hypotensive effect for the entire 4 h experimental period as previously reported (Lang et al., 1988). The combination of a bolus and continuous iv infusion of PAF has been shown to produce changes of glucose metabolism as well as elevations of stress hormones in plasma similar to those seen during endotoxemia (Lang et al., 1988). We presumed that PAF infusion would simulate the complex hemodynamic, hormonal, and metabolic changes associated with endotoxemia and septic shock, and therefore, constitute an appropriate model to study the role of PAF as a possible mediator of the alterations of protein and amino acid metabolism during these stress states.

Effect of PAF on Whole-Body and Skeletal Muscle Protein Metabolism

This study shows that an exogenous infusion of PAF mediates alterations of whole body and skeletal muscle protein metabolism in the rat, *in vivo*. PAF decreased whole body protein synthesis and leucine flux. The increase in the percentage of leucine flux oxidized and the ratio of leucine oxidation to protein synthesis indicate that the portion of leucine flux attributed to leucine oxidation was increased at the expense of protein synthesis in PAF-treated rats. These findings are similar to that seen during burn injury

(Karlstad et al., 1988) and TNF infusion (Flores et al., 1989; Sakurai et al., 1994). The fractional rate of protein synthesis (FSR) was measured for three skeletal muscles of different fiber type compositions because sepsis has been shown to have differential effects on myofibrillar protein and energy metabolism in red and white muscles (Angeras et al., 1991; Hall-Angeras et al., 1991). The effect of PAF on protein synthesis, however, appeared to be independent of fiber type composition in this study, since PAF caused a similar reduction (~30%) of protein synthesis in EDL, soleus, and rectus abdominus muscles. These early acute changes in protein metabolism may be due, in part, to a PAF-induced attenuation of system A amino acid transport in skeletal muscle (Boruff and Karlstad, 1993; Buripakdi and Karlstad, 1994). This is because an attenuation of amino acid transport may restrict amino acid availability for cellular metabolism and limit protein synthesis in skeletal muscle (Biolo et al., 1992). The overall decrease in skeletal muscle protein synthesis would contribute to the decrease in whole-body protein synthesis since skeletal muscle constitutes approximately 45% of body mass (Haverberg et al., 1975).

The rate of protein synthesis is variable and sensitive to a variety of factors (Garlick et al., 1973; Millward et al., 1976) and may be the result of changes in the content and activity of RNA (Millward et al., 1973). Previous studies have shown that both the content and activity of muscle RNA is reduced in conditions that depress protein synthesis (Millward et al., 1973; Fong et al., 1991). RNA activity, which reflects the regulation of protein

synthesis at the post-transcriptional level (Jepson et al., 1988; Fong et al., 1989), declined substantially in rectus abdominus muscle of PAF-treated rats, whereas the content of total RNA was not significantly different between the groups. This indicates that the PAF-induced reduction of FSR in skeletal muscle was due to a down-regulation of protein synthesis at the post-transcriptional level similar to that seen during the administration of endotoxin and cytokines (TNF- α or IL-1 α) (Fong et al., 1989). In contrast, the regulation of protein synthesis in skeletal muscle has been suggested to be at the transcriptional level (mRNA content) in simple starvation or dietary manipulation (Jepson et al., 1988; Fong et al., 1989).

Effect of PAF on Skeletal Muscle Myofibrillar Proteins

We investigated the distribution of protein in different fractions of rectus muscle according to solubility and found that PAF significantly decreased protein content in the Triton-X-100-soluble fraction (membrane protein pool). Western blot showed that myosin was qualitatively reduced and actin was unchanged in the membrane, cytoskeletal and total tissue fractions of muscle from PAF-treated rats. Myosin constitutes ~12% of total protein content in muscle (Fulton, 1984). Therefore, the major reduction of myosin-to-actin ratio in the total tissue extract of rectus abdominus muscle induced by PAF would be equivalent to a minor overall reduction of total protein content. Myosin was recently suggested to be a major muscle protein subjected to net catabolism

during sepsis (Ahmad et al., 1994). However, this study did not reveal any changes that would indicate an apparent increase of myosin proteolysis in muscle, such as fragmented proteins that represent proteolytic products on the SDS-PAGE and western blot, or a notable rise of 3-m-histidine muscle content that represents myofibrillar protein breakdown (Young and Munro, 1978).

Myosin synthesis has been shown to occur locally at the site of the cytoskeleton (Isaacs and Fulton, 1987). It is, therefore, conceivable that the decrease of myosin distribution to the membrane and cytoskeleton portions of skeletal muscle is due to a disruption of this process and/or deficit in the efficiency of myosin synthesis.

Effect of PAF on Plasma Amino Acids

Derangements of protein metabolism in sepsis have been shown to lead to changes in the free amino acid pattern in plasma (Jeevanandam et al., 1989; Petersson et al., 1992). PAF caused the amino acid profile of plasma to change in association with alterations of protein metabolism. The individual plasma BCAA and total plasma concentrations of BCAA were substantially reduced in PAF-treated rats as compared with controls. The increase in the percentage of leucine flux oxidized had the expected effect of lowering the plasma leucine concentration in PAF-treated rats. The demand for BCAA in severe injury is thought to be increased relative to that for the other amino acids (Birkhahn et al., 1986). Animal and human studies have shown that with

BCAA infusions, nitrogen retention and protein synthesis are improved in rats and nitrogen retention becomes positive earlier in traumatized patients (Hammarqvist et al., 1988). It has been reported that BCAA (Hammarqvist et al., 1988), in particular, leucine (Millward et al., 1976; Goldberg, 1983), stimulate skeletal muscle protein synthesis and inhibit breakdown. In this study, the reduction in muscle protein synthesis may be related to the decrease in the plasma concentrations of BCAA to a level that was rate-limiting for protein synthesis in PAF-treated rats.

Effect of PAF on Liver Protein Metabolism

The decrease in whole-body protein synthesis in PAF-treated rats was not due to a decrease in liver protein synthesis since liver FSR was maintained at control levels in PAF-treated rats. However, the small reduction (7%) in total liver protein content would suggest an elevated rate of liver protein loss in PAF-treated rats as compared with controls. The amino acid profile in liver showed small reductions in BCAA levels, similar to that in plasma, and indicates minor effects of PAF on liver amino acid metabolism. The small decrease in plasma protein levels in PAF-treated rats may be the direct effect of an increase in breakdown (consumption/albumin requirements) or an indirect effect of an increase in the permeability of the microvasculature to fluid and protein (Peter et al., 1972). Any increase in the distribution space would tend to dilute total body protein pools and reduce plasma protein levels. Alterations

in the amino acid pattern in liver would be expected in the protein catabolic stage during severe injury (Jepson et al., 1986; Hasselgren et al., 1988; Karlstad et al., 1988). We did not find any other major change in the liver amino acid profile except for the reduction in leucine, valine and sum BCAA which might have reflected the changes in plasma BCAA. A slight decrease in BCAA levels in liver were suggested to be contributed by plasma in early sepsis where the pattern of free amino acids in liver was not disturbed (Roth et al., 1985).

Conclusion of the Effect of PAF on Protein Metabolism

In this study we found that the iv infusion of PAF induced net protein catabolism by reducing protein synthesis in rectus abdominus, soleus, and EDL muscles. This reduction of protein synthesis in skeletal muscle might be due to suppressed efficiency at the post-transcriptional level in PAF-treated rats. Alterations in the plasma amino acid profile reflected abnormalities in tissue protein and amino acid metabolism. These abnormalities reflect the cumulative effects of PAF infusion which we have shown to a) increase oxidation of leucine (and maybe other BCAAs) at the expense of protein synthesis, b) reduce protein synthesis in skeletal muscle, and c) attenuate amino acid uptake via system A as we previously reported (Boruff and Karlstad, 1993; Buripakdi and Karlstad, 1994). Results from this study indicate that PAF has a significant effect on amino acid and protein metabolism in skeletal muscle,

whereas there was not such an impact on liver. Although the myofibrillar proteolysis was not prominent, an exclusive reduction of myosin relative abundance suggesting that myosin may be an immediate target for net degradation in skeletal muscle during shock. Thus, the release of PAF during shock and sepsis may be a crucial event for the initiation of the net protein catabolism of skeletal muscle.

VII. SUMMARY

SUMMARY

The data presented in this dissertation is divided into two parts to demonstrate the effect of PAF on skeletal muscle amino acid transport and whole-body and tissue protein metabolism in rats during shock. The PAF-induced shock model used in this dissertation was achieved by an iv infusion of 2 or 4 $\mu\text{g/kg}$ PAF which caused an immediate and severe hypotension similar to that observed in hypotensive shock. A constant infusion of PAF following a bolus injection was necessary to maintain a 35% reduction of MABP to approximately 75 mmHg for the entire infusion period. The combination of a bolus and continuous iv infusion of PAF has been shown to produce changes of glucose metabolism and elevations of stress hormones in plasma similar to those seen during endotoxemia (Lang et al., 1988).

The first part of this dissertation attempted to demonstrate the role of PAF in the attenuation of amino acid transport via system A in skeletal muscle during endotoxic shock. This was achieved by measuring the transport rate of 1- ^{14}C - α -aminoisobutyric acid (radioisotope-labeled system A amino acid analog, non-metabolizable) into soleus and EDL muscles of rats that received i.v. injections of endotoxin, PAF, or WEB 2086 (PAF antagonist)/endotoxin. The findings from this part of the dissertation have suggested that 1) PAF is, in part, responsible for the attenuation of system A amino acid transport in

skeletal muscle during endotoxic shock; 2) PAF attenuates amino acid transport in skeletal muscle due to conditions and/or factors released in plasma that may be similar to those existing during endotoxic shock; and 3) the direct inhibition of amino acid transport *in vitro* by PAF depends upon previous exposure of skeletal muscle to those conditions (hyperglycemia and hyperlactacidemia) or factors (such as catecholamines, glucagon, and TNF) generated by PAF *in vivo*.

The second part of this dissertation was devoted to further explore the role of PAF as a possible mediator of protein catabolism during shock. This was achieved by determining L-[1-¹⁴C]leucine kinetics in whole-body, liver, soleus, EDL, and rectus abdominous muscles from rats receiving a 4-h constant i.v. infusion of PAF together with L-[1-¹⁴C]leucine after a priming bolus i.v. (4 µg/kg) of PAF. In addition, amino acid profiles, using HPLC, in plasma, liver, soleus, EDL and rectus abdominous muscle from PAF-treated and control rats were determined. Finally, the content and activity of RNA for protein synthesis and the relative abundance of myofibrillar protein in rectus abdominous muscle were also determined. The findings from the second part of this dissertation suggested that PAF mediates a reduction in whole-body protein synthesis by attenuating protein synthesis in skeletal muscle which may result from 1) inhibition of amino acid uptake by skeletal muscle; 2) a shift in the utilization of BCAA, especially leucine, in favor of oxidation over incorporation into protein; 3) the overall alteration of the amino acid profiles in

plasma and skeletal muscle; and 4) a possible reduction in the efficiency for protein synthesis in skeletal muscle. Although the rate of whole-body protein breakdown remained at the basal level, results from this part of the dissertation have revealed a decrease in myosin relative abundance in skeletal muscle suggesting a selective breakdown of muscle protein. The reduction of muscle protein synthesis and the susceptibility of myosin for catabolism, would suggest a possible explanation for the decrease in skeletal muscle protein contents if PAF in the systemic circulation is elevated during shock.

Thus, PAF is a potential mediator of protein catabolism by causing an attenuation of amino acid uptake and reduction of protein synthesis in skeletal muscle during shock as illustrated in Figure 14.

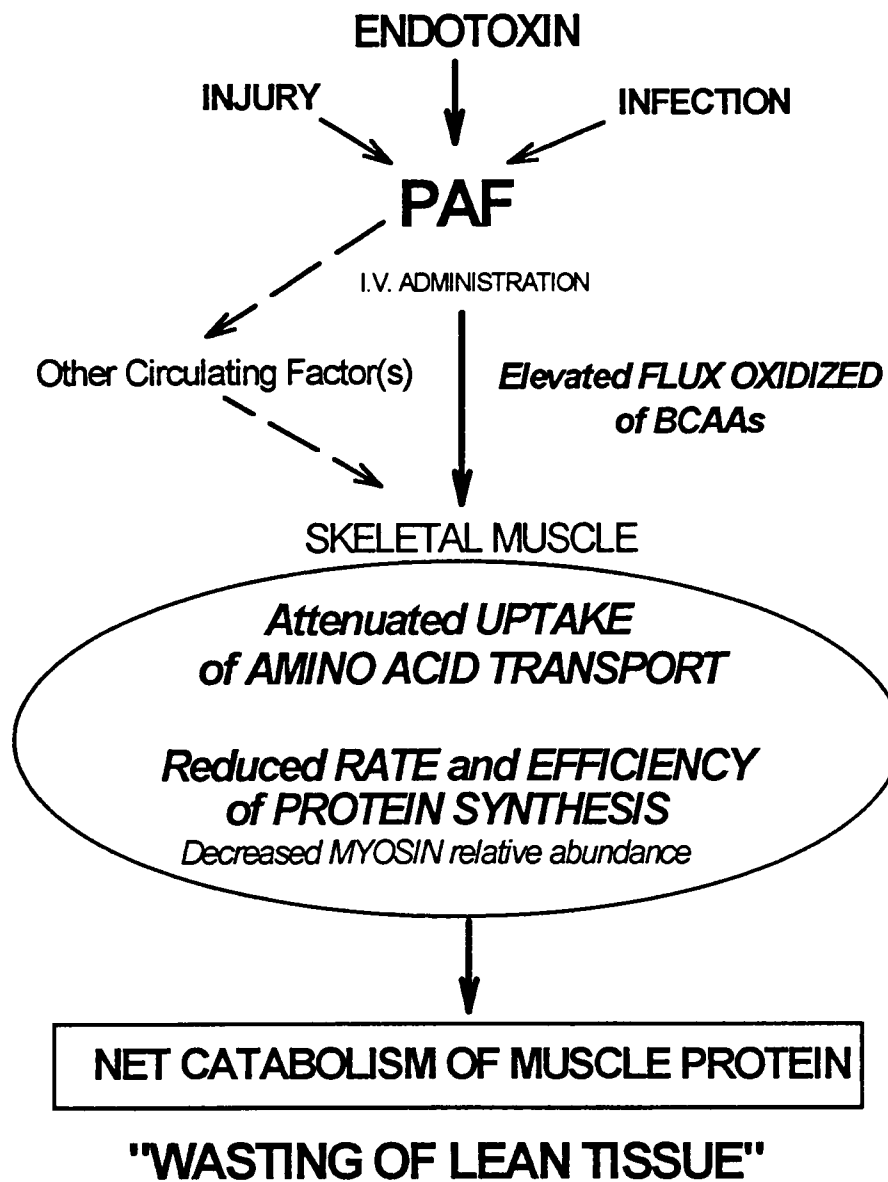


Figure 14. The effect of PAF on protein and amino acid metabolism. The findings from this dissertation are represented in italicized characters.

VIII. CONCLUSIONS AND PERSPECTIVES

CONCLUSION

This dissertation was initiated with the aim of understanding the role of PAF as a potential mediator of the protein catabolic response to shock and sepsis. Previous studies have given considerable insight into the nature of protein catabolism during sepsis and injury. Thus, major characteristics of protein catabolism include reductions of protein synthesis and net amino acid uptake and elevation of protein breakdown in lean tissue, mainly skeletal muscle, which then leads to wasting of muscle protein and loss of body nitrogen. The clinical management of protein metabolism during sepsis and injury has been difficult because of the complexity of the regulation of metabolism by various mediators released during sepsis and injury. The specific contributions of the individual mediators to the overall protein metabolic response, therefore, remains to be investigated.

Among several mediators released during sepsis and injury, PAF, a biologically active phospholipid, has been indicated as a potential factor that mediates sepsis- and injury-induced shock, bowel necrosis, lung injury, multi-organ failure and death. Previous studies have characterized some effects of PAF including hemodynamics, cardiac functions, and glucose metabolism. Thus, PAF can cause hypotension, microvasculature leakage, tachycardia, and elevation of plasma glucose and lactate levels in experimental animals.

An interesting finding of this dissertation was the ability of PAF to attenuate amino acid transport and protein synthesis in skeletal muscle. In accordance with the initial observations that PAF induced alterations of hemodynamics, cardiovascular function and glucose metabolism, PAF was shown to be effective at induction of amino acid and protein metabolic derangements which were similar to those that occur during sepsis and injury. Another important aspect of this dissertation was that most of the metabolic effects that PAF exerted on amino acid and protein metabolism in skeletal muscle and in the whole-body occurred within a relatively short period of time (1-5 h following PAF i.v. administration). This has confirmed the hypothesis of this dissertation that PAF may be one of the key mediators that potentiate amino acid and protein catabolic responses to trauma, injury, sepsis and shock (see Figure 14 for an illustration).

PERSPECTIVES

Further studies suggested by this dissertation would be the investigation of the molecular mechanisms involved in the reduction of the efficiency for protein synthesis in skeletal muscle and the transduction of cellular signals following PAF-receptor binding in the muscle micro-environment. An interesting *in vivo* project would be to study a potential therapeutic approach through pharmacologic blockade of PAF to manage the early amino acid and protein metabolic disturbances induced in the early phase of sepsis and injury.

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APPENDIX

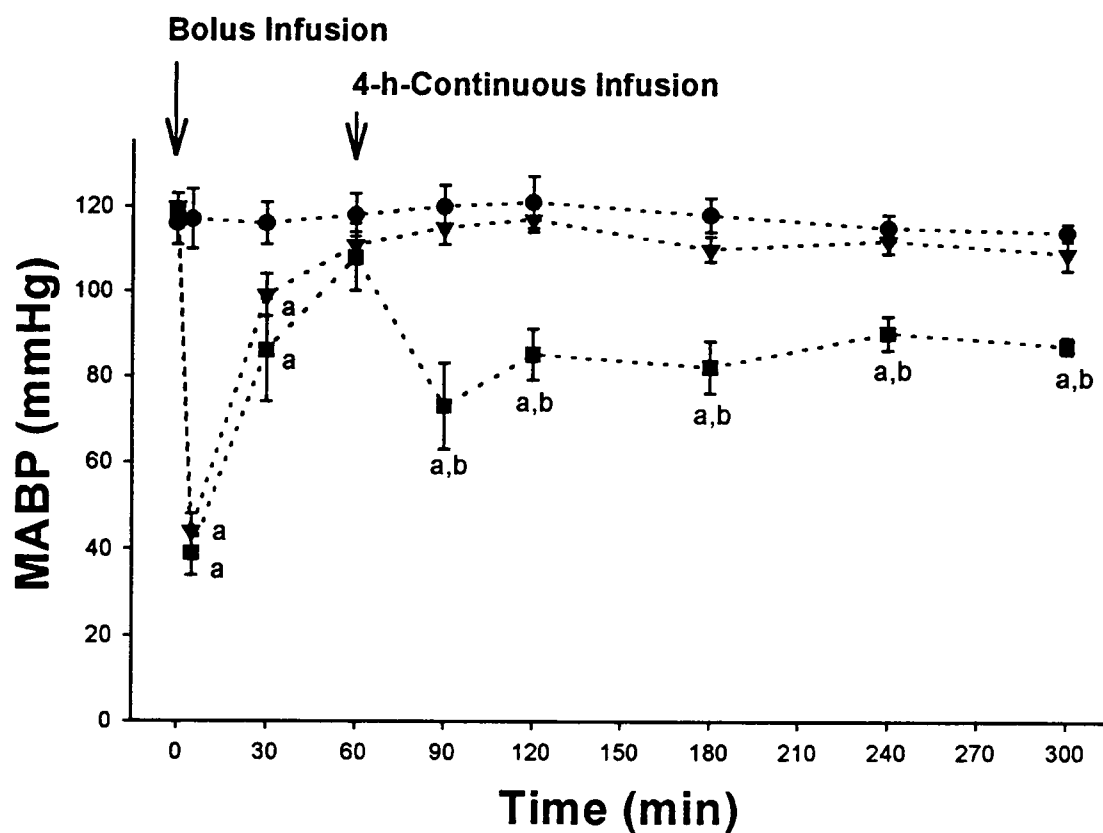


Figure 15. Alterations in mean arterial blood pressure (MABP) after bolus PAF infusion (PAF-B, solid triangle) or bolus PAF infusion and a 4-h-continuous infusion of PAF 1 h later (PAF-B/C, solid square). Values are means $\pm$ SE; $n = 5-6$ rats/group; a and b designate $p \leq 0.05$ versus time-matched control (solid circle) and PAF-B, respectively. All significant changes in PAF-B and PAF-B/C also reflect the same significant differences as compared with time-zero within each group. The dose of bolus PAF infusion is $2 \mu\text{g/kg}$ and the rate of continuous PAF infusion is $2 \mu\text{g/kg/h}$.

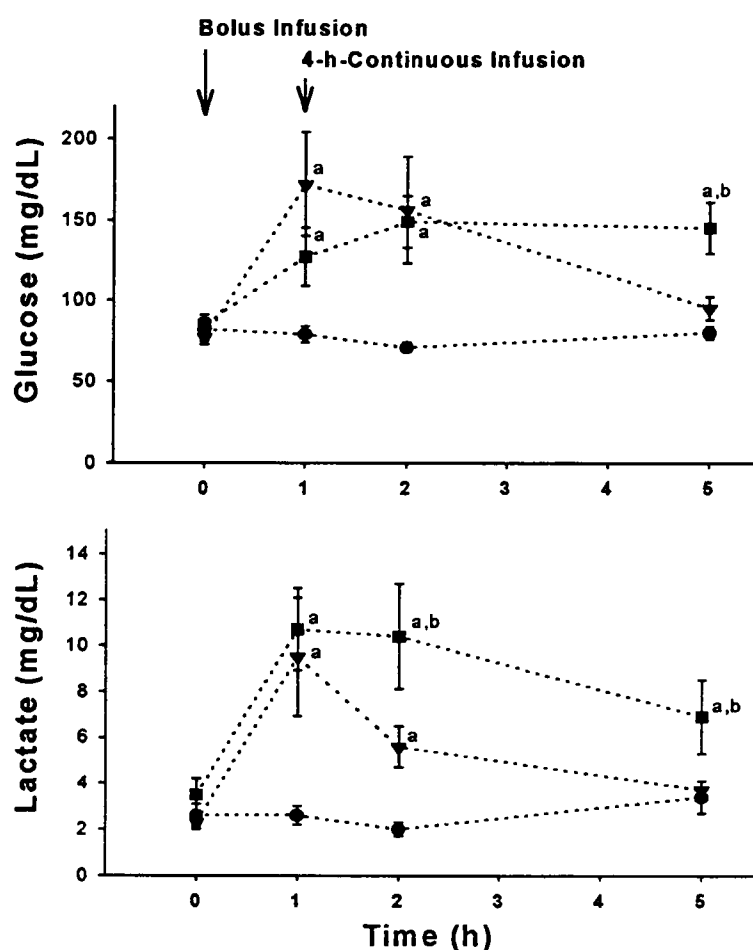


Figure 16. Alterations in plasma glucose (upper panel) and lactate (lower panel) concentrations after bolus PAF infusion (PAF-B, solid triangle) or bolus PAF infusion and a 4-h-continuous infusion of PAF 1 h later (PAF-B/C, solid square). Values are means $\pm$ SE; $n = 5-6$ rats/group; a and b designate $p \leq 0.05$ versus time-matched control (solid circle) and PAF-B, respectively. All significant changes in PAF-B and PAF-B/C groups also reflect the same significant differences as compared with time-zero within each group. The dose of bolus PAF infusion is 2 $\mu\text{g/kg}$ and the rate of continuous PAF infusion is 2 $\mu\text{g/kg/h}$.

Table 9. Alterations in hematological parameters after bolus PAF infusion (PAF-B) or bolus PAF infusion and 4-h continuous infusion of PAF 1 h later (PAF-B/C).

Parameter	Control	PAF-B	PAF-B/C
Hematocrit (%PCV)			
0-h	37 ± 2	39 ± 2	39 ± 3
1-h	36 ± 2	46 ± 1 ^a	43 ± 4 ^a
2-h	35 ± 2	38 ± 1 ^a	45 ± 3 ^{a,b}
5-h	37 ± 2	32 ± 2 ^a	34 ± 2 ^a
WBC ($\times 10^3$ Cell/μL)			
0-h	6.2 ± 0.8	7.1 ± 1.0	8.2 ± 0.9
1-h	6.8 ± 0.6	8.4 ± 1.7	7.2 ± 0.9
2-h	9.7 ± 2.1	14.8 ± 1.8 ^a	11.8 ± 2.0 ^b
5-h	8.5 ± 1.1	12.8 ± 2.0 ^a	16.8 ± 1.8 ^{a,b}
Lymphocytes ($\times 10^3$ Cell/μL)			
0-h	5.4 ± 0.8	4.2 ± 0.4	6.5 ± 1.0
1-h	5.7 ± 0.6	7.6 ± 1.6	6.3 ± 0.9
2-h	5.2 ± 1.5	5.5 ± 0.9	8.3 ± 1.6 ^{a,b}
5-h	4.2 ± 0.5	3.3 ± 0.8	6.1 ± 0.7
PMN ($\times 10^3$ Cell/μL)			
0-h	0.8 ± 0.3	2.9 ± 1.0	1.7 ± 0.8
1-h	1.1 ± 0.5	0.8 ± 0.3	0.9 ± 0.2
2-h	4.5 ± 1.1	9.4 ± 0.9 ^a	3.5 ± 1.1 ^b
5-h	4.3 ± 0.9	9.4 ± 1.5 ^a	10.7 ± 1.2 ^a

Values are means $\pm$ SE; n = 5-6 rats/group; ^a $p \leq 0.05$ versus time-matched control; ^b $p \leq 0.05$ versus time-matched PAF-B. All significant changes in PAF-B and PAF-B/C also reflect the same significant differences as compared with time-zero within each group, except PMN values in the control group at 2- and 5-h are significantly different as compared with time-zero. The dose of bolus PAF infusion is 2 μ g/kg and the rate of continuous PAF infusion is 2 μ g/kg/h.

VITA

Darunee Buripakdi was born in Phattalung, a southern province of Thailand, on May 6, 1966. Prior to pursuing a graduate study in the United States of America, she completed all her formal educations in Thailand. In 1970 she began her pre-elementary schooling at the Arunpradith School in Petchaburi province and then at the La-Or-Udhit School as her family moved to Bangkok in 1971 and live there ever since. From 1972 to 1978, she attended the Demonstrating Elementary School of Suansunandha Teaching College (currently, Suansunandha Campus of the Ratchapat Institute). She graduated from Bordindeja High School (Singha Singha-Saenee), in March of 1984. The following August she attended Chulalongkorn University and received the Bachelor of Science degree in Biochemistry in July 1988. Shortly after that, she was awarded a full scholarship to study abroad in advance degree from the Royal Thai Government via the Development and Promotion of Science and Technology Talents Project (DPST) of the Institute for Promotion of Science and Technology in Thailand. She decided to come to the United State of America and enrolled at the University of Tennessee, Knoxville, in January 1989 . She received a Doctor of Philosophy degree from the Life Sciences Intercollegiate Program in Physiology in December 1997.

Darunee plans to go back to Thailand and pursue an academic and research career in physiology and medical research at the Mahidol University.