Grisdale, Barbara S.

THE EFFECTS OF CATECHOLAMINES ON PROTEIN METABOLISM IN PIGS AND SHEEP

Iowa State University PH.D. 1986

The effects of catecholamines on protein metabolism in pigs and sheep

by

Barbara S. Grisdale

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Animal Nutrition

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1986

TABLE OF CONTENTS

GENERAL INTRODUCTION

The study of the naturally occurring catecholamines, epinephrine (EPI) and norepinephrine (NOR), has played an important part in the present understanding of endocrinology. As reviewed by Tepperman (24), EPI was the first hormone to be chemically identified and synthesized in 1905. Furthermore, receptor theories were first conceptualized using agents which blocked the binding of catecholamines to receptors. Lastly, the ubiquitous second messenger, cyclic AMP (cAMP), was discovered by an EPI investigator.

The adrenergic receptors were originally classified by Ahlquist (1) as alpha and beta, according to their responsiveness to EPI, NOR and other sympathomimetic amines. Subsequently, these receptor types were further classified into the subtypes α_1, α_2, β_1 and β_2, based on pharmacological criteria (23).

EPI has stimulating effects on all four receptor types, depending on the tissue. NOR elicits responses through α_1, α_2 and β_1, but has essentially no β_2 effects (8, 23). The adrenergic receptor types, their target tissues and responses, as reviewed by Exton (8) and Weiner and Taylor (26), are listed in Table 1. Each type of receptor elicits responses in numerous tissues. Furthermore, tissues such as smooth muscle and the pancreas may be affected positively by one type of receptor and negatively by another (Table 1). Tepperman (24) notes though, that the predominant effect usually masks the effects of the other receptors.

A major response to stress involves stimulation of the sympathetic nervous system, mediated by the neurotransmitter NOR. Stimulation of

Table 1. Adrenergic receptors and their target tissues and responses[a]

Receptor	Tissue	Response
α_1-Adrenergic	Smooth muscle (vascular, iris, ureter, pilomotor, uterus, gastrointestinal and bladder sphincters)	Contraction
	Smooth muscle (gastrointestinal)	Relaxation
	Liver	Glycogenolysis
	Heart	Increased force
	Salivary glands	K^+, H_2O secretion
	Sweat glands (localized)	Secretion
	Kidney (proximal tubule)	Gluconeogenesis, Na^+ reabsorption
	Brain	Neurotransmission
α_2-Adrenergic	Adrenergic nerve endings	Inhibition of norepinephrine release
	Platelets	Aggregation, granule release
	Adipose tissue	Inhibit lipolysis
	Endocrine pancreas	Inhibit insulin release
	Vascular smooth muscle	Contraction
	Kidney	Inhibit renin release
	Brain	Neurotransmission

[a]Adapted from Exton (8) and Weiner and Taylor (26).

Table 1, continued.

Receptor	Tissue	Response
$_1$-Adrenergic	Heart	Increased rate, conduction velocity, contractility
	Adipose tissue	Lipolysis
$_2$-Adrenergic	Liver	Glycogenolysis, gluconeogenesis
	Skeletal muscle	Glycogenolysis, lactate release, increased contractility, K^+ uptake
	Smooth muscle (bronchi, uterus, vascular, gastrointestinal, skeletal muscle, spleen capsule)	Relaxation
	Endocrine pancreas	Insulin secretion
	Salivary glands	Amylase secretion

the adrenal medulla also occurs, resulting in release of EPI and NOR into the general circulation (26). These actions result in simultaneous responses over the entire body. Weiner and Taylor (26) state that the result of this is that:

> "The heart rate is accelerated; the blood pressure rises; red blood cells are poured into the circulation from the spleen (in certain species); blood flow is shifted from the skin and splanchnic region to the skeletal muscles; blood glucose rises; the bronchioles and pupils dilate; and, on the whole, the organism is better prepared for 'fight or flight.'"

Galbo et al. (9) and Tepperman (24) have described resting plasma concentrations of EPI and NOR in man to be about 0.05 ng/ml and 0.2 to 0.4 ng/ml, respectively. In contrast, the stress of myocardial infarction (24) has been reported to increase plasma concentrations of EPI to 0.27 ng/ml and NOR to 4.1 ng/ml. Heavy exercise in man (9) increased the concentration of epinephrine to as high as 0.42 ng/ml and that of NOR to 2.22 ng/ml. Kozlowski et al. (14) reported that food deprivation in dogs increased the concentration of EPI from approximately 0.3 ng/ml after 4 h to about 0.7 ng/ml after 96 h. The concentration of NOR similarly rose from approximately 0.9 ng/ml to 2.8 ng/ml during the fast.

The second messengers involved in the response to the catecholamines include both Ca^{2+} and cAMP. The β_1- and β_2-adrenergic receptors elicit responses through mechanisms involving activation of adenylate cyclase, while α_2 receptors inhibit adenylate cyclase (23). The activation of adenylate cyclase promotes the accumulation of intracellular cAMP, which then leads to the activation of other enzymes such as cAMP-dependent protein kinase. These kinases phosphorylate and activate other enzymes which catalyze the final physiological response.

The initial step in α_1-adrenergic receptor action involves the breakdown of phosphatidylinositol-4,5-bisphosphate in the plasma membrane of target cells, producing myo-inositol-1,4,5-triphosphate (IP) and 1,2-diacylglycerol (8). The IP acts to rapidly mobilize intracellular stores of Ca^{2+}, which then bind to calmodulin. The physiological response is produced when the Ca^{2+}-calmodulin complex activates calmodulin-dependent protein kinases (8). The diacylglycerol

is thought to activate a Ca^{2+}-phospholipid-dependent protein kinase which also phosphorylates certain enzymes and generates subsequent physiological responses (8).

As indicated in Table 1, which was adapted from recently published, comprehensive tables of Exton (8) and Weiner and Taylor (26), listing the adrenergic receptors and their responses in effector organs, no mention is given of effects of adrenergic agonists on protein turnover in any tissue. A 1955 review (22) of the hormonal control of amino acid metabolism indicates though, that acute effects of EPI injections on protein metabolism were recognized as long as 50 years ago. The injections of EPI in rats resulted in a decrease in plasma amino nitrogen concentration and an increase in urea nitrogen excretion (22).

The breakdown of protein has been shown in vitro to be influenced by adrenergic compounds. Garber et al. (10) reported decreased rates of release of alanine and glutamine from preparations of rat epitrochlaris muscle, when EPI, NOR or isoproterenol (non-specific β-agonist) were added to the incubation media. The addition of the α-adrenergic blocker, phentolamine, to the media did not prevent the inhibitory effect of EPI on amino acid release, whereas the addition of propranolol, a β-adrenergic antagonist, resulted in a reversal of EPI's effect. The authors concluded from these studies that the effect of the catecholamines on protein degradation was mediated by β-adrenergic receptors. It has been noted though, that these results could be the result of either a reduction in protein degradation, as the authors suggested, and/or an increase in protein synthesis. An increase in protein synthesis would reduce the availability of substrate for the de

novo synthesis of alanine and glutamine, and thus the concentration of these amino acids in the media would be lower (7, 15). Li and Jefferson (15) also reported that perfusion of the isolated rat hemicorpus with isoproterenol resulted in a decrease in the rate of protein degradation, as measured by the dilution of ^{14}C-phenylalanine specific radioactivity in the media by unlabelled phenylalanine released from protein.

Evidence of an adrenergic effect on protein degradation in vivo was described by Miles et al. (18). In their study, post-absorptive humans were given a constant infusion of EPI sufficient to increase plasma EPI concentrations to stress levels. During this experiment, a decrease in the rate of appearance of leucine carbon in the plasma was indicative of reduced proteolysis.

Effects of adrenergic compounds on protein synthesis have also been described. EPI and NOR have been demonstrated to reduce the incorporation of ^{14}C-histidine into isolated rat diaphragms (27). However, there was no effect of isoproterenol on phenylalanine uptake into rat gastrocnemius muscles (15). In contrast to these in vitro studies showing decreases or no change in protein synthesis, chronic administration of the β_2-adrenergic agonists clenbuterol and fenoterol to rats for 16 to 19 d (7), resulted in increases in protein synthesis in the gastrocnemius muscle, of 34 and 26%, respectively.

The effect of adrenergic stimulation on amino acid oxidation has not been well studied. In dogs fasted for 24 h and treated for 4 h with a constant infusion of 0.1 or 0.5 $\mu g*kg^{-1}*min^{-1}$ EPI (16), the urea production rate did not significantly change from the control period. Liddell et al. (16) used the urea production rate as an indicator of net

protein breakdown or protein oxidation. An in vitro analysis of the effect of EPI on oxidation of amino acids in rat hemidiaphragms was reported by Buse et al. (4). It was determined that EPI could only stimulate the rate of leucine oxidation when the diaphragms were from fasted animals and when glucose and pyruvate were omitted from the media. Alanine oxidation was also stimulated in diaphragms from fasted rats treated with EPI, but no effect was found on histidine oxidation. The authors noted that the stimulation of branched chain amino acid oxidation in muscles from fasted animals may be secondary to the lipolytic effect of EPI in the fasted state, since branched chain amino acid oxidation is inhibited by fatty acids.

The first experiments illustrating effects of chronic administrations of EPI on storage of protein and fat in the intact animal may be those of Cunningham and associates (5). These authors showed that daily subcutaneous injections of 0.125 mg/kg EPI in corn oil for 6 weeks, significantly increased the percentage protein in the carcasses of young pigs, while reducing the fat content. Further investigations into the effects of adrenergic compounds on body composition were not reported until 1983. The renewed interest in these compounds may be due to the increased demand by consumers for leaner meat products.

In recent years, the effects of β-agonists on animal performance and carcass muscle and fat content have been well-illustrated. A summary of the performance and carcass composition data from representative experiments in studies in which β-agonists or EPI were fed or injected into animals is given in Table 2. Clenbuterol (7) and

Table 2. Effects of feeding and injections of adrenergic agonists on animal performance and carcass composition

Animal	Agonist	Dosage	Initial Weight	Time on Study	% Change from control			
					ADG[a]	G/F[b]	LMA[c]	Fat depth
Steers[d]	CLEN[e]	10 ppm	350 kg	98 d	-8	-1	+11*	-35**[f]
Lambs[g]	CLEN	2 ppm	41 kg	8 wk	+24**	+19**	+41**	-37**[f]
Lambs[h]	CIM[i]	10 ppm	17 kg	6 wk	-3	+6	+26*	-48*[j]
Swine[k]	CIM	1 ppm	60 kg	49 d	+4	+12*	+12*	-10*[l]
Swine[m]	EPI[n]	.125 mg/kg[o]	8 kg	6 wk	-17	-21	+5*[p]	-10[q]
Broilers[r]	CLEN	1 ppm	786 g	49 d	+5**[s]	+5**[s]	+2*[t]	-7*[u]
Rats[v]	CLEN	1 mg/kg[w]	150 g	16 d	+27**		+22**[x]	-5[y]

[a]Average daily gain.
[b]Gain to feed ratio.
[c]Longissimus muscle area.
[d]Ricks et al. (21).
[e]Clenbuterol.
[f]12th rib.
[g]Baker et al. (2).
[h]Beerman et al. (3).
[i]Cimaterol.
[j]Average of 2 measures over the longissimus and 2 over the rib.

[k] Jones et al. (13).
[l] Last rib.
[m] Cunningham et al. (5).
[n] Epinephrine.
[o] Subcutaneous injection once daily.
[p] Carcass protein as a percentage of carcass dry matter.
[q] Percentage carcass fat.
[r] Dalrymple et al. (6).
[s] Females and males combined.
[t] Percentage carcass protein in females.
[u] Percentage carcass fat in females.
[v] Emery et al. (7).
[w] Subcutaneous injections given twice daily.
[x] Body protein content.
[y] Body fat content.
* P<0.05 Denotes significantly different from controls.
** P<0.01 Denotes significantly different from controls.

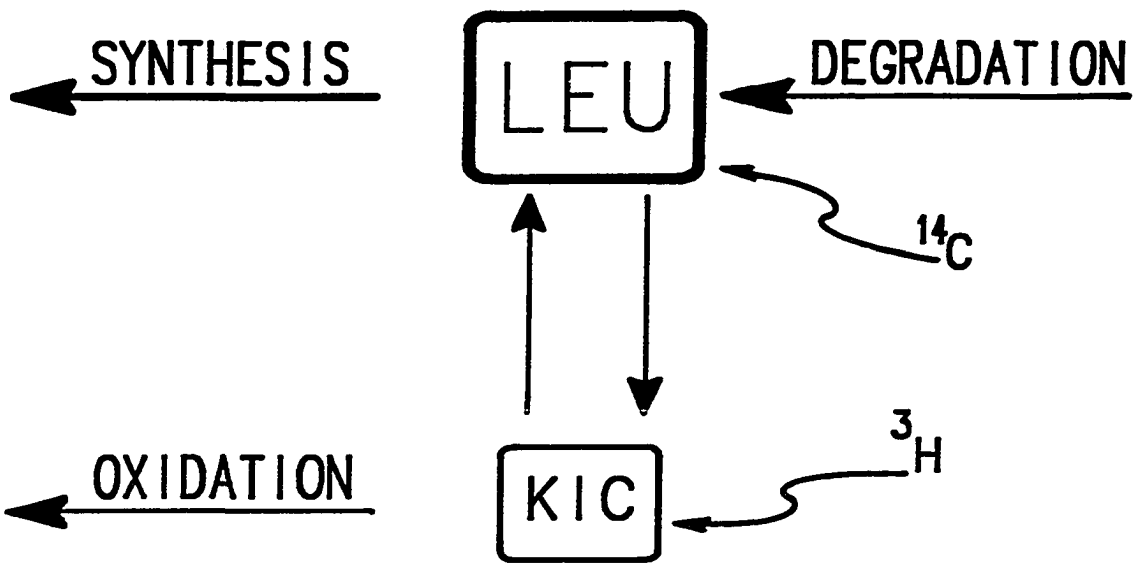
cimaterol (R. H. Dalrymple, American Cyanamid, Princeton, NJ, personal communication) are regarded as β_2-adrenergic agonists. The feeding of these compounds at low levels produces significant changes in the body composition of steers (21), lambs (2, 3), pigs (13), poultry (6) and rats (7). Significant increases in muscle size or percentage carcass protein was seen in all species tested, and a decrease in fat content was observed in all animals except rats. The effects of these compounds on body composition can be influenced by sex. Dalrymple et al. (6) reported that the response to clenbuterol in female broilers was greater than that in males.

Animal performance is not improved as consistently as body composition (Table 2) and may be influenced by animal maturity. In experiments involving lambs put on test at three different weights, Baker et al. (2) reported inconsistent average daily gain and feed conversion efficiency results when clenbuterol was fed to lambs put on test at weights of 33 and 37 kg, in comparison to the results obtained with lambs put on test at 41 kg. At high levels, these compounds can also negatively affect average daily gain (21).

These studies indicate that adrenergic compounds seem to influence muscle protein metabolism. In an attempt to better understand the details of the changes which may occur in protein metabolism during administration of adrenergic compounds, a two-pool model of protein turnover has been used. Several two-pool models involving the essential amino acid leucine and its keto-acid, α-ketoisocaproate (KIC), have been proposed (11, 17, 20). Practical and theoretical considerations of such models has been discussed by Helland et al. (12).

The initial step in protein turnover (Fig. 1) is the liberation of amino acids from the tissue protein pool. As in the case of leucine, the liberated amino acid can then be used to resynthesize protein or be catabolized (oxidized) for energy or used in the production of other substrates. In this model, [^{14}C]-leucine is used to label the body leucine pool and [^{3}H]-KIC is used to label the body KIC pool. In the fed state, this model is complicated by leucine entering the body pool from absorption of the diet. The dietary contribution can be estimated if an additional label, such as [^{13}C]-leucine, is utilized in the feed (20).

Figure 1. Whole-body leucine metabolism model. [^{14}C]leucine and [^{3}H]KIC infused


SYNTHESIS
LEU
DEGRADATION
^{14}C
^{3}H
OXIDATION
KIC

EXPLANATION OF DISSERTATION FORMAT

This dissertation is presented in the alternate format, as outlined in the Iowa State Graduate College Thesis Manual. Use of the alternate format allows for the preparation of independent sections that are suitable for submission to scientific journals.

Two separate papers have been prepared from the data collected from research performed to partly fulfill requirements for the Ph.D. degree. Each paper is complete in itself and has an abstract, introduction, materials and methods, results, discussion and reference section. The closeness of the subject matter of the two papers allowed a general discussion to be prepared.

ANIMAL CARE

The animals used in the following trials were treated in accordance with the Iowa State University animal care guidelines.

SECTION I. EFFECTS OF EPINEPHRINE AND NOREPINEPHRINE ON LEUCINE METABOLISM IN PIGS

EFFECTS OF EPINEPHRINE AND NOREPINEPHRINE ON LEUCINE METABOLISM IN PIGS[1]

Barbara Grisdale-Helland, Ståle J. Helland and Steven Nissen

Department of Animal Science,
Iowa State University,
Ames, Iowa 50011

[1]This project was supported in part by National Institute of Health Grant 31470 and by the Iowa Agriculture and Home Economics Experimental Station.

ABSTRACT

Whole-body leucine and α-ketoisocaproate (KIC) metabolism was estimated in young pigs during infusions of stress levels of epinephrine (EPI, 0.1 $\mu g*kg^{-1}*min^{-1}$), norepinephrine (NOR, 2.0 $\mu g*kg^{-1}*min^{-1}$) or the saline vehicle. A constant infusion of [1-^{14}C]leucine and [4,5-^{3}H]KIC was employed to estimate the rates of whole-body protein degradation, protein synthesis, KIC oxidation and interconversion of leucine and KIC. EPI infusion resulted in decreases in proteolysis and protein synthesis of 15 and 18%, respectively. NOR infusion resulted in decreases in proteolysis, protein synthesis and oxidation of 25, 25 and 24%, respectively. Leucine and KIC interconversion also decreased during the NOR infusion. Free fatty acid concentrations increased threefold and glucose concentrations increased twofold during the NOR infusion. Glucose was also elevated by 30% during the EPI infusion. Insulin concentrations did not change during any treatment, but glucagon and cortisol concentrations were increased by NOR. These data suggest that the liberation of energy-producing substrates from glycogen and adipose tissue was sufficient to reduce the need for amino acid oxidation during the NOR infusion, but was insufficient to decrease oxidation during the EPI infusion. Therefore, in stress situations, decreases in protein turnover will conserve energy and thus, augment the desired increase in readily available energy.

INTRODUCTION

Epinephrine (EPI) and norepinephrine (NOR) have an important function in mobilizing readily available energy for body tissues during periods of stress. This is achieved by enhancing glucose availability through increased glycogenolysis in liver and muscle (7, 13), and the augmentation of lipolysis (35) for the providing free fatty acids (FFA) for energy and glycerol for gluconeogenesis. The muscle protein also represents a potential energy store which could be liberated during periods of stress, although it is regarded as a less efficient energy source, because of the energy requirement associated with detoxification of the nitrogen waste (15). Furthermore, since protein synthesis, and perhaps protein degradation, are energy requiring processes (24), the energy expenditure of the animal can be influenced by changes in protein metabolism.

Several in vitro experiments have indicated that perfusion of rat muscle with EPI or NOR results in a decrease in the rate of release of amino acids (6) and a decrease in the rate of amino acid incorporation (34). An in vivo study with humans, in which EPI was infused for 3 h (20), also showed decreased appearance of leucine carbon in plasma, indicative of reduced proteolysis. The rate of amino acid oxidation has been shown to increase upon perfusion of rat diaphragm muscle with EPI (4), although oxidation was suppressed when pyruvate or glucose was added to the media. When EPI was infused into fasted dogs (19), however, no influence on the rate of protein oxidation was observed. These studies suggest that EPI and NOR influence certain aspects of protein metabolism. It was not clear from this information, however,

what the magnitude of these effects would be in relation to each other, since only one aspect of protein metabolism was usually analyzed. Thus, the objectives of the present experiment were to study the influence of constant infusions of EPI and NOR on leucine metabolism (protein synthesis, protein degradation, leucine and α-ketoisocaproate (KIC) interconversion and leucine oxidation) at the whole-animal level, and also to observe changes occurring in the concentrations of several metabolites and hormones as a result of these infusions, in pigs.

MATERIALS AND METHODS

L-[1-^{14}C]leucine (57 mCi/mmol) was purchased from Amersham and L-[4,5-^{3}H]leucine (58 Ci/mmol) was purchased from ICN Biomedicals. [^{3}H]KIC was enzymatically prepared from [4,5-^{3}H]leucine (27). EPI and NOR (arterenol hydochloride) were obtained from Sigma Chemical. The drugs were dissolved in a 0.9% sodium chloride solution containing 0.2% serum albumin.

Six female pigs (23 kg) were used in a cross-over design. Each animal was surgically implanted with an infusion catheter in the left ventricle via the carotid artery and a sampling catheter in the right ventricle via the internal jugular vein, 4 to 6 days before experimentation.

The pigs were fasted for 24 h before the start of each experiment. Two d of normal feeding separated each experiment day. A primed-dose constant infusion of [^{3}H]KIC and [^{14}C]leucine (1.5 and 0.6 $\mu Ci*min^{-1}$, respectively) was started at approximately 8 am. The priming dose was ten times the constant infusion dose for one min. The start of the treatment infusions was delayed for 2 h to allow for equilibration of the isotopes. Before the start of the treatments, four blood samples were taken in the latter 15 min of the basal steady state period. At zero time, a 2 h constant infusion of EPI (0.1 $\mu g*kg^{-1}*min^{-1}$) or NOR (2.0 $\mu g*kg^{-1}*min^{-1}$) was started. Two of the pigs were also studied during infusion of the saline-albumin vehicle. After the start of the treatment, blood was sampled at 10, 20, 30, 45, 60, 75, 90, 105 and 120 minutes. The blood was collected into tubes containing EDTA and cooled immediately on ice. One ml of whole blood was added to tubes containing

3 ml 1.5 N perchloric acid and frozen at -80 C for later analysis. The remaining blood was centrifuged and the plasma was collected and stored at -80 C until analyzed for hormones and metabolites. Trasylol (500 U/ml of plasma) was added to the samples which were to be analyzed for glucagon.

Whole-blood leucine and KIC concentrations were determined by high-performance liquid chromatography (HPLC, 22). The HPLC effluent corresponding to the leucine or KIC peaks was collected and the ^{14}C and ^{3}H radioactivity determined by dual isotope liquid scintillation counting.

Plasma glucose concentrations were determined using the glucose oxidase method (Worthington Diagnostics). Free fatty acid concentrations were analyzed enzymatically (29). Radioimmunoassays were used to measure insulin (33), glucagon (8) and cortisol (Gammacoat [^{125}I] Cortisol Radioimmunoassay Kit, Travenol-Genetech Diagnostics).

Rates of entry (proteolysis) and exit (protein synthesis) of leucine, exit of KIC (oxidation), and interconversion of leucine and KIC were calculated as described by Helland (9) for a reversible two-pool model. The specific radioactivities of ^{14}C-leucine and ^{14}C-KIC were found to be nonsignificantly different from each other and therefore, the average specific radioactivity of these two components was used in the flux calculations (9, 10).

It was subsequently determined that the plasma concentrations and specific radioactivities of leucine and KIC were constant between 75 and 120 min after the start of the hormone infusions, and thus a new steady state had been established. The Student's t-test (30) was used to

determine whether the change from the basal (mean of -15 to 0 min) to the final period (mean of 75 to 120 min), for each variable, was statistically different from zero. Because there were only 2 saline-infused animals, comparisons between treatments were not made. Data are presented as means $\pm$ SE.

RESULTS

The whole-blood concentrations of leucine and KIC in the basal and final periods are presented in Table 1. Leucine concentrations decreased significantly during the EPI infusion, while KIC concentrations decreased significantly during the NOR infusion.

Rates of proteolysis, protein synthesis, interconversion of leucine and KIC, and KIC oxidation, in the basal and final periods, for each treatment, are also presented in Table 1.

Both EPI and NOR infusions resulted in significant decreases in proteolysis (15 and 25%, respectively). Similarly, the rate of protein synthesis decreased by 18% during the EPI infusion and 25% during the NOR infusion. Leucine and KIC interconversion was not affected by EPI infusion, but during the NOR infusion deamination of leucine to KIC decreased by 29% and reamination was reduced by 30%. Only the infusion of NOR resulted in a significant change in KIC oxidation (0.82 to 0.62 $\mu mol*kg^{-1}*min^{-1}$).

No significant effects of the infusion of EPI were seen in the plasma concentrations of FFA in these pigs (Fig. 1). The infusion of NOR, however, resulted in a trebling of FFA concentrations by 20 min. This level was maintained throughout the infusion, resulting in a significant increase from the basal to the final period (592 $\pm$ 109 to 1870 $\pm$ 85 nmol/ml).

The infusion of EPI resulted in a significant change in glucose concentrations from the basal to the final period (91 $\pm$ 1 to 119 $\pm$ 7 mg/dl). This change was more gradual than that observed with the

Table 1. Leucine and KIC concentrations and leucine metabolism before, and during, infusion of saline, epinephrine or norepinephrine

	Time	Saline (N=2)	Epinephrine (N=6)	Norepinephrine (N=6)
Concentrations[a]				
Leucine	B[b]	125	137 ± 13	152 ± 12
	F[c]	129	108 ± 8*	136 ± 5
KIC	B	29.4	21.7 ± 1.6	28.6 ± 1.9
	F	26.6	27.4 ± 1.4	20.8 ± 1.1*
Fluxes[d]				
Proteolysis	B	4.32	4.90 ± 0.28	5.32 ± 0.31
	F	3.93	4.17 ± 0.23**	3.99 ± 0.23**
Synthesis	B	3.54	4.19 ± 0.25	4.50 ± 0.31
	F	3.17	3.44 ± 0.17*	3.37 ± 0.23**
Leu -> KIC	B	2.49	3.46 ± 0.30	3.85 ± 0.52
	F	2.68	3.50 ± 0.33	2.72 ± 0.33**
KIC -> Leu	B	1.72	2.75 ± 0.33	3.02 ± 0.50
	F	1.92	2.77 ± 0.31	2.10 ± 0.31**
Oxidation	B	0.78	0.71 ± 0.10	0.82 ± 0.07
	F	0.76	0.73 ± 0.09	0.62 ± 0.07*

[a]Units: μM ± SE.

[b]Basal time period (-15 to 0 min).

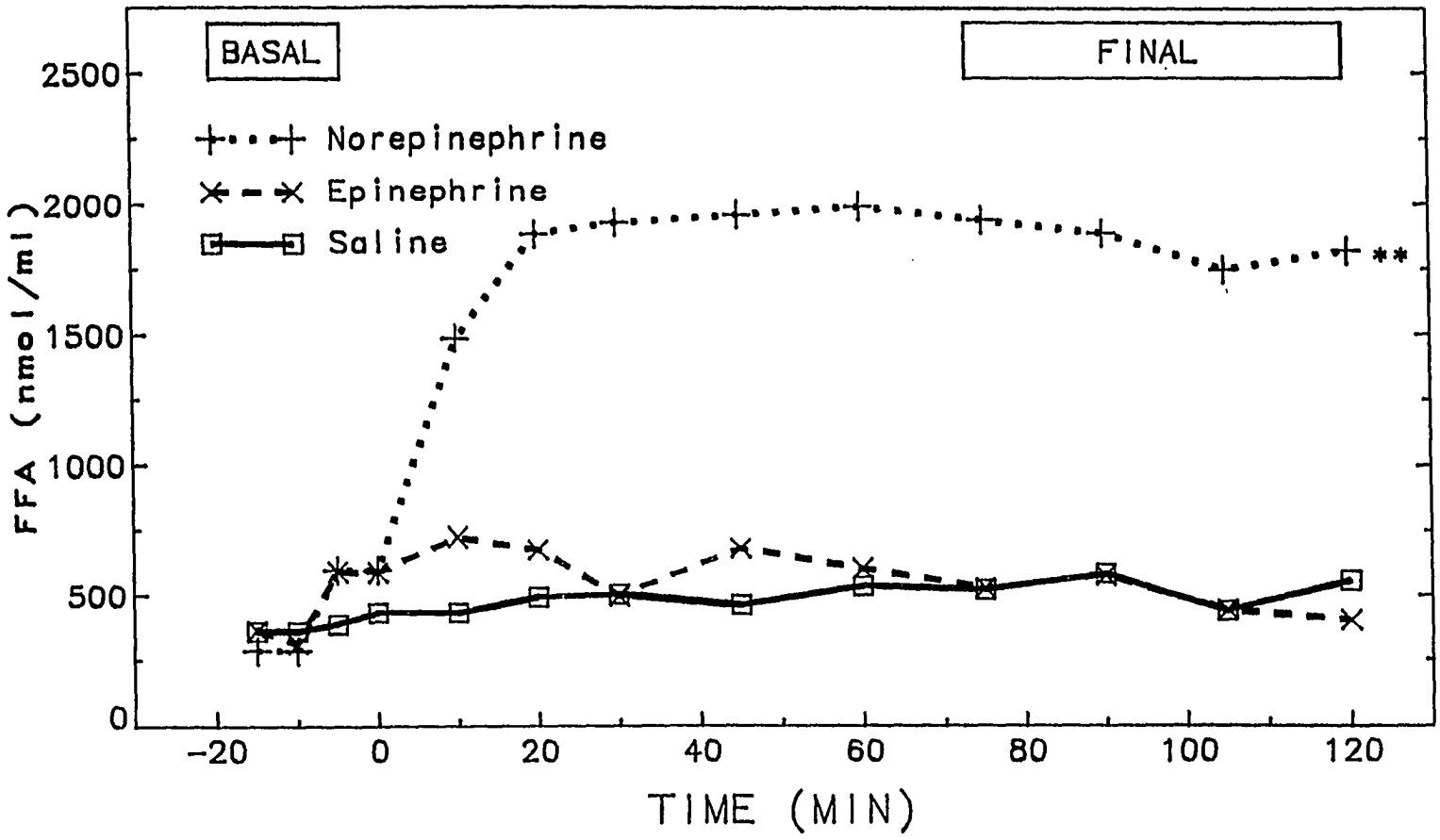
[c]Final time period (75 to 120 min).

[d]Units: μmol leucine or KIC*kg^{-1}*min^{-1} ± SE.

* Change from basal to final period significantly different from zero ($P<0.05$).

** Change from basal to final period significantly different from zero ($P<0.01$).

Figure 1. Plasma free fatty acid concentrations in young pigs before, and during infusion of saline, epinephrine or norepinephrine. Significance between basal and final periods is denoted by **P<0.01, within a treatment.


BASAL
FINAL
Norepinephrine
Epinephrine
Saline
FFA (nmol/ml)
2500
2000
1500
1000
500
0
-20
0
20
40
60
80
100
120
TIME (MIN)
**

infusion of NOR though, where glucose concentrations had doubled by 20 min after the start of the infusion (Fig. 2). Glucose concentrations reached a plateau at about 45 min after the start of the NOR infusion. This level was maintained through to the end of the infusion, resulting in a significant change from the basal to the final period (89 $\pm$ 5 to 198 $\pm$ 25 mg/dl).

There were no significant changes in the plasma concentrations of insulin during any of the infusions (overall mean 0.15 $\pm$ 0.03 ng/ml).

After an initial rise in glucagon concentrations during the EPI infusion (Fig. 3), the level returned to baseline and no significant change was observed from the basal to the final period. Plasma glucagon concentrations increased rapidly upon the start of the NOR infusion, reaching a peak by 10 min. From the basal to the final period, NOR infusion resulted in a significant increase in the plasma concentrations of glucagon from 142 $\pm$ 11 to 367 $\pm$ 79 pg/ml.

No significant effects on plasma cortisol concentrations were observed during the EPI infusion (Fig. 4). Plasma cortisol concentrations increased slowly during the infusion of NOR, peaking at 90 min. Overall, NOR resulted in a significant increase in cortisol concentrations from a basal level of 9.5 $\pm$ 1 to a final level of 21.1 $\pm$ 2.0 μg/dl ($P<.05$).

Figure 2. Plasma glucose concentrations in young pigs before, and during infusion of saline, epinephrine or norepinephrine. Significance between basal and final periods is denoted by **P<0.01, within a treatment.

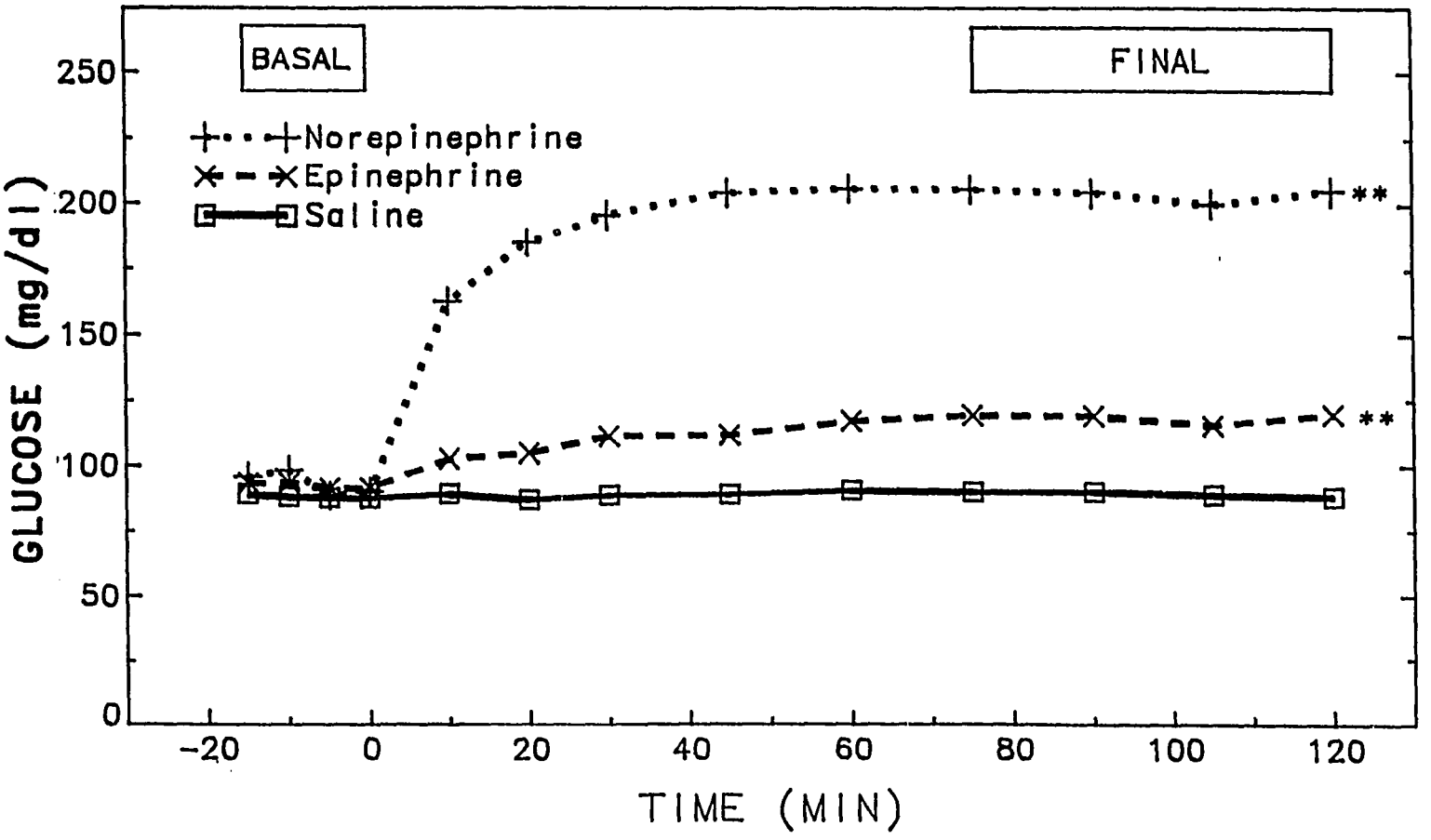

BASAL
FINAL
Norepinephrine
Epinephrine
Saline
GLUCOSE (mg/dl)
250
200
150
100
50
0
-20
0
20
40
60
80
100
120
TIME (MIN)
**
**

Figure 3. Plasma glucagon concentrations in young pigs before, and during infusion of saline, epinephrine or norepinephrine. Significance between basal and final periods is denoted by *P<0.05, within a treatment.

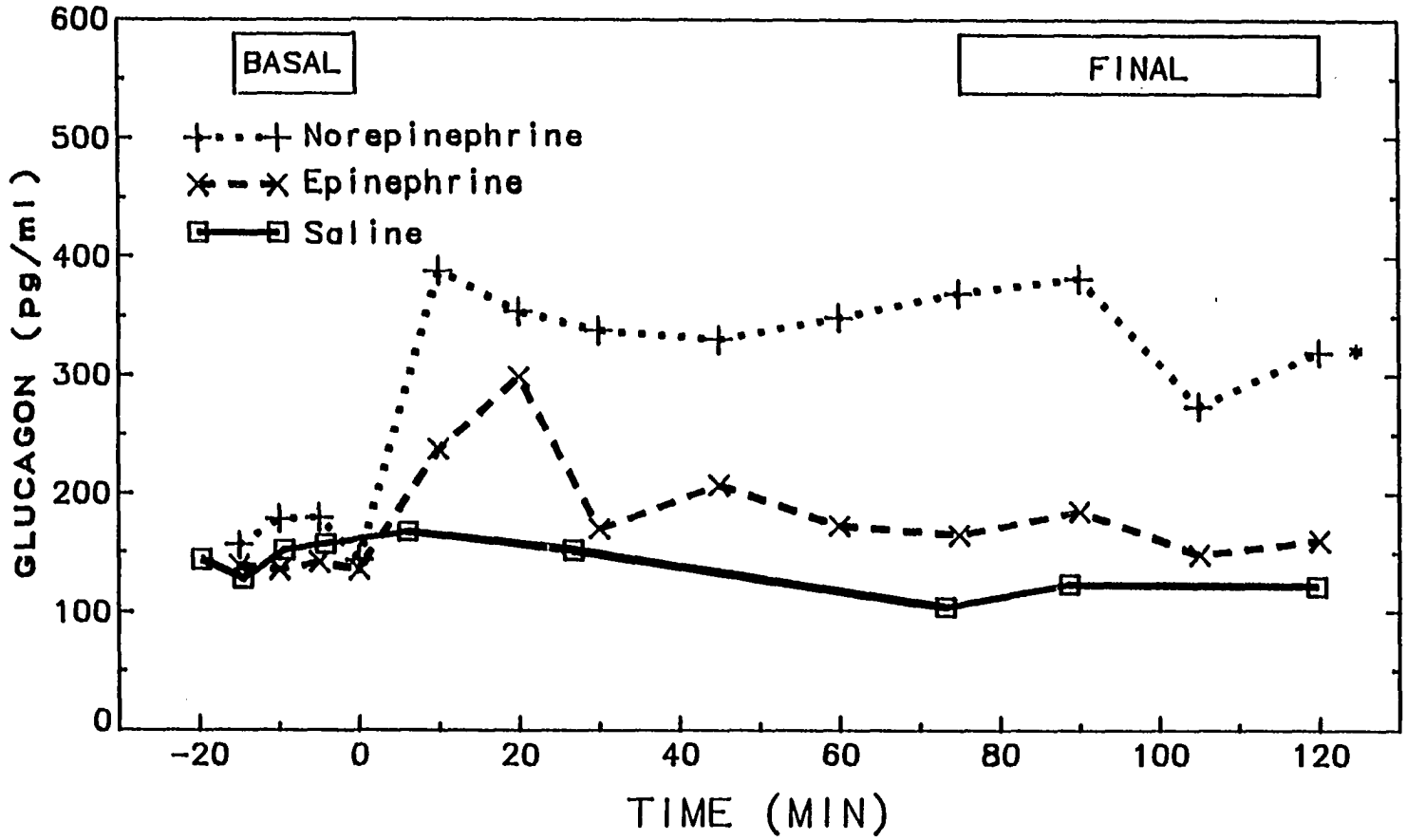
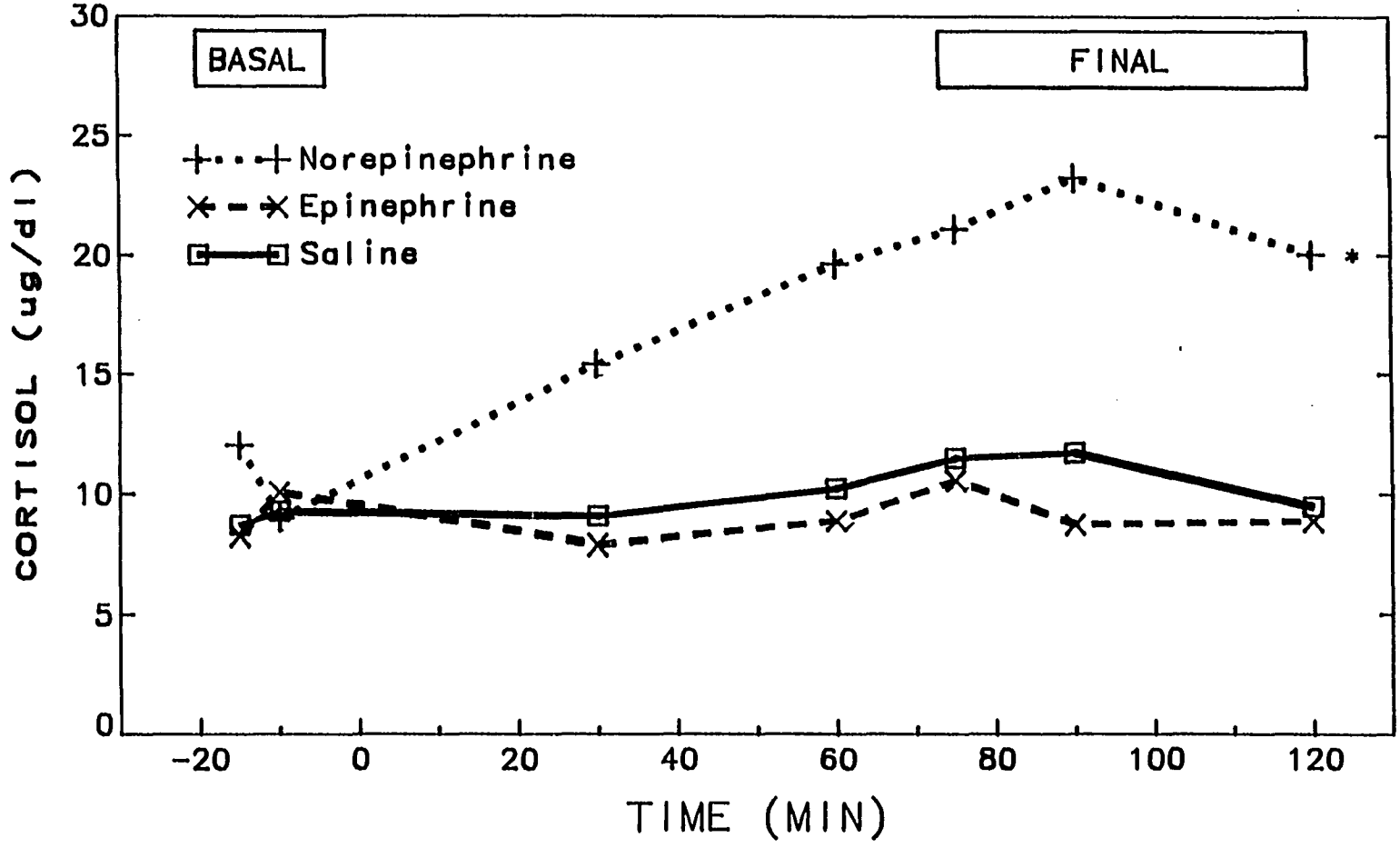

BASAL
FINAL
Norepinephrine
Epinephrine
Saline
GLUCAGON (pg/ml)
600
500
400
300
200
100
0
-20
0
20
40
60
80
100
120
TIME (MIN)

Figure 4. Plasma cortisol concentrations in young pigs before, and during infusion of saline, epinephrine or norepinephrine. Significance between basal and final periods is denoted by *P<0.05, within a treatment.


BASAL
FINAL
Norepinephrine
Epinephrine
Saline
CORTISOL (ug/dl)
30
25
20
15
10
5
0
-20
0
20
40
60
80
100
120
TIME (MIN)

DISCUSSION

The infusion rates of EPI and NOR used in the present studies were designed to mimic levels which would be expected in stress situations. Blum et al. (3) noted that an infusion of approximately 72 $ng*kg^{-1}*min^{-1}$ EPI in fed cattle resulted in a plasma EPI concentration of about 1500 pg/ml. An infusion of 50 $ng*kg^{-1}*min^{-1}$ EPI in humans (25) resulted in an increase in the plasma EPI concentration from 34 to 776 pg/ml and no change in plasma NOR concentration. Stresses such as exercise in man (5) and food deprivation in dogs (17) have been reported to result in EPI concentrations of 400 to 700 pg/ml.

Infusions of 40 (28) and 80 $ng*kg^{-1}min^{-1}$ (14) NOR in humans have been reported to raise plasma NOR concentrations to 1500 to 1800 pg/ml. Because these NOR levels were not as high as those which have been reported to occur during stresses (5, 17, 32) such as exercise (2200 pg/ml), food deprivation (2800 pg/ml) or myocardial infarction (4100 pg/ml), an infusion rate of 2000 $ng*kg^{-1}*min^{-1}$ was chosen. The levels of EPI and NOR infused are markedly different and thus, no direct comparisons between the effects of the two hormones can be made.

The infusion of 0.1 $\mu g*kg^{-1}*min^{-1}$ EPI into the pigs produced significant decreases in whole-body protein degradation. This result agrees with in vitro work, in which rat epitrochlaris muscle (6) was perfused with EPI, and decreased release of amino acids was observed. This finding is also in agreement with a human study (20), in which a 3 h infusion of 50 $ng*kg^{-1}*min^{-1}$ of EPI resulted in a decrease in the rate of appearance of leucine carbon in the plasma.

The infusion of 2.0 $\mu g*kg^{-1}*min^{-1}$ NOR into the fasted pigs also

resulted in a decreased rate of proteolysis in the whole body. This observation is consistent with an in vitro study, in which perfusion of rat epitrochlaris muscle with NOR decreased the release of glutamine and alanine into the media (6).

The rate of protein synthesis was significantly decreased during the infusion of EPI in the fasted animals. This result is supported by research in which subcutaneous injections of EPI in rats, 2 h before killing, resulted in a decrease in the incorporation of phenylalanine into muscle protein (34). When EPI was included in the incubation media of rat diaphragms (34), the incorporation of labelled amino acids was also decreased. Other in vitro research, however, has shown no effects of EPI on the uptake of amino acids into rat diaphragms (23). The decrease in protein synthesis in the pigs infused with EPI was of the same magnitude as that in protein degradation. Thus, it is not clear from this information whether EPI has a direct effect on synthesis or if the decrease in synthesis was a secondary result of the reduced amino acid availability caused by decreased proteolysis, and reflected in the decreased leucine concentration.

Similar to EPI, the infusion of NOR also resulted in decreased whole-body protein synthesis, which is consistent with in vitro work demonstrating NOR decreases amino acid incorporation into muscle (34).

The rates of interconversion of leucine and KIC significantly decreased during the infusion of NOR. No effects on leucine and KIC interconversion though, were found during the infusion of EPI. This is in contrast to a study involving humans (20), in which EPI infusion increased the rate of transfer of leucine nitrogen to alanine, while

decreasing the rate of irreversible loss of leucine carbon, indicative of an increase in leucine-KIC transamination.

Two h of NOR infusion into the fasted pigs resulted in decreased KIC oxidation. This may be the result of decreased availability of substrate, which is reflected in the decreased KIC concentrations during the NOR infusion.

In the present study, the rate of leucine oxidation was not significantly affected when EPI was infused. Previous studies in dogs (19) infused with 0.1 or 0.5 $\mu g*kg^{-1}*min^{-1}$ of EPI also forced no change in amino acid catabolism, as estimated by measuring urea production. The incubation of diaphragm muscle from both fed and fasted rats with EPI (4), however, enhanced branched-chain amino acid oxidation when pyruvate and glucose were omitted from the media.

The observation that no significant alterations occurred in the concentrations of FFA during the infusion with EPI was surprising, because continuous infusions of EPI over periods of 30 min to 6 h have previously been shown to significantly increase FFA concentrations in cattle (3), humans (20), sheep (1, 12), pigs (11) and dogs (19). Differences in dosages and conditions of the animals in each experiment may account for this apparent discrepancy.

The increase in FFA concentrations resulting from the infusion of NOR in the pigs agrees with previous research in cattle (3), sheep (1) and humans (14).

Glucose concentrations have been shown to increase significantly during EPI infusions in humans (20), pigs (11), sheep (1, 12) and cattle (3). Those results agree with that found in the present study. It is

not possible to tell from the methods used in the present experiment whether the increased glucose is the result of increased production of glucose and/or decreased removal of glucose from the plasma. A glucose turnover study in fasted humans (26) indicated though, that a 3 h infusion of EPI, at half the rate used in the present experiment, resulted in an initial increase in the rate of glucose appearance in the plasma from 15 to 75 min. This was followed by a return to the basal rate. The rate of glucose clearance, however, was significantly lower than the basal rate during the period from 30 to 180 min after the start of the EPI infusion (26). These flucuations in turnover resulted in increased plasma glucose levels from 60 to 180 min. Results observed when dogs were infused with EPI (13) indicated, however, that despite increased hepatic glucose production and peripheral glycogenolysis, the rate of glucose clearance was not significantly altered.

Infusions of NOR have been shown to result in increases in glucose concentrations in humans (14), while having no effect in sheep (1). The present results indicate that pigs also respond to NOR infusion with increases in glucose concentrations.

The combined results for glucose and FFA during the EPI and NOR infusions, support the general concept that these hormones increase the supply of readily available energy in stress situations.

In agreement with the present experiment, higher levels of EPI than that used in the present study, also produced no effect on insulin concentration in pigs fasted for 18 h (11). Experiments involving the infusion of EPI into humans (20, 24, 25), at lower levels than that used in the present experiment, however, have shown EPI increases insulin

concentrations. Thus, the effect of EPI on insulin may be related to the dosage infused, condition of the subjects and/or species differences.

The infusion of 2 $\mu g*kg^{-1}*min^{-1}$ NOR produced no change in insulin concentrations in the pigs. The infusion of 0.04 $\mu g*kg^{-1}*min^{-1}$ NOR into humans (28), though, has been shown to cause a decrease in insulin concentrations during the first 30 min of infusion. This was followed, however, by a return to basal levels during the next 30 min of infusion. Since the insulin levels observed during the pig experiment were near the minimum detection limit of the assay, any possible decrease in insulin would be difficult to detect.

In agreement with our results, infusions of 50 $ng*kg^{-1}*min^{-1}$ EPI in humans have been shown to result in transient increases in plasma glucagon concentrations (25, 26). Other results with humans, however, have shown no significant changes in glucagon concentrations (20). In contrast though, the primed-dose constant infusion of 625 $ng*kg^{-1}*min^{-1}$ EPI in rabbits (16) produced a sustained increase in glucagon concentrations.

Infusions of NOR have also produced conflicting glucagon responses. In agreement with our results, the infusion of 0.04 $\mu g*kg^{-1}min^{-1}$ NOR in humans (28) produced a significant increase in glucagon levels within 30 min. The infusion of 0.08 $\mu g*kg^{-1}*min^{-1}$ for 170 min in humans, however, had no effect on glucagon concentrations (14).

Cortisol is generally considered to be a catabolic hormone (18). Thus, an increase in cortisol levels may be expected to increase protein degradation. The continuous infusion of NOR into the pigs approximately

doubled cortisol concentrations. The finding that NOR infusion decreased the rate of proteolysis though, despite elevated cortisol concentrations, may indicate an overriding influence of NOR on protein metabolism.

The increase in cortisol concentrations during the NOR infusion was similar to that which occurred after a bolus infusion of 1000 μg/kg NOR in dogs (31). Bolus injections of 10 or 100 μg/kg NOR in the dogs, however, failed to alter cortisol concentrations.

A 20 min infusion of 40 to 1000 $ng*kg^{-1}*min^{-1}$ EPI in rats (2) has been reported to produce a significant dose-related increase in plasma corticosterone levels. The infusion of 500 $ng*kg^{-1}*min^{-1}$ EPI for one hour in dogs (31), however, produced no changes in cortisol concentrations. Since the infusion of 100 $ng*kg^{-1}*min^{-1}$ EPI into the pigs in the present experiment also failed to influence cortisol concentrations, these results may indicate species-related effects.

Glucocorticoids are thought to be slow-acting, long-term regulators of blood glucose, maintaining for several hours the increase in glucose concentrations caused by EPI or glucagon (21). This may be accomplished by inhibiting insulin secretion and stimulating glucagon secretion (21). It is possible that the stress level achieved during the NOR infusion in the pigs, may have been sufficient to stimulate a cortisol effect on insulin and glucagon and aid in the maintenance of high glucose levels.

Assuming the decrease in oxidation of the ketogenic amino acid leucine is reflected in the pattern of metabolism of the gluconeogenic amino acids, then the results from the NOR infusion suggest that liberation of energy-producing substrates from glycogen and adipose

tissue was adequate to meet the needs imposed by the artificial stress situation. The decrease in endogenous proteolysis, despite the fact that the animals were fasting, also emphasizes the reduced need for available amino acids.

During the EPI infusion, the increases in the metabolites and hormones measured were not as dramatic as those which occurred during the NOR treatment. As a result, the rate of oxidation of free amino acids may have been maintained at the pre-EPI infusion level to supplement the provision of energy. Amino acids stored as protein were not affected though, as the rate of protein degradation was not increased.

In conclusion, similar decreases in the rates of protein degradation and synthesis during the infusion of EPI, indicate an overall decrease in whole-body protein turnover, but no change in net protein synthesis. This should result in a conservation of energy, since protein turnover is an energy requiring process (24). Decreases in the rates of proteolysis and protein synthesis during the infusion of NOR should likewise result in a conservation of energy. Furthermore, the decrease in the rate of oxidation with NOR will result in a conservation of nitrogen. Therefore, the results of this study suggest that during periods of stress, the conservation of energy resulting from changes in protein metabolism, will augment the desired increase in readily available energy.

REFERENCES

1. Bassett, J. M. Metabolic effects of catecholamines in sheep. Aust. J. Biol. Sci. 23: 903-914, 1970.

2. Berkenbosch, F., I. Vermes, R. Binnekade, and F. J. H. Tilders. Beta-adrenergic stimulation induces an increase of the plasma levels of immunoreactive α-MSH, β-endorphin, ACTH and of corticosterone. Life Sci. 29: 2249-2256, 1981.

3. Blum, J. W., D. Froehli, and P. Kunz. Effects of catecholamines on plasma free fatty acids in fed and fasted cattle. Endocrinology 110: 452-456, 1982.

4. Buse, M. G., J. F. Biggers, C. Drier, and J. F. Buse. The effect of epinephrine, glucagon, and the nutritional state on the oxidation of branched chain amino acids and pyruvate by isolated hearts and diaphragms of the rat. J. Biol. Chem. 248: 697-706, 1973.

5. Galbo, H., J. J. Holst, and N. J. Christensen. Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. J. Appl. Physiol. 38: 70-76, 1975.

6. Garber, A. J., I. E. Karl, and D. M. Kipnis. Alanine and glutamine synthesis and release from skeletal muscle. IV. β-adrenergic inhibition of amino acid release. J. Biol. Chem. 251: 851-857, 1976.

7. Garceau, D., N. Yamaguchi, R. Goyer, and F. Guitard. Correlation between endogenous noradrenaline and glucose released from the liver upon hepatic sympathetic nerve stimulation in anesthetized dogs. Can. J. Physiol. Pharmacol. 62: 1086-1091, 1984.

8. Harris, V., G. R. Faloona and R. H. Unger. Glucagon. In Methods of

Hormone Radioimmunoassay, edited by B. M. Jaffe and H.R. Behrman. New York:Academic Press, 1979, p. 643-656.

9. Helland, S. J., R. C. Ewan, A. Trenkle and S. Nissen. In vivo metabolism of leucine and α-ketoisocaproate in the pig: Influence of dietary glucose or sucrose. J. Nutr. (In Press), 1986.
10. Helland, S. J., B. Grisdale-Helland, and S. Nissen. Effect of site of isotope infusion on the estimates of leucine metabolism in pigs and sheep. Submitted to Am. J. Physiol., 1986.
11. Hertelendy, F., I. Machlin, R. S. Gordon, M. Horino, and D. M. Kipnis. Lipolytic activity and inhibition of insulin release by epinephrine in the pig. Proc. Soc. Exp. Biol. Med. 121: 675-677, 1966.
12. Hertelendy, F., I. Machlin, and D. M. Kipnis. Further studies on the regulation of insulin and growth hormone secretion in the sheep. Endocrinology 84: 192-199, 1969.
13. Issekutz Jr., B. Effect of epinephrine on carbohydrate metabolism in exercising dogs. Metabolism 34: 457-464, 1985.
14. Keller, U., P. P. G. Gerber, and W. Stauffacher. Stimulatory effect of norepinephrine on ketogenesis in normal and insulin-deficient humans. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E732-E739, 1984.
15. Kleiber, M. Fire of Life. Huntington:Robert E. Krieger Publishing Co., Inc., 1975.
16. Knudtzon, J. Adrenergic effects on plasma levels of glucagon, insulin, glucose and free fatty acids in rabbits. Horm. Metabol. Res. 16: 415-422, 1984.

17. Kozlowski, S., K. Nazar, H. Kaciuba-Uscilko, D. Stevens, and Z. Brzezinska. Increased plasma catecholamine levels in response to reduced energy intake in the dog. Horm. Metabol. Res. 15: 108-109, 1983.

18. Liddle, G. W. The adrenals. In Textbook of Endocrinology, edited by R. H. Williams. Philadelphia:W. B. Saunders Co., 1981, p. 249-292.

19. Liddell, M. J., A. M. Daniel, L. D. MacLean, and H. M. Shizgal. The role of stress hormones in the catabolic metabolism of shock. Surg. Gynecol. Obstet. 149: 822-830, 1979.

20. Miles, J. M., S. L. Nissen, J. E. Gerich, and M. W. Haymond. Effects of epinephrine infusion on leucine and alanine kinetics in humans. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E166-E172, 1984.

21. Munck, A., P. M. Guyre, and N. J. Holbrook. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5: 25-44, 1984.

22. Nissen, S. L., C. Van Huysen, and M. W. Haymond. Measurement of branched chain α-ketoacids in plasma by high-performance liquid chromatography. J. Chromatogr. 232: 170-175, 1982.

23. Nutting, D. F. Anabolic effects of catecholamines in diaphragm muscle from hypohysectomized rats. Endocrinology 110: 307-317, 1982.

24. Reeds, P. J., M. F. Fuller, and B. A. Nicholson. Metabolic basis of energy expenditure with particular reference to protein. In Substrate and Energy Metabolism in Man, edited by J. S. Garrow and D. Halliday. London:John Libbey, p. 46-56, 1985.

25. Rizza, R. A., P. E. Cryer, M. W. Haymond, and J. E. Gerich. Adrenergic mechanisms of catecholamine action on glucose homeostasis in man. Metabolism 29 (Suppl. 1): 1155-1163, 1980.

26. Rizza, R., M. Haymond, P. Cryer, and J. Gerich. Differential effects of epinephrine on glucose production and disposal in man. Am. J. Physiol. 237 (Endocrinol. Metab. Gastrointest. Physiol. 6): E356-E362, 1979.

27. Rudiger, H. W., U. Langenbeck, and H. W. Goedde. A simplified method for the preparation of ^{14}C-labeled branched-chain β-oxoacids. Biochem. J. 126: 445-446, 1972.

28. Schade, D. S. and R. P. Eaton. The regulation of plasma ketone body concentration by counter-regulatory hormones in man. III. Effects of norepinephrine in normal man. Diabetes 28: 5-10, 1979.

29. Shimizu, S., K. Inoue, Y. Tani and H. Yamada. Enzymatic microdetermination of serum free fatty acids. Anal. Biochem. 98: 341-345, 1979.

30. Snedecor, G. W. and W. G. Cochran. Statistical Methods. Ames:Iowa State University Press, 1979, 593 pp.

31. Takahashi, K., T. Tsushima, and M. Irie. Effect of catecholamines on plasma growth hormone in dogs. Endocrinol. Japon. 20: 323-330, 1973.

32. Tepperman, J. Metabolic and Endocrine Physiology: An Introductory Text. Chicago:Yearbook Medical Publishers, 1980.

33. Trenkle, A. Radioimmunoassay of plasma hormones: Review of plasma insulin in ruminants. J. Dairy Sci. 55: 1200-1211, 1972.

34. Wool, I. G. Incorporation of C^{14}-amino acids into protein of

isolated diaphragms: effect of epinephrine and norepinephrine. Am. J. Physiol. 198: 54-56, 1960.

35. Zapf, J., M. Waldvogel, and E. R. Froesch. Increased sensitivity of rat adipose tissue to the lipolytic action of epinephrine during fasting and its reversal during re-feeding. FEBS Lett. 76: 135-138, 1977.

SECTION II. EFFECTS OF EPINEPHRINE, NOREPINEPHRINE AND CIMATEROL ON LEUCINE METABOLISM IN SHEEP

EFFECTS OF EPINEPHRINE, NOREPINEPHRINE AND CIMATEROL ON LEUCINE METABOLISM IN SHEEP[1]

Barbara Grisdale-Helland, Ståle J. Helland, Allen Trenkle and Steven Nissen

Department of Animal Science
Iowa State University
Ames, Iowa 50011

[1]This project was supported in part by National Institute of Health Grant AM 32540 and by the Iowa Agriculture and Home Economics Experimental Station, Project No. 2614.

ABSTRACT

Using a constant infusion of [1-^{14}C]leucine and [4,5-^{3}H] α-ketoisocaproate (KIC), the rates of whole-body leucine and KIC metabolism were estimated in young female lambs during acute infusions of the β_2-adrenergic agonist cimaterol (CIM, 34 $\mu g*kg^{-1}*min^{-1}$), and during acute infusions of stress levels of epinephrine (EPI, 0.1 $\mu g*kg^{-1}*min^{-1}$) and norepinephrine (NOR, 2.0 $\mu g*kg^{-1}*min^{-1}$). No significant changes in leucine metabolism resulted from saline or NOR infusions. EPI infusion decreased the rates of leucine entry (proteolysis and absorption) and protein synthesis by 10 and 13%, respectively ($P<0.01$), while CIM infusion decreased these rates by 34 and 38%, respectively ($P<0.1$). Leucine and KIC interconversion was increased during the EPI infusion and reamination was decreased during the CIM infusion. NOR infusion significantly increased the plasma concentrations of free fatty acids (FFA), glucose, insulin and cortisol. FFA, glucose and insulin were increased and glucagon was decreased as a result of the EPI infusion. The infusion of CIM resulted in increases in glucose and insulin and a decrease in glucagon concentration. These data indicate that, in addition to providing readily available energy through the liberation of glucose and FFA, the adrenergic compounds, EPI and CIM, reduce the rate of amino acid turnover but do not affect amino acid catabolism.

INTRODUCTION

Daily injections of epinephrine (EPI) have been shown to influence nitrogen retention and body fat stores in pigs (9). This effect may be the result of stimulation of the β-adrenergic receptors, since chronic administrations of specific β-agonists to sheep (2, 6), pigs (19), cattle (27), poultry (11) and rats (12) have recently been shown to significantly alter body composition, producing an increase in muscle mass and a decrease in fat content.

Catecholamines can be classified according to their stimulatory effects on α_1-, α_2-, β_1- and β_2-adrenergic receptors. As reviewed by Exton (13), epinephrine (EPI) stimulates all four receptor types, while norepinephrine (NOR) elicits responses only through α_1-, α_2- and β_1-adrenergic receptors. Skeletal muscle is generally considered to respond to β_2-receptor stimulation with increased glycogenolysis and subsequent lactate release (13, 34). Several studies have indicated, however, that muscle protein turnover may also be specifically influenced by adrenergic stimulation. Perfusion of rat muscle with EPI or NOR decreases the rate of release of amino acids (15) and the rate of amino acid incorporation into protein (40). Perfusion of rat gastrocnemius muscle (22) with isoproterenol (β-agonist) decreases the rate of protein degradation, but has no effect on protein synthesis. In vivo studies also indicate that EPI infusion decreases the rate of endogenous proteolysis (24), but has no effect on oxidation (22). Experiments involving rats treated for 7 d with injections of either clenbuterol or fenoterol (specific β_2-agonists), have shown that this type of agonist increases the rate of protein synthesis in skeletal

muscle (12). No measurements were made, however, on other aspects of protein turnover which may influence total body protein content. Thus, the objective of the present experiment was to study the acute effects of infusions of EPI, NOR and cimaterol (CIM; a specific β_2-agonist) on whole-body leucine metabolism (leucine entry from proteolysis and absorption, protein synthesis, leucine and α-ketoisocaproate (KIC) interconversion, and leucine oxidation), in sheep. Since the effects of these compounds on protein turnover may be indirectly influenced by concomitant changes in hormones and metabolites, plasma concentrations of insulin, glucagon, cortisol, free fatty acids (FFA) and glucose were also measured.

MATERIALS AND METHODS

L-[1-^{14}C]leucine (57 mCi/mmol) was purchased from Amersham and L-[4,5-^{3}H]leucine (58 Ci/mmol) was purchased from ICN Biochemicals. [^{3}H]KIC was enzymatically prepared from [4,5-^{3}H]leucine (29). The $NaH^{14}CO_2$ used was obtained from Calatomic. EPI and NOR (arterenol hydochloride) were obtained from Sigma Chemical. CIM was received as a gift from American Cyanamid. The drugs were dissolved in a 0.9% sodium chloride solution containing 0.2% serum albumin. $NaH_2PO_4 \cdot H_2O$ was added to the CIM at 1.5 times its weight to aid in dissolving the drug in the saline (R.H. Dalrymple, American Cyanamid, Princeton, NJ, personal communication).

On the day before the first isotope infusion, four young female sheep (40 kg) were fitted with an infusion catheter in the right ventricle via the right jugular vein, and a sampling catheter in the right atrium via the left jugular vein. Each sheep was studied once with each of the four treatments (EPI, NOR, CIM and saline). One day of normal feeding separated each experiment day. Feed was removed approximately 12 h before the start of each experiment and water was available at all times.

Two h before the start of the hormone infusions, a priming dose of 5 μCi $NaH^{14}CO_2$ (1) was given, and a primed-dose constant infusion of [^{3}H]KIC and [^{14}C]leucine (1.2 and 0.5 μCi/min, respectively) was started to allow for isotope equlilibration. The priming dose was ten times the constant infusion dose for one minute.

Before the start of the hormone infusions, blood samples were taken during the basal steady state period at -30, -20, -10 and 0 min. At

zero time, a 3 h constant infusion of epinephrine (0.1 $\mu g*kg^{-1}*min^{-1}$), norepinephrine (2.0 $\mu g*kg^{-1}*min^{-1}$), cimaterol (34 $ng*kg^{-1}*min^{-1}$) or the saline-albumin vehicle was started. Blood was then sampled at 60, 90, 120, 150 and 180 min. The blood was collected into tubes containing EDTA and cooled immediately on ice. One ml of whole blood was added to tubes containing 3 ml 1.5 N perchloric acid and frozen at -80 C for later analysis. The remaining blood was centrifuged and the plasma was collected and stored at -80 C until analyzed for hormones and metabolites. Trasylol (500 U/ml of plasma) was added to the samples which were to be analyzed for glucagon.

Whole-blood leucine and KIC concentrations were determined by high-performance liquid chromatography (25). The HPLC effluent corresponding to the leucine or KIC peaks was collected and the ^{14}C and ^{3}H radioactivity determined by dual isotope liquid scintillation counting.

Plasma glucose concentrations were determined using the glucose oxidase method (Worthington Diagnostics, Freehold, NJ). Free fatty acids were analyzed enzymatically (31). Radioimmunoassays were used to measure insulin (38), glucagon (16) and cortisol (Gammacoat [^{125}I] Cortisol Radioimmunoassay Kit, Travenol-Genetech Diagnostics).

Rates of leucine entry (proteolysis and absorption), protein synthesis, KIC oxidation and interconversion of leucine and KIC were calculated as described by Helland et al. (17) for a reversible two-pool model. An independent estimate of the rate of KIC oxidation was also determined through collection of breath $^{14}CO_2$. Expired air was collected in 30 liter latex balloons (Warren E. Collins, Inc.) for 2-3 min periods at -30, 0, 90, 150 and 180 min. The air was slowly

aspirated through 250 ml of a 1 M ethanolamine solution. Triplicate 2 ml samples of this solution were added to vials containing 15 ml of scintillation cocktail and the ^{14}C radioactivity determined in a liquid scintillation counter. CO_2 flux was calculated as ($^{14}CO_2$ DPM expired*min^{-1})*(^{14}C-KIC specific radioactivity^{-1})*(kg^{-1}) and corrected for 20% CO_2 fixation in the body (1).

It was subsequently determined that the plasma specific radioactivities of leucine and KIC were constant between 90 and 180 min after the start of the hormone infusions, and thus a new steady state had been established. The F test (32) was used to test for homogeneity of variance of the flux estimates. The variance between treatments was not homogeneous and therefore, the analysis of variance was not a valid test of differences between the treatments. The Student's t-test (32) was used to determine whether the change from the basal steady state period (mean of -30 to 0 min) to the final steady state period (mean of 90 to 180 min), for each variable, was statistically different from zero. All data are expressed as means $\pm$ SE.

RESULTS

Whole-blood concentrations of leucine and KIC in the basal and final periods are presented in Table 1. Leucine concentrations decreased during the infusions of EPI ($P<0.05$) and CIM ($P<0.1$). No changes were observed in the whole-blood concentrations of KIC.

The rates of the sum of leucine entry from proteolysis and absorption, protein synthesis, leucine and KIC interconversion, KIC oxidation and breath CO_2 flux for each treatment, are presented in Table 1. No significant changes were found in any of the flux estimates when the sheep were infused with either saline or NOR for three hours.

The infusion of EPI resulted in decreases of 10 and 13% in the rates of leucine entry and protein synthesis, respectively ($P<0.01$). In addition, deamination was increased by 18% ($P<0.1$) and reamination was increased by 28% ($P<0.01$). There were no significant effects of EPI infusion on KIC oxidation measured with either the two-pool model or breath CO_2.

CIM infusion decreased the estimate of leucine entry by 34% ($P<0.1$) and the estimate of protein synthesis by 38% ($P<0.1$). The rate of reamination of KIC to leucine was also decreased by the CIM infusion ($P<0.1$). No effects of this compound were observed on leucine deamination or either measurement of KIC oxidation.

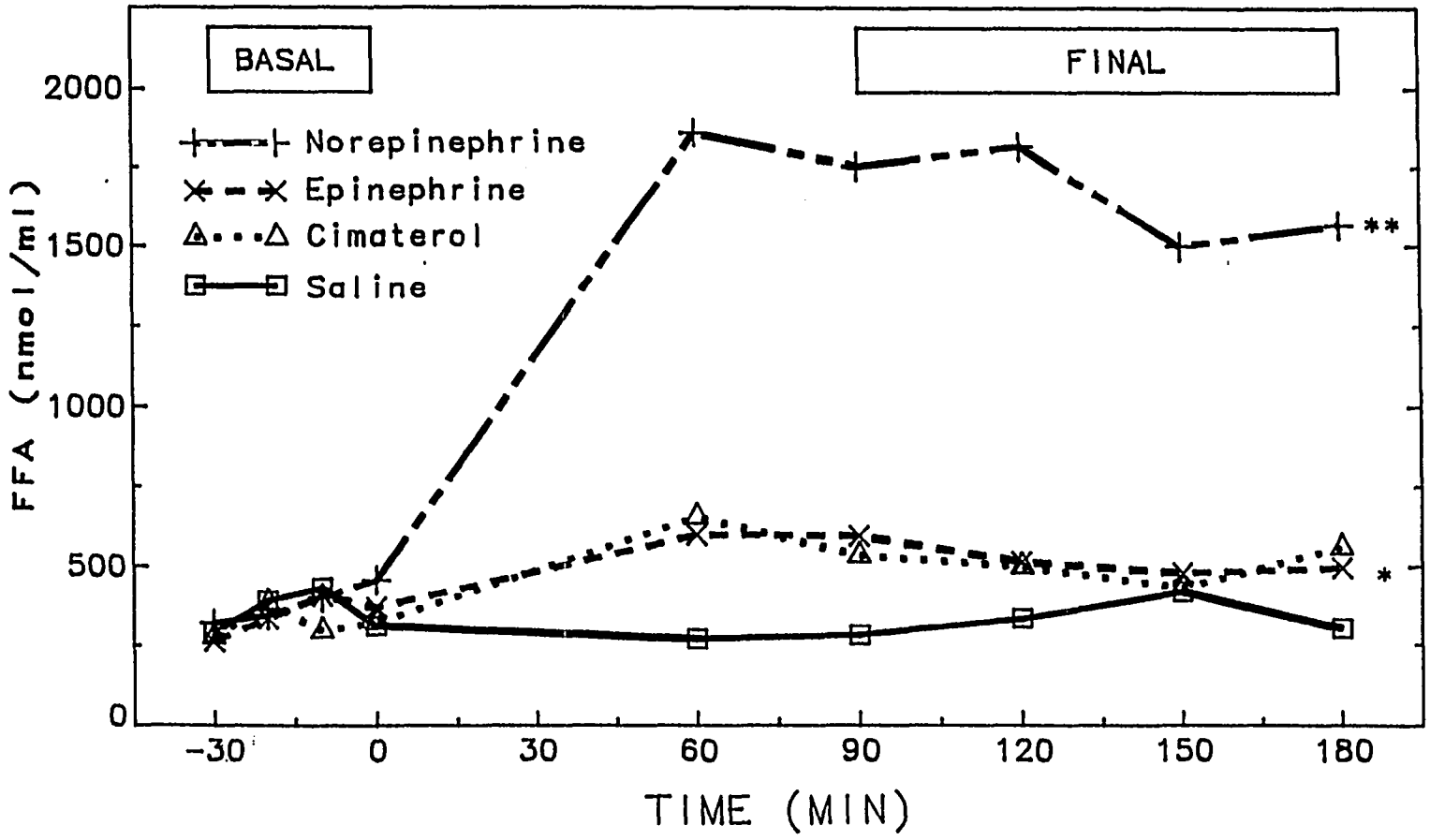
Plasma FFA concentrations rapidly increased during the infusion of NOR, from 380 $\pm$ 55 nmol/ml to a plateau concentration of 1658 $\pm$ 91 nmol/ml (Fig. 1). The infusions of EPI and CIM also increased the concentrations of FFA, although the change from the basal to the final period was only significant with the EPI treatment (342 $\pm$ 10 to 521 $\pm$ 45

Table 1. Leucine and KIC concentrations and metabolism in sheep before, and during, infusion of saline, epinephrine, norepinephrine or cimaterol

		Saline (N=4)	Epinephrine (N=4)	Norepinephrine (N=4)	Cimaterol (N=4)
Concentrations[a]					
Leucine	B[b]	179 ± 18	204 ± 15	140 ± 8	163 ± 18
	F[c]	177 ± 20	157 ± 10*	144 ± 5	89 ± 5+
KIC	B	13.9 ± 1.0	15.0 ± 1.6	14.3 ± 0.6	12.7 ± 0.8
	F	13.9 ± 0.9	12.5 ± 0.5	10.0 ± 1.1	9.9 ± 0.5
Fluxes[d]					
Leucine entry	B	3.99 ± 0.47	4.40 ± 0.49	4.45 ± 0.60	4.92 ± 1.38
	F	3.50 ± 0.03	3.96 ± 0.44**	5.53 ± 1.53	3.24 ± 0.57+
Synthesis	B	3.47 ± 0.43	3.86 ± 0.41	3.96 ± 0.61	4.31 ± 1.21
	F	3.01 ± 0.07	3.35 ± 0.39**	5.09 ± 1.55	2.69 ± 0.48+
Leu -> KIC	B	0.66 ± 0.08	0.72 ± 0.13	0.67 ± 0.08	0.86 ± 0.30
	F	0.60 ± 0.06	0.85 ± 0.15*	0.85 ± 0.15	0.66 ± 0.13
KIC -> Leu	B	0.14 ± 0.04	0.18 ± 0.06	0.17 ± 0.03	0.25 ± 0.14
	F	0.12 ± 0.01	0.23 ± 0.08**	0.41 ± 0.14	0.11 ± 0.03+
Oxidation	B	0.52 ± 0.04	0.54 ± 0.08	0.50 ± 0.06	0.61 ± 0.17
	F	0.49 ± 0.06	0.61 ± 0.09	0.44 ± 0.07	0.54 ± 0.10
CO_2 Flux	B	0.65 ± 0.15	0.73 ± 0.18	0.54 ± 0.06	0.56 ± 0.15
	F	0.45 ± 0.09	0.44 ± 0.08	0.81 ± 0.19	0.39 ± 0.09

[a] Units: μM ± SE.
[b] Basal time period (-30 to 0 min).
[c] Final time period (90 to 180 min).
[d] Units: μmol leucine or KIC*kg^{-1}*min^{-1} ± SE.
+ Change from basal to final period significantly different from zero (P<0.1).
* Change from basal to final period significantly different from zero (P<0.05).
** Change from basal to final period significantly different from zero (P<0.01).

Figure 1. Plasma free fatty acid concentrations in sheep before, and during, infusion of saline, epinephrine, norepinephrine or cimaterol. Significance between basal and final periods is indicated by ** $P<0.01$ and * $P<0.05$, within a treatment.


BASAL
FINAL
Norepinephrine
Epinephrine
Cimaterol
Saline
FFA (nmol/ml)
2000
1500
1000
500
0
**
*
-30
0
30
60
90
120
150
180
TIME (MIN)

nmol/ml, $P<0.05$).

Glucose concentrations (Fig. 2) were significantly increased from the basal to the final period by the infusions of EPI, NOR and CIM (89 ± 6 to 141 ± 7, 97 ± 4 to 264 ± 15, 84 ± 4 to 125 ± 8 mg/dl, respectively). The most rapid increase in glucose concentrations occurred during the NOR infusion, in which a plateau was reached by approximately 60 min. The other treatments caused more gradual changes in glucose levels. During the saline infusion, glucose decreased from 105 ± 10 to 93 ± 7 mg/dl ($P<0.01$).

During the infusion of saline into the sheep, a significant decrease in the plasma insulin concentration, from 0.71 ± 0.11 in the basal period to 0.54 ± 0.08 ng/ml in the final period, was observed (Fig. 3). In contrast, infusions of EPI, NOR and CIM all significantly increased plasma insulin concentrations (0.90 ± 0.11 to 1.57 ± 0.35, $P<0.1$, 1.10 ± 0.07 to 2.06 ± 0.20, $P<0.01$, 0.50 ± 0.06 to 2.73 ± 0.85 ng/ml, $P<0.05$, respectively). Although insulin concentrations were similar at 150 min after the start of the NOR and CIM infusions, the peak in insulin occurred one hour earlier during the CIM infusion than during the NOR infusion.

No significant changes in glucagon concentrations occurred during the infusions of either saline or NOR (Fig. 4). The infusions of EPI and CIM, however, resulted in decreases in the plasma concentrations of glucagon from the basal to the final period (442 ± 54 to 363 ± 67, $P<0.05$, 393 ± 108 to 265 ± 41 pg/ml, $P<0.1$, respectively).

The insulin to glucagon ratio (I/G) was affected by all treatments (Fig. 5). The saline infusion produced a decrease in I/G from $1.55 \pm$

Figure 2. Plasma glucose concentrations in sheep before, and during, infusion of saline, epinephrine, norepinephrine or cimaterol. Significance between basal and final periods is indicated by ** $P<0.01$ and + $P<0.1$, within a treatment.

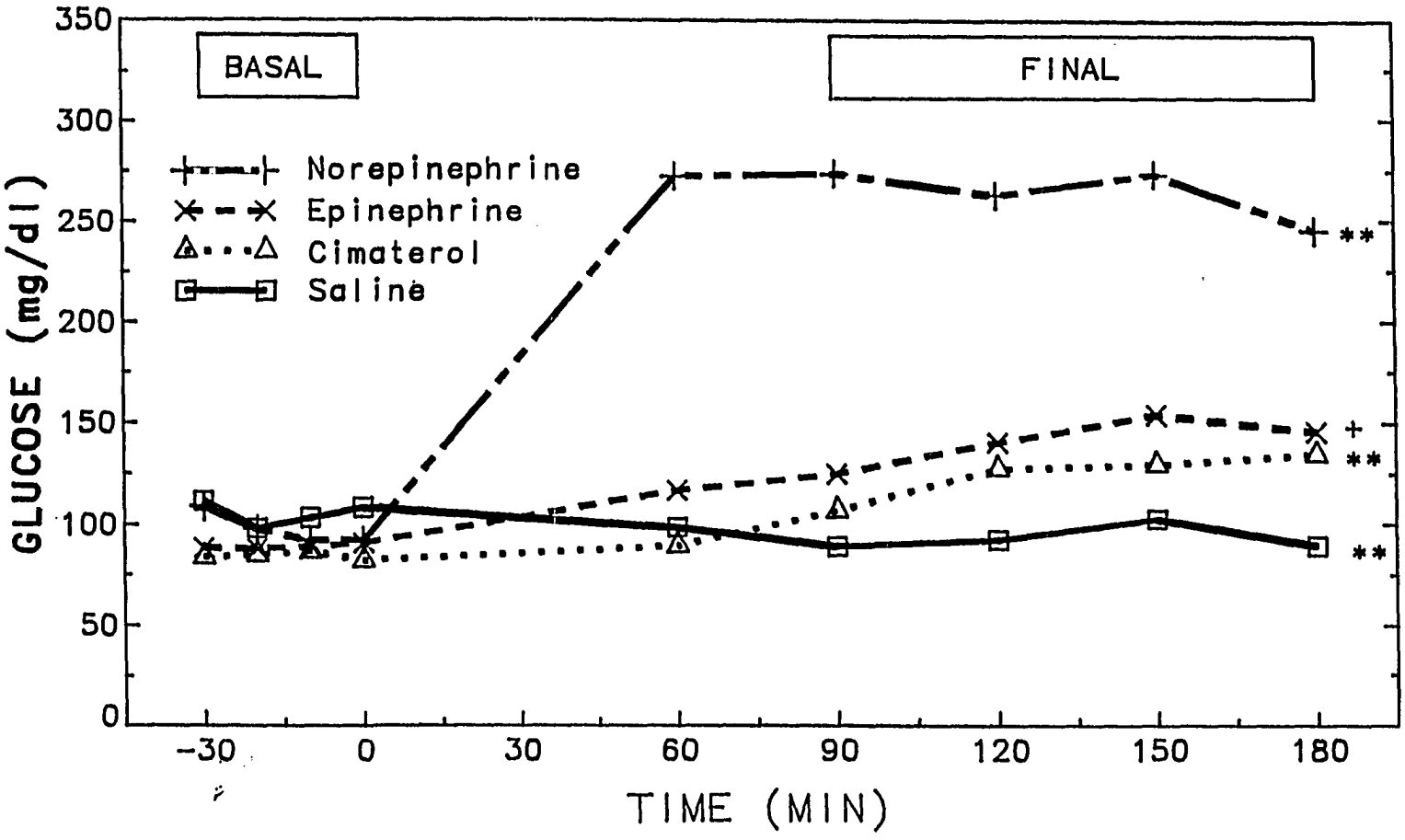

BASAL
FINAL
Norepinephrine
Epinephrine
Cimaterol
Saline
GLUCOSE (mg/dl)
350
300
250
200
150
100
50
0
-30
0
30
60
90
120
150
180
TIME (MIN)
**
**
+
**

Figure 3. Plasma insulin concentrations in sheep before, and during, infusion of saline, epinephrine, norepinephrine or cimaterol. Significance between basal and final periods is indicated by ** $P<0.01$, * $P<0.05$ and + $P<0.1$, within a treatment.

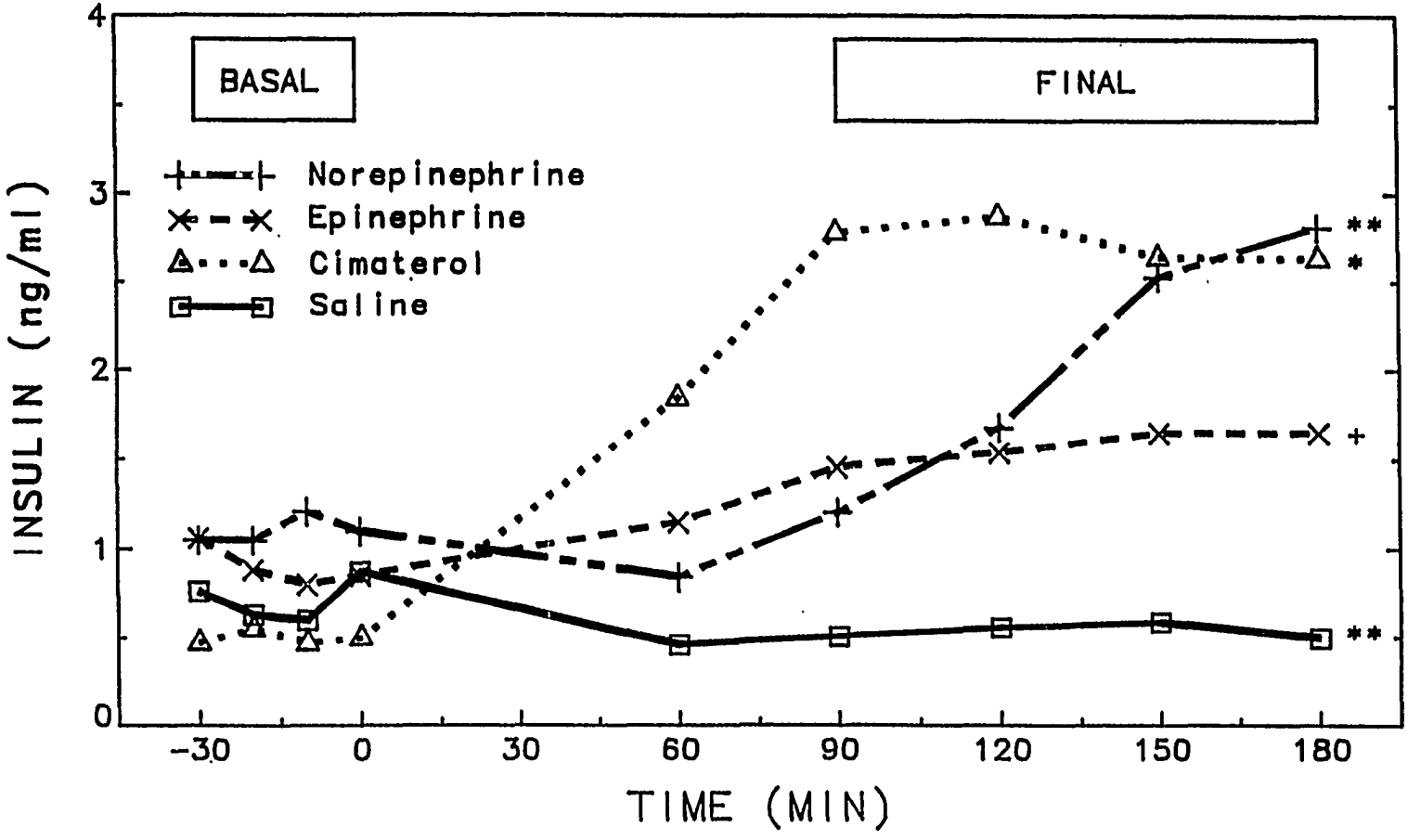

BASAL
FINAL
Norepinephrine
Epinephrine
Cimaterol
Saline
INSULIN (ng/ml)
TIME (MIN)
0
1
2
3
4
-30
0
30
60
90
120
150
180
**
*
+
**

Figure 4. Plasma glucagon concentrations in sheep before, and during, infusion of saline, epinephrine, norepinephrine or cimaterol. Significance between basal and final periods is indicated by ** $P<0.01$ and + $P<0.1$, within a treatment.

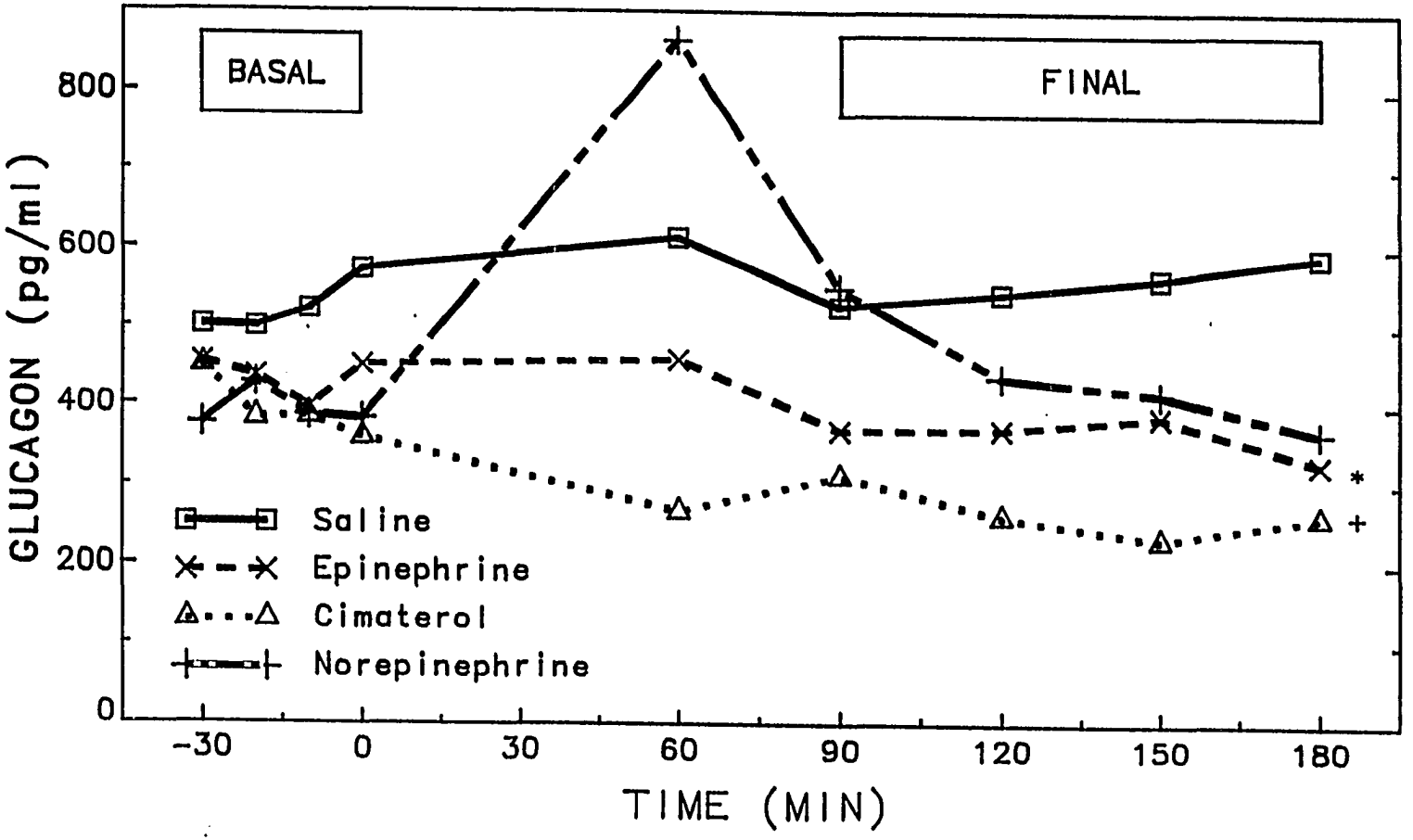
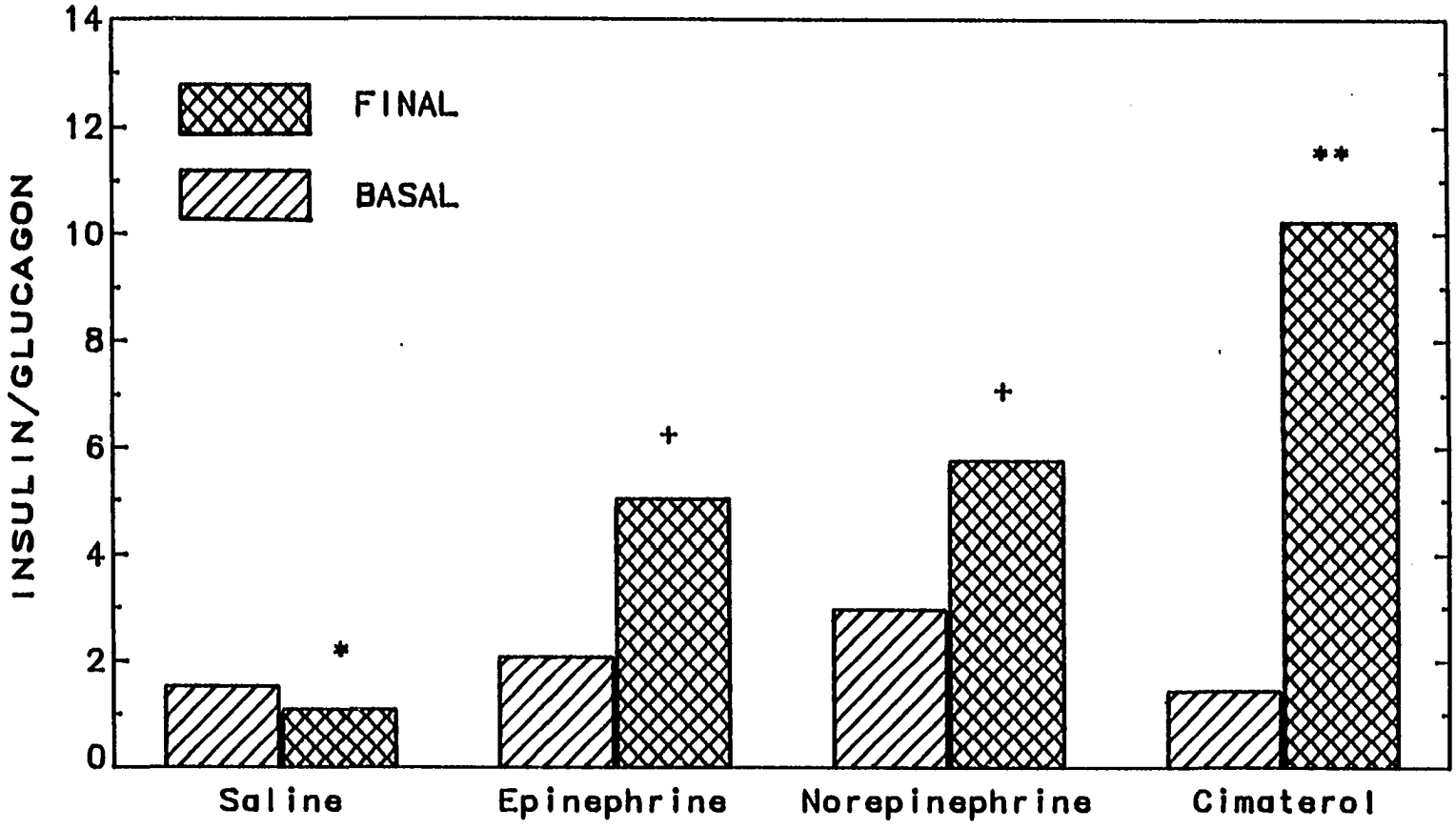

BASAL
FINAL
800
600
400
200
0
GLUCAGON (pg/ml)
Saline
Epinephrine
Cimaterol
Norepinephrine
-30
0
30
60
90
120
150
180
TIME (MIN)

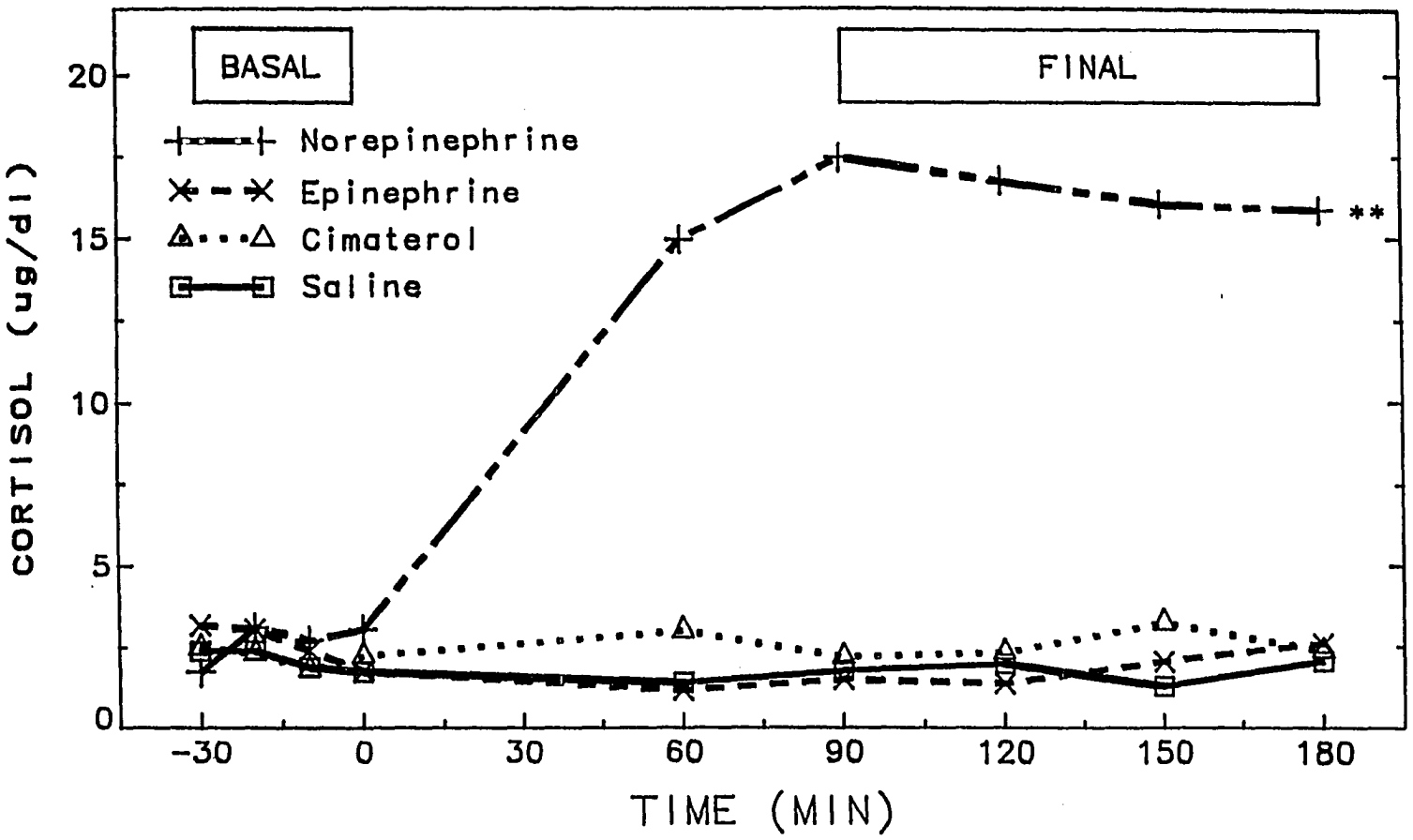
Figure 5. Insulin to glucagon ratios in sheep plasma before (basal), and during (final), infusions of saline, epinephrine, norepinephrine or cimaterol. Significance between basal and final periods is indicated by ** $P<0.01$, * $P<0.05$ and + $P<0.1$, within a treatment.


INSULIN/GLUCAGON
14
12
10
8
6
4
2
0
FINAL
BASAL
*
+
+
**
Saline
Epinephrine
Norepinephrine
Cimaterol

0.31 in the basal period to 1.13 ± 0.28 in the final period ($P<0.05$). The infusions of EPI and NOR tended to increase I/G (2.11 ± 0.25 to 5.05 ± 1.67, 2.99 ± 0.55 to 5.79 ± 1.23, $P<0.1$, respectively), while CIM infusion resulted in a dramatic increase in I/G from 1.48 ± 0.27 to 10.26 ± 1.93 ($P<0.01$).

Cortisol concentrations were significantly altered only during the infusion of NOR (Fig. 6), in which a rise from 2.69 ± 0.46 in the basal period to 16.56 ± 1.80 μg/dl ($P<0.01$) in the final period, was observed.

Figure 6. Plasma cortisol concentrations in sheep before, and during,. infusion of saline, epinephrine, norepinephrine or cimaterol. Significance between basal and final periods is indicated by ** $P<0.01$, within a treatment.


BASAL
FINAL
Norepinephrine
Epinephrine
Cimaterol
Saline
**
CORTISOL (ug/dl)
20
15
10
5
0
-30
0
30
60
90
120
150
180
TIME (MIN)

DISCUSSION

The infusion rates of EPI and NOR used in this experiment were designed to mimic levels which would be expected in stress situations. Blum et al. (7) noted that an infusion of approximately 72 $ng*kg^{-1}*min^{-1}$ EPI in fed cattle resulted in a plasma EPI concentration of about 1500 pg/ml. An infusion of 50 $ng*kg^{-1}*min^{-1}$ EPI in humans (28) resulted in an increase in the plasma EPI concentration from 34 to 776 pg/ml and no change in plasma NOR concentration. Stresses such as exercise in man (14) and food deprivation in dogs (21) have been reported to result in EPI concentrations of 400 to 700 pg/ml.

Infusions of 40 (30) and 80 $ng*kg^{-1}min^{-1}$ (20) NOR in humans have been reported to raise plasma NOR concentrations to 1500 to 1800 pg/ml. Because these NOR levels were not as high as those which have been reported to occur during stresses (14, 21, 35) such as exercise (2200 pg/ml), food deprivation (2800 pg/ml) or myocardial infarction (4100 pg/ml), an infusion rate of 2000 $ng*kg^{-1}*min^{-1}$ was chosen. The levels of EPI and NOR infused are markedly different and thus, no direct comparisons between the effects of the two hormones can be made.

The CIM infusion rate used was equal to the amount which, when administered parenterally to lambs by osmotic minipumps (10), produced significant changes in body composition.

The lack of a significant effect of NOR infusion on whole-body leucine metabolism in the sheep, is in contrast to the response observed in pigs (Section I), in which a 2 h NOR infusion caused decreases in proteolysis, protein synthesis, leucine and KIC interconversion and oxidation. Similar to pigs, rat muscle also responded to NOR perfusion

by decreasing the rate of release of glutamine and alanine into the medium (15) and the rate of amino acid incorporation into protein (40). These studies may indicate that the response of muscle in ruminants to noradrenergic stimulation is different than that in nonruminants.

The concentrations of glucose, FFA and glucagon during the NOR infusion all peaked at 60 min, while insulin concentrations were just beginning to rise. This indicates that the effect of NOR infusion on insulin may have been of a suppressive nature initially (3), while subsequent changes may have been the result of alterations in metabolites or other hormones such as glucagon (4).

The rise in the plasma concentrations of FFA, concomitant with an increase in glucagon, during the NOR infusion, is consistent with reports of lipolytic actions of NOR (33) and glucagon (4). This effect seemed to be obscured after 60 min, however, by the rise in the antilipolytic hormone insulin.

NOR infusion increased the plasma cortisol concentration approximately sixfold in the sheep. Because no significant change in protein degradation was observed during the NOR infusion, despite elevated levels of the catabolic hormone cortisol, it is possible that NOR and cortisol had offsetting effects on proteolysis.

EPI infusion in the sheep produced significant decreases in both leucine entry (proteolysis and absorption) and protein synthesis. These results agree with those found in pigs during infusions of EPI (Section I). Other research has also indicated that EPI caused decreased release of amino acids from muscle in vitro (15) and decreased appearance of leucine carbon in the plasma of humans (24).

The infusion of EPI produced no significant changes in the rate of KIC oxidation in the sheep. This is consistent with the results obtained with EPI infusion in pigs (Section I) and dogs (23). In vitro research (8) has indicated though, that perfusion of rat diaphragm muscle with EPI increased the rate of amino acid catabolism when glucose and pyruvate were omitted from the medium.

EPI infusion resulted in increased ($P<0.01$) reamination of KIC to leucine and a marginal increase ($P<0.1$) in leucine deamination. Interconversion between the branched-chain amino acids and their keto acids is catalyzed by a specific transaminase which occurs in a variety of tissues (18). Since the leucine model used in this study reflects the sum of changes in interconversion in the whole-body though, it is not possible to determine the magnitude of changes in any particular organ, or to predict which organs may have been influenced by the increased shuttling of leucine and KIC.

The plasma concentrations of glucose, FFA and insulin all increased from the start of the EPI infusion. FFA concentrations peaked at 60 min, however, while the levels of glucose and insulin continued to rise until 150 min. Glucagon concentrations were stable until 60 min, but subsequently decreased until the end of the experiment. These data suggest that EPI directly influenced glucose and FFA metabolism and the release of insulin from the pancreas, because the increases were observed during the early infusion period. The effects of EPI on glucose homeostasis may be the result of β-adrenergic stimulation of muscle and liver glycogenolysis and liver gluconeogenesis (13).

The infusion of the β_2-agonist CIM in the sheep, produced marginal

decreases ($P<0.1$) in the rates of leucine entry and protein synthesis. Because no significant changes occurred in the rate of KIC oxidation, however, no effect would be expected in the net protein synthesis rate (synthesis - proteolysis).

During the CIM infusion, the I/G ratio dramatically increased, indicating a more anabolic situation. Inconsistent with this was an increase in glucose concentrations from 60 to 120 min. It has been previously noted, however, that glucose and insulin concentrations in ruminants are not well related (37).

In contrast to the increase in insulin which occurred during the acute infusion of the β-agonist CIM, Beerman et al. (5) indicated in a preliminary report, that the chronic feeding of CIM to 17 kg lambs, for 6 or 12 weeks, resulted in decreases in insulin concentrations of 50%. Insulin was also found to decrease following injections of fenoterol for 19 d in rats (12), although no change in insulin was observed following 16 d of clenbuterol injections. Tepperman (35) notes that chronic stimulation can cause down-regulation of β-receptors. The differences in insulin response to acute versus chronic stimulation suggest that the latter may cause down-regulation of receptors in the pancreas.

FFA concentrations during the CIM infusion increased initially but were not changed significantly between the basal and final periods. Other research has indicated that both acute (7) and chronic (5) β-receptor stimulation result in increases in FFA levels in ruminants.

There may be species-related differences in the response of glucocorticoids to adrenergic stimulation. In rats, EPI produced a dose-related increase in corticosterone and ACTH (36), but in the

present experiment, no changes in cortisol were observed during either the EPI or CIM infusions. The response to EPI in rats (36) could be blocked with propranolol (β-adrenergic antagonist) and mimicked with isoproterenol (β-adrenergic agonist), indicating a β-stimulated response. Because isoproterenol exhibits both β_1 and β_2 actions (39) and no effect on cortisol was seen following acute (Fig. 6) or chronic (5) administrations of CIM (β_2-agonist), these data suggest that the effect is mediated via β_1-receptors. This could also account for the NOR effect since, as it was previously noted, NOR has β_1 but little β_2 action (13).

Previous research has indicated that the rate of protein synthesis in rat skeletal muscle is increased upon chronic treatment of the animals with β_2-agonists (12). Furthermore, numerous experiments have shown that chronic administration of β_2-agonists causes dramatic increases in the muscle content and decreases in the quantity of adipose tissue in several species (2, 6, 11, 12, 19, 27), including sheep. A major reason why the present acute study may not have duplicated effects seen in the chronic studies, is that the acute study only covers a short time period during one part of the absorptive stage. Diurnal changes not reflected in this experiment may influence the responsiveness of the animals to these compounds. Other factors which may cause differences between the chronic and acute studies include alterations in the relative changes in levels of various hormones or metabolites, as affected by down-regulation, and changes in blood flow which could influence the partitioning of substrates to each tissue.

In conclusion, these experiments indicate that in sheep, acute

elevations of EPI to levels seen in stress, may cause a decrease in the rate of whole-body protein turnover, but no change in catabolism. Similarly, infusion of the β-agonist CIM also results in a decrease in protein turnover. A reduced requirement for energy will be associated with these changes in protein metabolism (26) and thus, indirectly increase the supply of readily available energy for other metabolic processes.

REFERENCES

1. Allsop, J. R., R. R. Wolfe, and J. F. Burke. Tracer priming the bicarbonate pool. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45: 137-139, 1978.

2. Baker, P. K., R. H. Dalrymple, D. L. Ingle, and C. A. Ricks. Use of a β-adrenergic agonist to alter muscle and fat deposition in lambs. J. Anim. Sci. 59: 1256-1261, 1984.

3. Bassett, J. M. Metabolic effects of catecholamines in sheep. Aust. J. biol. Sci. 23: 903-914, 1970.

4. Bassett, J. M. The effects of glucagon on plasma concentrations of insulin, growth hormone, glucose, and free fatty acids in sheep: Comparison with the effects of catecholamines. Aust. J. Biol. Sci. 24: 311-320, 1971.

5. Beerman, D. H., W. R. Butler, D. E. Hogue, R. H. Dalrymple, and C. A. Ricks. Plasma metabolic hormone, glucose and free fatty acid concentrations in lambs fed the repartitioning agent, cimaterol (CL 263,780). J. Anim. Sci. 61 (Suppl. 1): 255, 1985.

6. Beerman, D. H., D. E. Hogue, V. K. Fishell, R. H. Dalrymple, and C. A. Ricks. Effects of cimaterol and fishmeal on performance, carcass characteristics and skeletal muscle growth in lambs. J. Anim. Sci. 62: 370-380, 1986.

7. Blum, J. W., D. Froehli, and P. Kunz. Effects of catecholamines on plasma free fatty acids in fed and fasted cattle. Endocrinology 110: 452-456, 1982.

8. Buse, M. G., J. F. Biggers, C. Drier, and J. F. Buse. The effect of epinephrine, glucagon, and the nutritional state on the oxidation

of branched chain amino acids and pyruvate by isolated hearts and diaphragms of the rat. J. Biol. Chem. 248: 697-706, 1973.

9. Cunningham, H. M., D. W. Friend, and J. W. G. Nicholson. Effect of epinephrine on nitrogen and fat deposition of pigs. J. Anim. Sci. 22: 632-636, 1963.

10. Dalrymple, R. H., P. K. Baker, M. E. Doscher, D. L. Ingle, and C. A. Ricks. Effect of the repartitioning agent cimaterol (CL 263,780) on growth and carcass characteristics of lambs. J. Anim. Sci. 61 (Suppl. 1): 256, 1985.

11. Dalrymple, R. H., P. K. Baker, P. E. Gingher, D. L. Ingle, J. M. Pensack, and C. A. Ricks. A repartitioning agent to improve performance and carcass composition of broilers. Poult. Sci. 63: 2376-2383, 1984.

12. Emery, P. W., N. J. Rothwell, M. J. Stock, and P. D. Winter. Chronic effects of β_2-adrenergic agonists on body composition and protein synthesis in the rat. Biosci. Rep. 4: 83-91, 1984.

13. Exton, J. H. Mechanisms involved in α-adrenergic phenomena. Am. J. Physiol. 248 (Endocrinol. Metab. 11): E633-E647, 1985.

14. Galbo, H., J. J. Holst, and N. J. Christensen. Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. J. Appl. Physiol. 38: 70-76, 1975.

15. Garber, A. J., I. E. Karl, and D. M. Kipnis. Alanine and glutamine synthesis and release from skeletal muscle. IV. β-adrenergic inhibition of amino acid release. J. Biol. Chem. 251: 851-857, 1976.

16. Harris, V., G. R. Faloona and R. H. Unger. Glucagon. In Methods

of Hormone Radioimmunoassay, edited by B. M. Jaffe and H.R. Behrman. New York:Academic Press, 1979, p. 643-656.

17. Helland, S. J., B. Grisdale-Helland, and S. Nissen. Effect of the site of isotope infusion on the estimates of leucine metabolism in pigs and sheep. Submitted to Am. J. Physiol., 1986.

18. Ichihara, A., C. Noda, and K. Tanaka. Oxidation of branched chain amino acids with special reference to their transaminase. In Metabolism and Clinical Implications of Branched Chain Amino and Ketoacids, edited by M. Walser and J. R. Williamson. New York: Elsevier North Holland, 1981, p. 227-231.

19. Jones, R. W., R. A. Easter, F. K. McKeith, R. H. Dalrymple, H. M. Maddock, and P. J. Bechtel. Effect of the β-adrenergic agonist cimaterol (CL 263,780) on the growth and carcass characteristics of finishing swine. J. Anim. Sci. 61: 905-913, 1985.

20. Keller, U., P. P. G. Gerber, and W. Stauffacher. Stimulatory effect of norepinephrine on ketogenesis in normal and insulin-deficient humans. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E732-E739, 1984.

21. Kozlowski, S., K. Nazar, H. Kaciuba-Uscilko, D. Stevens and Z. Brzezinska. Increased plasma catecholamine levels in response to reduced energy intake in the dog. Horm. Metabol. Res. 15: 108-109, 1983.

22. Li, J. B. and L. S. Jefferson. Effect of isoproterenol on amino acid levels and protein turnover in skeletal muscle. Am. J. Physiol. 232 (Endocrinol. Metab. Gastrointest. Physiol. 1): E243-E249, 1977.

23. Liddell, M. J., A. M. Daniel, L. D. MacLean, and H. M. Shizgal. The role of stress hormones in the catabolic metabolism of shock. Surg. Gynecol. Obstet. 149: 822-830, 1979.

24. Miles, J. M., S. L. Nissen, J. E. Gerich, and M. W. Haymond. Effects of epinephrine infusion on leucine and alanine kinetics in humans. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E166-E172, 1984.

25. Nissen, S. L., C. Van Huysen, and M. W. Haymond. Measurement of branched chain α-ketoacids in plasma by high-performance liquid chromatography. J. Chromatogr. 232: 170-175, 1982.

26. Reeds, P. J., M. F. Fuller, and B. A. Nicholson. Metabolic basis of energy expenditure with particular reference to protein. In Substrate and Energy Metabolism in Man, edited by J. S. Garrow and D. Halliday, London:John Libbey, p. 46-56, 1985.

27. Ricks, C. A., R. H. Dalrymple, P. K. Baker, and D. L. Ingle. Use of a β-agonist to alter fat and muscle deposition in steers. J. Anim. Sci. 59: 1247-1255, 1984.

28. Rizza, R. A., P. E. Cryer, M. W. Haymond, and J. E. Gerich. Adrenergic mechanisms of catecholamine action on glucose homeostasis in man. Metabolism 29 (Suppl. 1): 1155-1163, 1980.

29. Rudiger, H. W., U. Langenbeck, and H. W. Goedde. A simplified method for the preparation of ^{14}C-labeled branched-chain β-oxoacids. Biochem. J. 126: 445-446, 1972.

30. Schade, D. S. and R. P. Eaton. The regulation of plasma ketone body concentration by counter-regulatory hormones in man. III. Effects of norepinephrine in normal man. Diabetes 28: 5-10, 1979.

31. Shimizu, S., K. Inoue, Y. Tani and H. Yamada. Enzymatic microdetermination of serum free fatty acids. Anal. Biochem. 98: 341-345, 1979.

32. Snedecor, G. W. and W. G. Cochran. Statistical Methods. Ames:Iowa State University Press, 1979, 593 pp.

33. Steffens, A. B., G. Damsma, J. Van Der Gugten, and P. G. M. Luiten. Circulating free fatty acids, insulin, and glucose during chemical stimulation of hypothalamus in rats. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E765-E771, 1984.

34. Stiles, G. L., M. G. Caron, and R. J. Lefkowitz. β-adrenergic receptors: Biochemical mechanisms of physiological regulation. Physiol. Rev. 64: 661-743, 1984.

35. Tepperman, J. Metabolic and Endocrine Physiology: An Introductory Text. Chicago:Yearbook Medical Publishers, 1980.

36. Tilders, F. J. H., F. Berkenbosch, and P. G. Smelik. Adrenergic mechanisms involved in the control of pituitary-adrenal activity in the rat: A β-adrenergic stimulatory mechanism. Endocrinology 110: 114-120, 1982.

37. Trenkle, A. Postprandial changes in insulin secretion rates in sheep. J. Nutr. 101: 1099-1103, 1971.

38. Trenkle, A. Radioimmunoassay of plasma hormones: Review of plasma insulin in ruminants. J. Dairy Sci. 55: 1200-1211, 1972.

39. Weiner, N. Norepinephrine, epinephrine and the sympathomimetic amines. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, edited by A. G. Gilman, L. S. Goodman, T. W. Rall and F. Murad. New York:Macmillan, 1985, p. 145-180.

40. Wool, I. G. Incorporation of C^{14}-amino acids into protein of isolated diaphragms: Effect of epinephrine and norepinephrine. Am. J. Physiol. 198: 54-56, 1960.

GENERAL DISCUSSION

Epinephrine (EPI) and norepinephrine (NOR) were infused at levels which were designed to mimic concentrations of these hormones in stress situations and thus, NOR was infused at a rate 20 times that of EPI. The level of cimaterol (CIM) used was one which had been found to affect muscle and fat deposition when chronically infused into lambs. Therefore, any comparison of the effects of these compounds is only relevant for the specific dosages employed.

A cross-species evaluation of the results from these experiments is dependent on the recognition of the inherent differences between ruminant and nonruminant animals. It was impractical to attempt these studies at the same stage of nutrient absorption because of the difficulty in fasting ruminants and because other work in our laboratory had shown that pigs refused feed during catecholamine infusions. Therefore, differences exist in the amount of time each species was without feed before measurements were begun. It is likely that the effects of these compounds on mobilization of body stores for energy are dependent, to some extent, on the absorption of substrates from the diet. Buse et al. (4) noted that in vitro stimulation of leucine oxidation by EPI only occurred in diaphragms from fasted animals, and when glucose and pyruvate were omitted from the media.

Given these reservations about comparisons among experiments, an evaluation of the results of these trials indicates a great number of similarities. Figure 1 summarizes the changes in protein metabolism and the plasma concentrations of the hormones and metabolites studied, as a result of infusion of each adrenergic substance. Disregarding the

Figure 1. Summary of changes in protein metabolism and the plasma concentrations of hormones and metabolites, during infusions of adrenergic compounds in pigs and sheep. Significance at $P<0.05$ denoted by closed arrows. Significance at $P<0.1$ denoted by open arrows.

	EPINEPHRINE		NOREPINEPHRINE		CIMATEROL
	PIGS	SHEEP	PIGS	SHEEP	SHEEP
LEUCINE ENTRY	↓	↓	↓		⇓
SYNTHESIS	↓	↓	↓		⇓
LEU -) KIC		⇑	↓		
KIC -) LEU		↑	↓		⇓
OXIDATION			↓		
GLUCOSE	↑	⇑	↑	↑	↑
FFA		↑	↑	↑	
CORTISOL			↑	↑	
GLUCAGON		↑	↑		⇓
INSULIN		⇑		↑	↑

magnitude of response, the changes which occurred were generally the same, both between species and among compounds. The only exceptions to this are the interconversions of leucine and KIC and the glucagon response. A decrease in interconversion of leucine and KIC during the NOR infusion in pigs and a decrease in reamination during the CIM infusion in sheep, contrasts with the increase in interconversion during the EPI infusion in sheep. In addition, contrary to the decrease in the glucagon concentration observed during the CIM infusion in sheep, positive glucagon responses were stimulated during the EPI infusion in sheep and the NOR infusion in pigs.

Overall, the protein kinetic data indicate that catecholamines affect the degradation of protein in the whole animal. It does not seem, however, that this decrease results in more efficient nitrogen conservation, because oxidation was not consistently decreased.

The decrease in whole-body protein turnover which occurred in all of the experiments, except the NOR study in sheep, should result in a conservation of energy. This will further supplement the energy availability from body stores and aid in the maintenance of the internal environment during periods of stress.

LITERATURE CITED

1. Ahlquist, R. P. A study of the adrenotropic receptors. Am. J. Physiol. 153: 586-600, 1948.

2. Baker, P. K., R. H. Dalrymple, D. L. Ingle, and C. A. Ricks. Use of a β-adrenergic agonist to alter muscle and fat deposition in lambs. J. Anim. Sci. 59: 1256-1261, 1984.

3. Beerman, D. H., D. E. Hogue, V. K. Fishell, R. H. Dalrymple, and C. A. Ricks. Effects of cimaterol and fishmeal on performance, carcass characteristics and skeletal muscle growth in lambs. J. Anim. Sci. 62: 370-380, 1986.

4. Buse, M. G., J. F. Biggers, C. Drier, and J. F. Buse. The effect of epinephrine, glucagon, and the nutritional state on the oxidation of branched chain amino acids and pyruvate by isolated hearts and diaphragms of the rat. J. Biol. Chem. 248: 697-706, 1973.

5. Cunningham, H. M., D. W. Friend, and J. W. G. Nicholson. Effect of epinephrine on nitrogen and fat deposition of pigs. J. Anim. Sci. 22: 632-636, 1963.

6. Dalrymple, R. H., P. K. Baker, P. E. Gingher, D. L. Ingle, J. M. Pensack, and C. A. Ricks. A repartitioning agent to improve performance and carcass composition of broilers. Poultry Sci. 63: 2376-2383, 1984.

7. Emery, P. W., N. J. Rothwell, M. J. Stock, and P. D. Winter. Chronic effects of β_2-adrenergic agonists on body composition and protein synthesis in the rat. Biosci. Rep. 4: 83-91, 1984.

8. Exton, J. H. Mechanisms involved in α-adrenergic phenomena. Am.

J. Physiol. 248 (Endocrinol. Metab. 11): E633-E647, 1985.

9. Galbo, H., J. J. Holst, and N. J. Christensen. Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. J. Appl. Physiol. 38: 70-76, 1975.

10. Garber, A. J., I. E. Karl, and D. M. Kipnis. Alanine and glutamine synthesis and release from skeletal muscle. IV. β-adrenergic inhibition of amino acid release. J. Biol. Chem. 251: 851-857, 1976.

11. Helland, S. J., R. C. Ewan, A. Trenkle and S. Nissen. In vivo metabolism of leucine and α-ketoisocaproate in the pig: Influence of dietary glucose or sucrose. J. Nutr. (In Press), 1986.

12. Helland, S. J., B. Grisdale-Helland, and S. Nissen. Effect of the site of isotope infusion on the estimates of leucine metabolism in pigs and sheep. (Submitted to Am. J. Physiol.), 1986.

13. Jones, R. W., R. A. Easter, F. K. McKeith, R. H. Dalrymple, H. M. Maddock, and P. J. Bechtel. Effect of the β-adrenergic agonist cimaterol (CL 263,780) on the growth and carcass characteristics of finishing swine. J. Anim. Sci. 61:905-913, 1985.

14. Kozlowski, S., K. Nazar, H. Kaciuba-Uscilko, D. Stevens and Z. Brzezinska. Increased plasma catecholamine levels in response to reduced energy intake in the dog. Horm. Metabol. Res. 15: 108-109, 1983.

15. Li, J. B. and L. S. Jefferson. Effect of isoproterenol on amino acid levels and protein turnover in skeletal muscle. Am. J. Physiol. 232 (Endocrinol. Metab. Gastrointest. Physiol. 1): E243-E249, 1977.

16. Liddell, M. J., A. M. Daniel, L. D. MacLean, and H. M. Shizgal. The role of stress hormones in the catabolic metabolism of shock. Surg. Gynecol. Obstet. 149: 822-830, 1979.

17. Matthews, D. E., D. M. Bier, M. J. Rennie, R. H. T. Edwards, D. Halliday, D. J. Millward, and G. A. Clugston. Regulation of leucine metabolism in man: A stable isotope study. Science 214:1129-1131, 1981.

18. Miles, J. M., S. L. Nissen, J. E. Gerich, and M. W. Haymond. Effects of epinephrine infusion on leucine and alanine kinetics in humans. Am. J. Physiol. 247 (Endocrinol. Metab. 10):E166-E172, 1984.

19. Nissen, S. and M. W. Haymond. Effects of fasting on flux and interconversion of leucine and α-ketoisocaproate in vivo. Am. J. Physiol. 241 (Endocrinol. Metab. 4): E72-E75, 1981.

20. Nissen, S. and M. W. Haymond. Changes in leucine kinetics during meal absorption; effects of dietary leucine availability. Am. J. Physiol. (In Press), 1986.

21. Ricks, C. A., R. H. Dalrymple, P. K. Baker, and D. L. Ingle. Use of a β-agonist to alter fat and muscle deposition in steers. J. Anim. Sci. 59: 1247-1255, 1984.

22. Russell, J. A. Hormonal control of amino acid metabolism. Federation Proc. 14:696-705, 1955.

23. Stiles, G. L., M. G. Caron, and R. J. Lefkowitz. β-adrenergic receptors: Biochemical mechanisms of physiological regulation. Physiol. Rev. 64: 661-743, 1984.

24. Tepperman, J. Metabolic and Endocrine Physiology: An Introductory

Text. Chicago:Yearbook Medical Publishers, 1980.

25. Weiner, N. Norepinephrine, epinephrine and the sympathomimetic amines. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, edited by A. G. Gilman, L. S. Goodman, T. W. Rall and F. Murad. New York:Macmillan, 1985, p. 145-180.

26. Weiner, N and P. Taylor. Neurohormonal transmission: The autonomic and somatic motor nervous systems. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, edited by A. G. Gilman, L. S. Goodman, T. W. Rall and F. Murad. New York:Macmillan, 1985, p. 66-99.

27. Wool, I. G. Incorporation of C^{14}-amino acids into protein of isolated diaphragms: effect of epinephrine and norepinephrine. Am. J. Physiol. 198: 54-56, 1960.

ACKNOWLEDGEMENTS

I would like to thank Dr. Steven Nissen for providing the opportunity for doing this work and for serving as my major professor. I would also like to thank Dr. Ted Huiatt for allowing me to experiment with muscle cell cultures and for serving on my advisory committee. Appreciation is also given to Drs. Allen Trenkle, Jerry W. Young and Malcolm Crump for serving on this committee and providing valuable suggestions.

Much appreciation is also extended to the technicians and fellow graduate students who helped with this work and provided an enjoyable environment.

Sincere thanks is given to my family for providing encouragement and understanding throughout the completion of my graduate studies.

APPENDIX

Table 1. Leucine metabolism in pigs during infusions of saline, epinephrine or norepinephrine[a]

	Saline (N=2)	Epinephrine (N=6)	Norepinephrine (N=6)
		%	
Proteolysis	-8.8 ± 5.1	-10.7 ± 1.9**	-24.8 ± 2.6**
Synthesis	-10.5 ± 1.4	-14.4 ± 3.8*	-26.3 ± 2.4**
Leu -> KIC	9.2 ± 20.3	5.9 ± 12.0	-31.1 ± 6.7**
KIC -> Leu	15.4 ± 21.5	6.6 ± 16.3	-35.0 ± 8.3**
Oxidation	-4.2 ± 21.2	80.0 ± 55.2	-21.0 ± 5.4*

[a]Percentage change between basal (-15 to 0 min) and final periods (75 to 120 min).

* Change from basal to final period significantly different from zero ($P<0.05$).

** Change from basal to final period significantly different from zero ($P<0.01$).

Table 2. Leucine metabolism in sheep during infusions of saline, epinephrine, norepinephrine or cimaterol[a]

	Saline (N=4)	Epinephrine (N=4)	Norepinephrine (N=4)	Cimaterol (N=4)
		%		
Leucine entry	-8.7 ± 10.5	-9.9 ± 1.0**	20.5 ± 18.2	-26.8 ± 10.4+
Synthesis	-9.7 ± 9.7	-13.5 ± 2.1**	24.0 ± 19.9	-30.5 ± 10.3+
Leu -> KIC	-1.6 ± 19.6	19.0 ± 7.4+	31.4 ± 25.9	-8.7 ± 14.7
KIC -> Leu	7.8 ± 30.8	31.0 ± 5.1**	160.4 ± 77.7	-33.4 ± 13.5+
Oxidation	-2.7 ± 17.5	15.0 ± 8.6	-12.9 ± 9.3	-0.6 ± 12.8

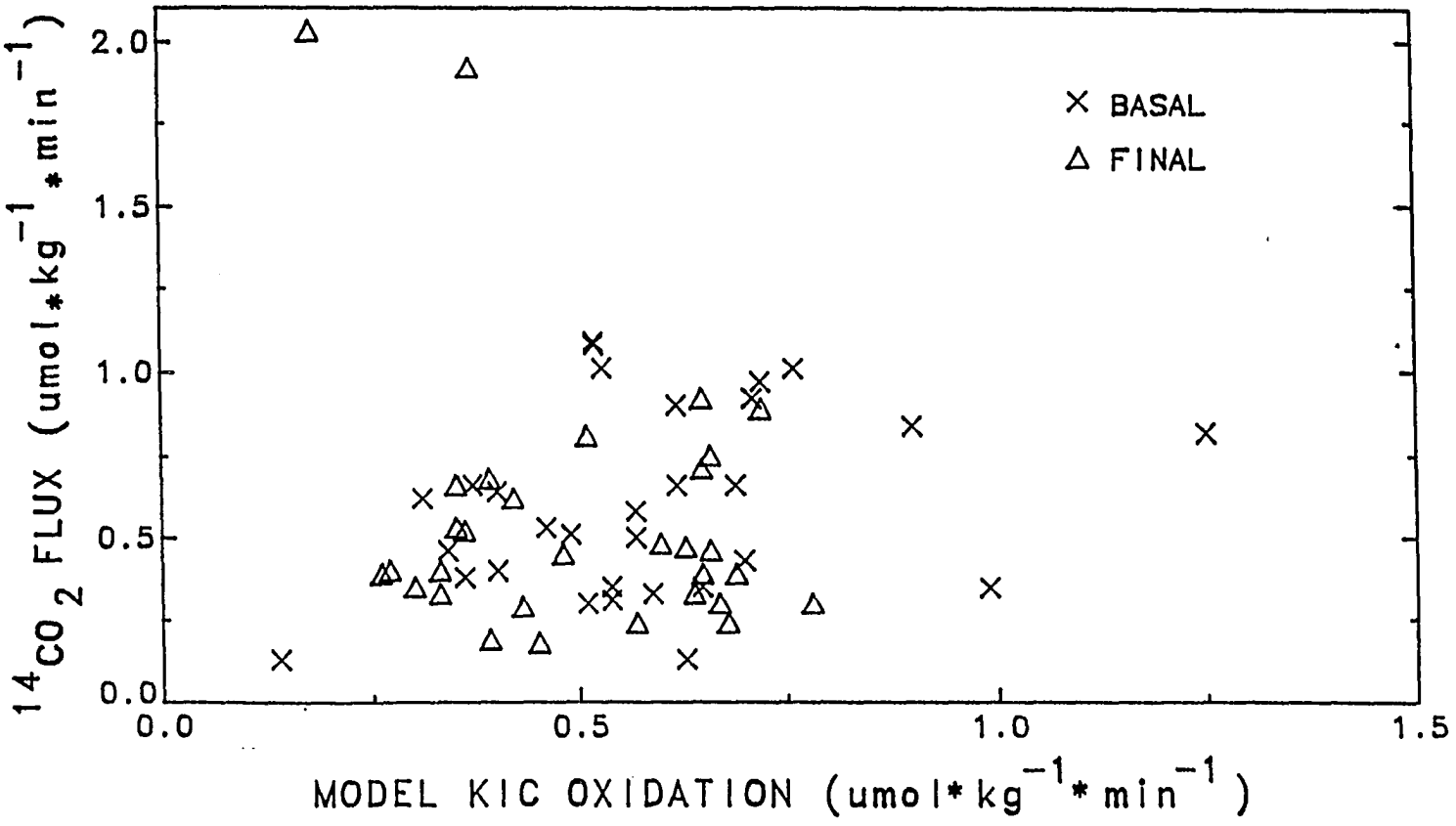
[a]Percentage change between basal (-30 to 0 min) and final periods (90 to 180 min).

+ Change from basal to final period significantly different from zero ($P<0.1$).

* Change from basal to final period significantly different from zero ($P<0.05$).

** Change from basal to final period significantly different from zero ($P<0.01$).

Figure 1. Breath $^{14}CO_2$ flux versus two-pool model KIC oxidation, at -30 and 0 min (basal) and 150 and 180 min (final), during all treatments, in sheep. $Y = 0.41 + 0.26*X$, $R^2 = 0.03$.


2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
BASAL
FINAL
$^{14}CO_2$ FLUX ($umol*kg^{-1}*min^{-1}$)
MODEL KIC OXIDATION ($umol*kg^{-1}*min^{-1}$)