NEUROENDOCRINE, METABOLIC AND CARDIOVASCULAR RESPONSES TO EXERCISE DIFFER AMONG HEALTHY MEN

1995

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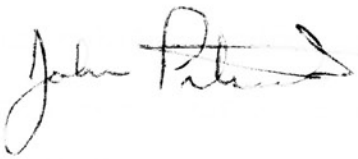

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ABSTRACT

Title of Dissertation: Neuroendocrine, Metabolic and Cardiovascular Responses To Exercise Differ Among Healthy Men

John S. Petrides, Doctor of Philosophy, 1995

Dissertation co-directed by:

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This dissertation shows that healthy, moderately trained males (19-42) may be divided into two groups based upon their unique endocrine and metabolic responses to a physiologic stressor, high-intensity treadmill exercise. Individuals exhibiting enhanced exercise-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis that was resistant to glucocorticoid suppression were designated as high-responders (≈ 36% of each population sample studied) and those showing normal exercise-induced HPA activation that was completely suppressed by glucocorticoid pretreatment were termed low-responders. Specifically, in phase I of this project, high-responders (n=4) exhibited a significant increase in exercise-induced plasma adrenocorticotropin (ACTH) concentrations following pretreatment with 4 mg of dexamethasone (DEX), a synthetic glucocorticoid. In contrast, the exercise-induced plasma ACTH response of low-responders (n=7) was completely suppressed by DEX administration. High-responders also showed significantly greater exercise-induced increases in heart rate and circulating concentrations of plasma ACTH, cortisol, lactate and glucose with placebo as compared to low-responders ($p < 0.05$). Interestingly, high-responders exhibited a markedly enhanced plasma

arginine vasopressin (AVP) response to exercise that was not attenuated by DEX pretreatment. Phase II of this project confirmed and extended these findings to include: an examination of equipotent doses of DEX (4 mg) and hydrocortisone (100 mg) on exercise-induced HPA activation; determination of individual anaerobic thresholds and pituitary-adrenal responses to both exogenous AVP stimulation and a 1 mg dexamethasone suppression test (DST). In addition, individuals underwent assessments of trait behavior. During exercise, high-responders (n=7) exhibited a greater sensitivity to the negative feedback actions of hydrocortisone, a mixed Type I and Type II glucocorticoid receptor ligand, as compared to DEX, a pure Type II receptor ligand ($p < 0.05$). In addition to differential neuroendocrine and metabolic responses to exercise, plasma ACTH responses to exogenous AVP were significantly greater in high- as compared to low-responders (n=8) ($p<0.05$). Both groups showed similar suppression of basal cortisol concentrations to the DST, further supporting the premise of differential regulation of stimulated HPA activity. Moreover, high-responders exhibited a tendency for increased arousibility as judged by the Spielberger Trait Anxiety Scale compared to low-responders. Finally, high-responders had lower anaerobic thresholds and enhanced exercise-induced lactate production, suggesting a role for lactate in mediating differences between low- and high-responders. However, *in vitro* studies investigating this possibility showed no direct actions of lactate on rat anterior pituitary β-endorphin and hypothalamic CRH release, an indication it may have actions on higher brain centers. In summary, these findings indicate that the increased responsiveness of the HPA axis in high-responders results, in part, from enhanced AVP release, increased sensitivity to AVP's actions and differential

sensitivity to negative feedback mediated by Type II glucocorticoid receptors. Moreover, fundamental differences in higher brain function also appear to play a role in defining the responsiveness of the HPA axis. Finally, exercise may provide a model for determining one's susceptibility to disorders involving dysregulation of HPA function, including major depression, anxiety and inflammatory disease.

Neuroendocrine, Metabolic and Cardiovascular Responses

to Exercise Differ Among Healthy Men

by

John S. Petrides

Dissertation submitted to the Faculty of the Department
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DEDICATION

I dedicate this dissertation to my grandfather and namesake, Mr. John Bassios. At 90 years young, he may not be able to read this dissertation, but can still understand every word. He has always been an inspiration as I strive towards a long, healthy and productive life.

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from the inquisitive and precise environment inherent to Dr. Mueller's lab and the honesty and ethics that Dr. Deuster brings to hers.

Lastly, I would like to thank my family and my wife's family for all of their love, support and encouragement through this tremendously difficult task. Most of all, the completion of this dissertation would not have been possible without the unconditional love and acceptance as well as the technical support both in the lab and while writing that I received from my wife, Tracey. She believed in me and struggled with me when there was only a flicker of light at the end of the tunnel. This is an accomplishment which we will celebrate together.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

$5HT_2$	5-hydroxytryptamine$_2$
AC	Adenylate cyclase
ACTH	Adrenocorticotropin
AL	Anterior lobe
ANF	Atrial natriuretic factor
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUC	Area under the curve
AVP	Arginine vasopressin
β-end	Beta-endorphin
BSA	Bovine serum albumin
cAMP	cyclic adenosine monophosphate
CNS	Central nervous system
CO	Cortisol
CO_2	Carbon dioxide
CREB	cAMP response element binding protein
CRH	Corticotropin releasing hormone
DAG	Diacyleglycerol
DEX	Dexamethasone
dl	Deciliter
DMEM	Dulbecco's Modified Eagles Medium
DST	Dexamethasone Suppression Test
EDTA	Ethylenediamine tetraacetic acid
fmol	Femtomole
GABA	Gamma-aminobutyric acid
GH	Growth hormone
GR	Glucocorticoid receptor

h	Hour
HCO	Hydrocortisone
HPA	Hypothalamic-pituitary-adrenal
HPLC	High pressure liquid chromatography
HR	Heart rate
Ht	Height
IL-1α	Interleukin-1α
IL-1β	Interleukin-1β
IL-2	Interleukin-2
IL-6	Interleukin-6
IP_3	Inositol triphosphate
IRMA	Immunoradiometric assay
IU	International units
Kg	Kilogram
Km	Kilometer
L	Liter
LC	Locus coeruleus
LSH	Lipotropin stimulating hormone
m	Mile
mg	Milligram
min	Minute
mIU	Milli-international units
ml	Milliliter
mmol	Millimole
mph	Miles per hour
MR	Mineralocorticoid receptor
mRNA	Messenger ribonucleic acid
MSH	Melanocyte stimulating hormone
nmol	Nanomole

NPY	Neuropeptide Y
O_2	Oxygen
P	Placebo
PIP_2	Phosphatidylinositol bisphosphate
pmol	Picomole
POMC	Pro-opiomelanocortin
POMS	Bi-polar Profile of Mood States
RIA	Radioimmunoassay
SAS	Statistical Analysis System
sec	Second
TFA	Trifluoroacetic acid
TNFα	Tumor necrosis factor α
$\dot{V}O_2$	Oxygen uptake or consumption
$\dot{V}O_{2max}$	Maximal oxygen uptake or consumption
°C	Degrees celsius

SIGNIFICANCE

I. INTRODUCTION

This dissertation presents a series of findings which show that normal healthy males may be divided into two groups based upon their unique endocrine and metabolic responses to a physiologic stressor, exercise. One group exhibits a very high plasma adrenocorticotropin (ACTH) response to exercise that does not completely suppress with dexamethasone (DEX), and a behavioral profile suggestive of greater arousal. These individuals also have a very pronounced plasma arginine vasopressin (AVP) response to exercise. The other group has much lower plasma ACTH and AVP responses to exercise, and a behavioral profile suggestive of a lower level of arousal. These findings may have implications for individual genetic differences in arousability and temperament, and for the susceptibility to a variety of disorders that are thought to be associated with dysfunction of the stress response, such as anxiety and depression.

This dissertation research project evolved from initial efforts to learn more about the physiology of the stress response, especially as it is activated by a physiological stressor, graded treadmill exercise. It is well known that treadmill exercise produces dose-dependent increases in plasma ACTH, catecholamines, growth hormone (GH), lactate, and glucose (Deuster et al., 1989; Farrell et al., 1983; Luger et al., 1988; Luger et al., 1987). In addition, exercise has the

advantage of producing responses that are independent of physical conditioning or experience on a treadmill (Deuster et al., 1989; Luger et al., 1987). The original purpose for this project was to learn more about individual factors that might control HPA activation and their relation to metabolic changes evoked by exercise. In particular, we wished to further clarify the role of AVP and cortisol in the physiologic control of pituitary-adrenal function.

One objective of our initial investigation was to systematically determine how AVP secreted during exercise correlated with the pituitary-adrenal response. Unexpectedly, we discovered that a subgroup of healthy controls showed greatly exaggerated plasma ACTH and AVP responses to exercise as compared to the rest of controls who showed normal responses. Close inspection of these data revealed that the controls who showed the highest plasma ACTH responses also showed the highest plasma AVP responses, and that these subjects systematically failed to completely suppress their plasma ACTH responses to exercise after receiving 4 mg of dexamethasone (DEX) 4 hours prior to exercise (Petrides et al., 1994). These findings raised questions about the role of AVP in regulating ACTH secretion and in mediating glucocorticoid resistance during exercise. However, they also presented the possibility that healthy controls could be divided into two subgroups: those who showed very robust neuroendocrine responses to exercise and those who did not. On the basis of these findings, we designed a specific, comprehensive study in healthy controls and asked the following questions: 1) Could we replicate the

findings of two groups of controls based on the suppression and escape of plasma ACTH responses during exercise from high dose DEX? 2) Would these findings tell us anything about individual differences in the relative role of CRH and other factors, such as AVP in the neuroendocrine response to exercise stress? 3) Did distinguishing low- from high-responders predict neuroendocrine responses obtained during exercise without DEX pretreatment (e.g., plasma ACTH, AVP, GH and catecholamine responses)? 4) Were there differences in the behavioral profiles of low- and high-responders? 5) Did these overall differences between the two groups have implications for individual genetic differences in stress responsiveness and possibly, in susceptibility to stress-related diseases like depression?

II. BACKGROUND

A. Physiologic Mechanisms Mediate Homeostasis

In all complex, multicellular organisms, there is a requirement for the maintenance of optimal internal physiologic conditions, or homeostasis. Failure to maintain this physiologic balance results in altered body functions that can be life threatening (Cannon, 1929; Selye, 1937). Thus, the physiologic construction of the body is such that it constantly regulates tissue oxygenation, temperature, pH, as well as electrolytes, nutrients and other vital constituents within narrow limits (Galbo, 1983).

Intricate physiologic mechanisms play a crucial role in maintaining homeostasis (Viru, 1985). The body's internal milieu is continually monitored to

detect any disturbances. If any are detected, a variety of physiologic signals are transmitted to the brain, which functions as the integrator of signals both from without and within. The primary means by which the brain preserves homeostasis is via neuroendocrine and autonomic signals. The neuroendocrine regulatory center within the brain, the hypothalamus, sends hormonal signals to the anterior pituitary, which in turn releases hormones into the peripheral circulation for transport to their effector target tissues. The autonomic control centers are thought to reside principally within the brain stem, and contribute to the regulation of homeostasis both by synaptic neurotransmitter regulation and by paracrine and endocrine mechanisms (Galbo, 1983; Viru, 1985). While the mechanisms for the neural and endocrine systems differ, both coordinate homeostatic changes within limits defined by physiologic set points. Thus, homeostasis is accomplished through the integration and coordination of these two regulatory systems.

B. Challenge of Exercise

The ability of the human body to maintain homeostasis is clearly evident in its physiological adaptation to exercise. Strenuous exercise is one of the most powerful, naturally occurring, metabolic challenges experienced by the human body. During exercise, the energy demand of working muscle dramatically increases. Consequently, tissue autoregulation and neuronal mechanisms increase blood supply to the muscle to meet the heightened nutrient and oxygen requirements (Galbo, 1986; Rowell, 1986). The increased delivery of substrates

enhances the glycolytic and β-oxidative capacities of the muscle, augmenting the production of intracellular adenosine-tri-phosphate (ATP), the primary intracellular energy substrate. Thus, this physiological response, in part, ensures an adequate fuel supply to meet the increased metabolic demands of exercise (Galbo, 1983; Galbo, 1985; Galbo, 1986; Viru, 1985).

Additional physiologic mechanisms are required to maintain fuel supplies to critical organs during exercise. Exercise-induced increases in pancreatic glucagon secretion as well as the activation of the sympatho-adrenal and hypothalamic-pituitary-adrenal (HPA) systems, result in the mobilization of fuel stores (Galbo, 1983; Galbo, 1985; Viru, 1985). These mechanisms serve to mobilize primarily glucose and free fatty acids. Ultimately, these metabolic adjustments are necessary to restore homeostatic balance and to sustain physical activity in the face of fuel deprivation.

The psychological perception of "stress", as well as the autoregulatory cardiovascular changes that accompany strenuous exercise, activates the body's sympathetic nervous system, ultimately causing the release of catecholamines (Galbo, 1983; Galbo, 1985; Mason et al., 1973; Viru, 1985). There are two sources for blood-borne catecholamines: postganglionic sympathetic neurons and adrenal medullary chromaffin cells. Postganglionic sympathetic nerve terminals directly innervating vascular smooth muscle release norepinephrine, whereas the adrenal medulla releases primarily epinephrine with some norepinephrine. Catecholamines activate β- and α-adrenergic receptors which, in turn, evoke the

mobilization of free fatty acids and glucose (Galbo, 1985; Issekutz, 1978; Wahren, 1977). Specifically, catecholamines enhance the hepatic actions of glucagon which stimulate glycogen phosphorylase and glucose-6-phosphatase and inhibit glycogen synthetase, the enzymes that control the rate of glycogen breakdown and glucose production, respectively (Himms-Hagen, 1972; Saccà, 1987; Wahren, 1977). Catecholamines also increase lipolysis by activating hormone sensitive lipase (Galbo, 1983; Galbo, 1985; Himms-Hagen, 1972). This brings about rapid increases in circulating free fatty acids and glycerol. In addition to their catabolic actions, catecholamines and subsequent increases in intracellular cAMP attenuate the anabolic actions of insulin in adipose and non-exercising muscle tissues (Himms-Hagen, 1972). Specifically, catecholamines decrease the number of insulin receptors available to bind to insulin as well as decrease their binding capacity (Smith et al., 1993). Catecholamines also decrease the number of glucose transporters available within the cell membrane thereby decreasing insulin-induced glucose transport through the cell membrane (Himms-Hagen, 1972; Saccà, 1987). Therefore, catecholamines ensure that an adequate supply of glucose is available for working muscle and other tissues where demand is increased.

The stress of exercise also activates the HPA axis. The end result of this activation is the release of glucocorticoids from the adrenal cortex. Cortisol is the primary glucocorticoid in humans, whereas, corticosterone serves the same function in rats. The actions of glucocorticoids are primarily permissive allowing

cells to achieve a higher catabolic state than would occur in its absence (Galbo, 1983; Munck et al., 1984; Saccà, 1987). Thus, the two systems, in combination with several other circulatory and hormonal mechanisms, serve to increase circulating fuel supplies and thereby, sustain the delivery of energy to exercising muscle (Galbo, 1983; Galbo, 1985).

C. Regulation of the HPA Axis - Overview

The HPA axis has been the subject of much investigation and it is well known that there are three major components involved in its regulation. One involves neuronal control mechanisms which mediate the circadian rhythm and pulsatile secretion of ACTH and thus, cortisol (Carnes et al., 1990; Krieger, 1977; Watabe et al., 1987). A second component involves a closed loop negative-feedback mechanism whereby cortisol feeds back negatively to inhibit hypothalamic CRH and pituitary ACTH release (Calogero et al., 1991; Keller-Wood and Dallman, 1984; Lan et al., 1984; Lundblad and Roberts, 1988). The third component involves an open-loop system which enables the activation of the HPA axis by central mechanisms which can override glucocorticoid negative feedback. This third mechanism for activation is most evident under conditions of extreme "stress" including fever, surgery, emotional trauma and strenuous exercise (Axelrod and Reisine, 1984; Bartanusz et al., 1993; Beyer et al., 1988; Calogero et al., 1992a; Cohen et al., 1991; Dallman et al., 1992; Deuster et al., 1989; Galbo, 1985; Gold, 1988a; Kemppainen et al., 1990; Luger et al., 1988; Udelsman et al., 1987). Overall, the three mechanisms regulating HPA activation involve

multiple effector systems which include the nervous, endocrine, and as more recently shown, the immune system. It has also been suggested that the HPA axis is regulated by products of metabolism (Luger et al., 1988; Petrides et al., 1994). It remains unclear, however, the extent to which metabolic factors such as lactate, glucose and free fatty acids contribute to HPA activation (Figure 1).

1. Neuroendocrine Regulation

a. CRH, AVP, ACTH and Cortisol

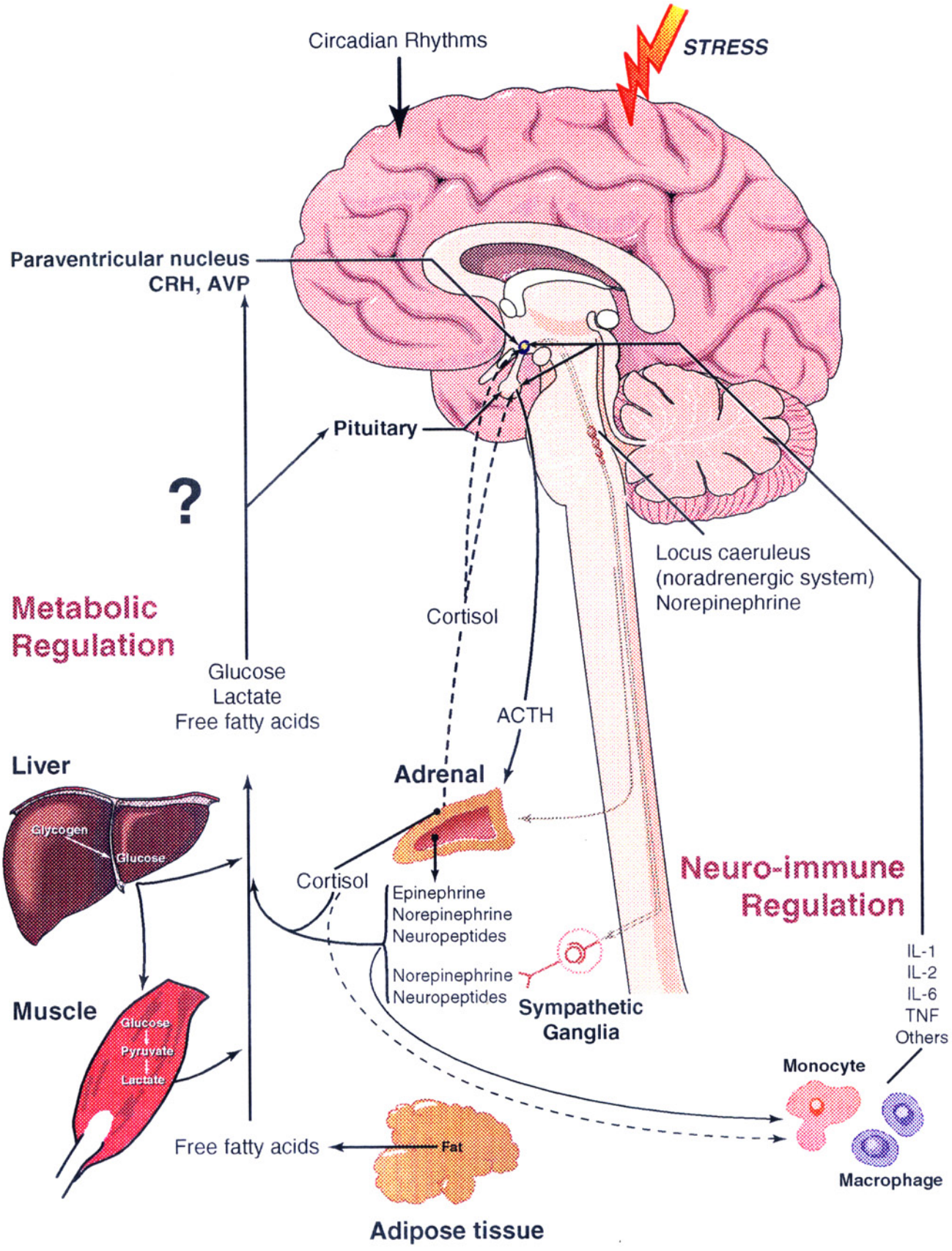
The primary hormonal components of the HPA axis are CRH, ACTH and cortisol. CRH released by parvocellular neurons projecting to the median eminence travels via the hypophyseal portal circulation to the anterior pituitary where it stimulates the synthesis and release of ACTH by corticotrophs (Antoni, 1986; Makura et al., 1984; Swanson et al., 1983) (Figure 2). Several investigators have shown that, in addition to CRH, AVP also mediates anterior pituitary ACTH release. AVP is synthesized within the hypothalamus by both parvocellular neurons of the paraventricular nucleus and magnocellular neurons of the paraventricular and supraoptic nuclei. Axonal projections of parvocellular neurons extend to the median eminence where AVP is released into the hypophyseal portal circulation and travels to anterior pituitary cells stimulating ACTH release (Kiss, 1988a; Whitnall, 1988; Whitnall, 1990; Whitnall, 1993). Magnocellular neurons project axonal terminals to the neurohypophysis of the pituitary gland where AVP is released into the general circulation (Antoni, 1993; Zimmerman, 1985). Therefore, it is predominately believed that parvocellular

Figure 1. Regulation of the Hypothalamic-Pituitary-Adrenal Axis

The paraventricular nucleus and the locus coeruleus (noradrenergic system) are shown along with component regulatory systems: the pituitary-adrenal, the adrenomedullary, the systemic-sympathetic, the immune and metabolic. Activation of the HPA axis involves the release of CRH and AVP by paraventricular neurons which synergistically stimulate the secretion of ACTH by pituitary corticotrophs. ACTH secreted into the peripheral circulation travels to the adrenal cortex to stimulate the synthesis of cortisol. Neuroendocrine regulation of HPA function involves: 1) neuronal control mechanisms which mediate the circadian rhythm of CRH and AVP release, 2) negative-feedback regulation whereby cortisol feeds back negatively to inhibit both CRH and ACTH release and 3) a central mediated "stress" mechanism that stimulates HPA function irrespective of glucocorticoid negative-feedback. Neuroimmune regulation of the HPA axis involves adrenomedullary and noradrenergic stimulation of cytokine (IL-1, IL-2, IL-6, TNF) release which in turn enhances HPA function. Increases in circulating cortisol then suppress cytokine release to regulate inflammation and immune function. It has been suggested that metabolic factors mobilized during physical challenges play a role in HPA regulation. Exercise-induced activation of the sympathoadrenal and HPA systems and subsequent release of norepinephrine, epinephrine and cortisol stimulate the mobilization of glucose, lactate and free fatty acids. These metabolic factors may positively feedback to hypothalamic and pituitary levels and enhance HPA function. Solid lines represent stimulatory mechanisms and dashed lines represent inhibitory mechanisms. Figure modified from Chrousos, 1995.

Regulation of the Hypothalamic-Pituitary-Adrenal Axis

Neuroendocrine Regulation



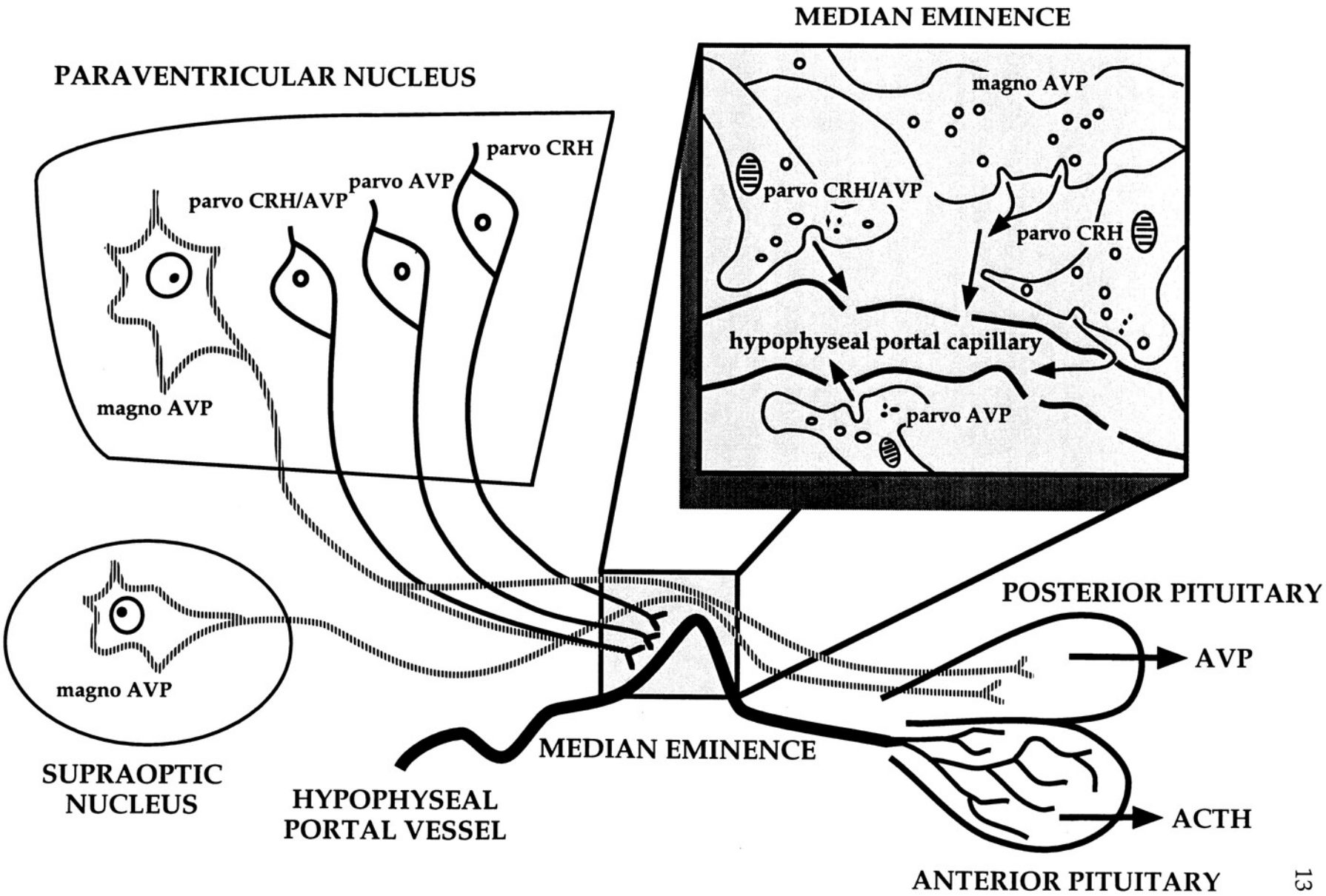
AVP, but not magnocellular, contributes to corticotroph ACTH release. However, anatomical data have shown that magnocellular neurons send collaterals to the median eminence to release AVP into the hypophyseal portal circulation (Figure 2). Thus, it appears that AVP originating from both parvocellular and magnocellular neurons may contribute to anterior pituitary ACTH release. Circulating ACTH, in turn, travels to the adrenal cortex where it stimulates the secretion of cortisol from cells of the zona fasciculata (Simpson and Waterman, 1988). The negative feedback actions of cortisol maintain the functions of the axis at the "set point" defined by the hypothalamus (Munck et al., 1984).

b. Hypothalamic Regulation

At each anatomical level of the HPA axis, multiple regulatory factors control the secretion of CRH, ACTH and cortisol. Secretion of hypothalamic CRH is regulated by a combination of neuronal and blood-borne factors. Noradrenergic, cholinergic, and serotonergic neurons innervate hypothalamic paraventricular neurons expressing CRH, and directly stimulate the release of CRH *in vitro* (Al-Damluji, 1988; Calogero et al., 1988a; Calogero et al., 1988b; Calogero et al., 1988c; Plotsky, 1987; Sawchenko et al., 1993; Swanson and Simmons, 1989; Tsagarakis et al., 1988). In addition, dopamine, angiotensin II and NPY have been shown to directly stimulate the release of CRH (Plotsky et al., 1988; Tsagarakis et al., 1989). Inhibition of CRH release is mediated by gamma-aminobutyric acid (GABA) (Calogero et al., 1988d) and endogenous

Figure 2. Hypothalamic Control of Pituitary ACTH and AVP Release

Schematic diagram showing hypothalamic control of pituitary ACTH and AVP release. Magnocellular (magno) neurons of the supraoptic and paraventricular nuclei synthesize AVP and release it from terminals in the posterior pituitary. Collateral neuronal projections from magnocellular neurons of the paraventricular nucleus release AVP into the hypophyseal portal capillaries at the median eminence. Similarly, parvocellular neurons of the paraventricular nucleus synthesize CRH and AVP either individually or together and release these peptides into hypophyseal portal capillaries at the median eminence. Colocalization of AVP and CRH also occurs in these parvocellular neurons. AVP and CRH travel in the hypophyseal portal circulation to the anterior pituitary and stimulate ACTH release. Figure modified from Antoni, 1993.


MEDIAN EMINENCE
PARAVENTRICULAR NUCLEUS
magno AVP
parvo CRH/AVP
parvo AVP
parvo CRH
magno AVP
parvo CRH/AVP
parvo CRH
hypophyseal portal capillary
parvo AVP
POSTERIOR PITUITARY
AVP
magno AVP
SUPRAOPTIC NUCLEUS
MEDIAN EMINENCE
HYPOPHYSEAL PORTAL VESSEL
ACTH
ANTERIOR PITUITARY

opioid systems as well as glucocorticoids, atrial natriuretic factor (ANF), and substance P (Faria et al., 1990; Ibanez-Santos et al., 1990; Makino et al., 1995d; Plotsky, 1986; Takao et al., 1988). Overall, results from human studies investigating the effects of these factors on HPA function tend to agree with the *in vitro* observations. However further study is required to clarify their roles as prominent regulators of CRH *in vivo*.

c. Pituitary Regulation

Although CRH (Chrousos et al., 1985; Chrousos et al., 1984; Orth et al., 1983; Rivier and Plotsky, 1986; Schulte et al., 1985; Schürmeyer et al., 1984; Taylor and Fishman, 1988; Vale et al., 1981) is the primary regulator stimulating corticotroph ACTH release, there is considerable evidence that other mediators participate as well. These include adrenal and sympathetic catecholamines (Al-Damluji, 1988; Axelrod and Reisine, 1984; Jones and Gillham, 1988; Vale et al., 1983), AVP (Antoni, 1993; Bagdy et al., 1990; Bilezikjian et al., 1987b; Hauger and Aguilera, 1993; Kjaer, 1993), angiotensin II (Rivier and Vale, 1983), ANF (Jones and Gillham, 1988) and possibly other neuropeptides including vasoactive intestinal peptide (VIP) and NPY (Jones and Gillham, 1988). Additionally, AVP has been demonstrated to act synergistically with CRH in potentiating the release of ACTH *in vitro* and *in vivo* (Antoni et al., 1983; Bilezikjian and Vale, 1987a; Debold et al., 1985; Gillies et al., 1982). Glucocorticoids negatively feedback at the pituitary level to inhibit expression and release of ACTH by corticotrophs (Munck et al., 1984; Plotsky, 1989a).

d. Adrenal Regulation

In contrast to the multiple factor regulation of CRH and ACTH, cortisol secretion from the adrenal cortex is predominately regulated by circulating ACTH (Sala et al., 1979; Simpson and Waterman, 1983). In addition, ACTH has a profound trophic influence on the adrenal cortex and thus, has a permissive role in the secretion of all adrenocortical hormones. *In vitro* studies have shown that CRH and α-MSH directly stimulate the release of corticosterone by the adrenocortical cells (Andreis et al., 1992; Vinson et al., 1988). It has been suggested that the regulation of cortisol secretion involves paracrine stimulation by adrenomedullary catecholamines as well as neuronal stimulation by neuropeptides including NPY and VIP (Hinson, 1990; Ottenweller and Meier, 1982). However, the overall contribution of these peptides in mediating *in vivo* cortisol secretion remains to be determined.

2. Neuro-immune Regulation

Recently, a relationship has been demonstrated between the immune and neuroendocrine systems. Microbial infections have been shown to activate the HPA axis via cytokines. It has also been shown that cytokine regulation may be linked to HPA activation (Chrousos, 1995; Dunn, 1994; Imura et al., 1991). Catecholamines, CRH, ACTH and β-endorphin have been shown to stimulate cytokine release (Bellinger et al., 1992; Cross et al., 1980; Dunn, 1994; Imura et al., 1991; Leu and Singh, 1992) whereas glucocorticoids cause marked suppression of cytokine release and inflammation (Boumpas et al., 1993; Chrousos and Gold,

1992b; Cronstein et al., 1992; Zitnik et al., 1994). Recent research has shown that interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) all influence the HPA axis (Berkenbosch et al., 1987; Bernardini et al., 1990; Naitoh et al., 1988; Patchev et al., 1992; Perretti et al., 1993; Rivier et al., 1989; Sapolsky et al., 1987). The predominate stimulatory cytokines increasing HPA activity are IL-1, IL-6, and TNF. These cytokines can function both alone or in combination with one another to synergistically activate the HPA axis (Perlstein et al., 1993). Their primary mode of action appears to be within the CNS (Berkenbosch et al., 1987; Bernardini et al., 1988; Dunn, 1988; Matta et al., 1992; Sapolsky et al., 1987; Sternberg et al., 1990). For example, it has been shown that infusion of IL-1 elicits a febrile reaction in humans (Fontana et al., 1984). In addition, *in vitro* studies have shown direct stimulation by both IL-1 and IL-6 of CRH release from hypothalamic cultures (Sapolsky et al., 1987). Furthermore, the infusion of IL-6 into human subjects elicits an ACTH response that is greater than that seen with CRH (Mastorakis et al., 1993). Thus, cytokines also may stimulate the release of AVP or other pituitary secretogogues in addition to CRH (Mastorakis et al., 1994). Also, direct cytokine stimulation of ACTH and cortisol release has been demonstrated at the pituitary and adrenocortical levels, respectively, yet their actions are weak and slow to develop as compared to those mediated through the hypothalamus (Roh et al., 1987; Salas et al., 1990; Spangelo et al., 1989; Uehara et al., 1987). In contrast, *in vitro* studies have shown that these cytokines also

possess the ability to suppress the HPA axis. IL-6 and TNFα have been shown to inhibit CRH induced ACTH release from pituitary cell culture (Bernardini et al., 1988; Imura et al., 1991). In addition, TNFα is a potent inhibitor of ACTH-induced cortisol secretion by adrenocortical cells. Therefore, the nature of the physiologic interaction between cytokines and HPA regulation remains unclear but appears to be critical to better understanding autoimmune disorders which accompany HPA dysregulation.

D. Mechanisms Mediating HPA Activation

The mechanisms involved in HPA activation have been extensively studied utilizing *in vitro* preparations and the administration of numerous physiologic and pharmacologic agonists and antagonists. Cellular mechanisms regulating the activity of the HPA axis have been clearly shown to involve two fundamental endocrine mechanisms: 1) stimulation of cell surface receptors by peptide and amine hormones with subsequent activation of intracellular second messenger systems and 2) steroid hormone-induced intracellular mechanisms involving nuclear transcription and protein synthesis.

1. Regulation of CRH Release

The secretion of CRH by parvocellular neurons of the paraventricular nucleus is mediated via a host of cell surface ligands that induce multiple intracellular second messenger systems. Results from *in vitro* investigations suggest that CRH secretion is mediated, in part, by the adenylate cyclase-cAMP system and the activation of both protein kinase A and C (Adler et al., 1990;

Adler et al., 1988; Majzoub et al., 1993; Seaholtz et al., 1988). For example, catecholaminergic stimulation of CRH secretion appears to be mediated primarily by the activation of β-adrenergic receptors with a possible contribution from α-receptors, and appears to involve cAMP-induced activation of protein kinase A (Calogero et al., 1988c; Majzoub et al., 1993; Plotsky et al., 1989b; Tsagarakis et al., 1988). Acetylcholine-induced CRH release is mediated predominantly by activation of muscarinic and some nicotinic receptors with subsequent depolarization of the cell membrane (Calogero et al., 1989b; Emanuel et al., 1990; Tsagarakis et al., 1988). In addition, it has been suggested that acetylcholine-stimulated CRH release involves the activation of protein kinase C (Majzoub et al., 1993). It is been shown that serotonin stimulates the release of CRH through 5-hydroxytryptamine$_2$ ($5HT_2$) receptors although the physiologic importance of this action is not yet understood (Calogero et al., 1990). Inhibitory regulation of CRH release is also mediated by multiple cell surface and intracellular mechanisms. While inhibitory neurotransmitters including GABA and substance P may play physiological roles in the suppression CRH release (Calogero et al., 1988d; Faria et al., 1990; Tsagarakis et al., 1990), the majority of CRH suppression occurs through the actions of glucocorticoids and is discussed in more detail below (Section D, #4).

2. ACTH Release

a. Activation by CRH and cAMP

Stimulated secretion of pituitary ACTH is mediated primarily by the

action of CRH released from the hypothalamus. Interestingly, ACTH secretion involves separate and distinct intracellular second messenger pathways. Extensive *in vitro* investigations have revealed that CRH acts though specific high-affinity membrane receptors which activate adenylate cyclase via guanyl nucleotide binding proteins (Antoni, 1986; Jones and Gillham, 1988). Increases in intracellular cAMP then elicit a cascade of events including: activation of cAMP dependent protein kinase A, increases in intracellular Ca^{2+} (Guild and Resine, 1987; Luini et al., 1985; Won and Orth, 1990a), increases in transcription of the ACTH precursor gene encoding pro-opiomelanocortin (POMC) (Levin and Roberts, 1991; Lundblad and Roberts, 1988), and membrane depolarization and active exocytosis (Figure 3).

b. Activation by AVP and Inositol triphosphate

In addition to CRH, AVP directly stimulates pituitary ACTH release. It is believed that AVP-induced secretion of ACTH by pituitary corticotrophs occurs through the activation of the V_{1b} receptor subtype (Antoni, 1989). Whereas CRH activates the adenylate cyclase second messenger system, AVP, after binding to its V_{1b} receptor, activates the inositol triphosphate (IP_3) second messenger system (Raymond et al., 1985; Todd and Lightman, 1987). Specifically, binding of AVP to the V_{1b} receptor activates phospholipase C enzyme via a G-protein couple. Phospholipase C then hydrolyzes phosphatidylinositol (PIP_2) into two active products: IP_3 and diacyleglycerol (DAG). IP_3 and DAG in turn increase intracellular Ca^{2+} (Won and Orth, 1990b) and activate protein kinase C,

respectively (Bilezikjian et al., 1987b; Carvallo and Aguilera, 1989; Oki et al., 1990). Both of these responses are thought to contribute to the increased release of ACTH stimulated by AVP (Figure 3). The direct effects of AVP on ACTH secretion are limited, however, it has been clearly demonstrated that AVP acts synergistically with CRH to potentiate the release of ACTH by pituitary corticotrophs (Antoni, 1986; Antoni, 1993; Debold et al., 1985; Gillies et al., 1982; Kjaer, 1993).

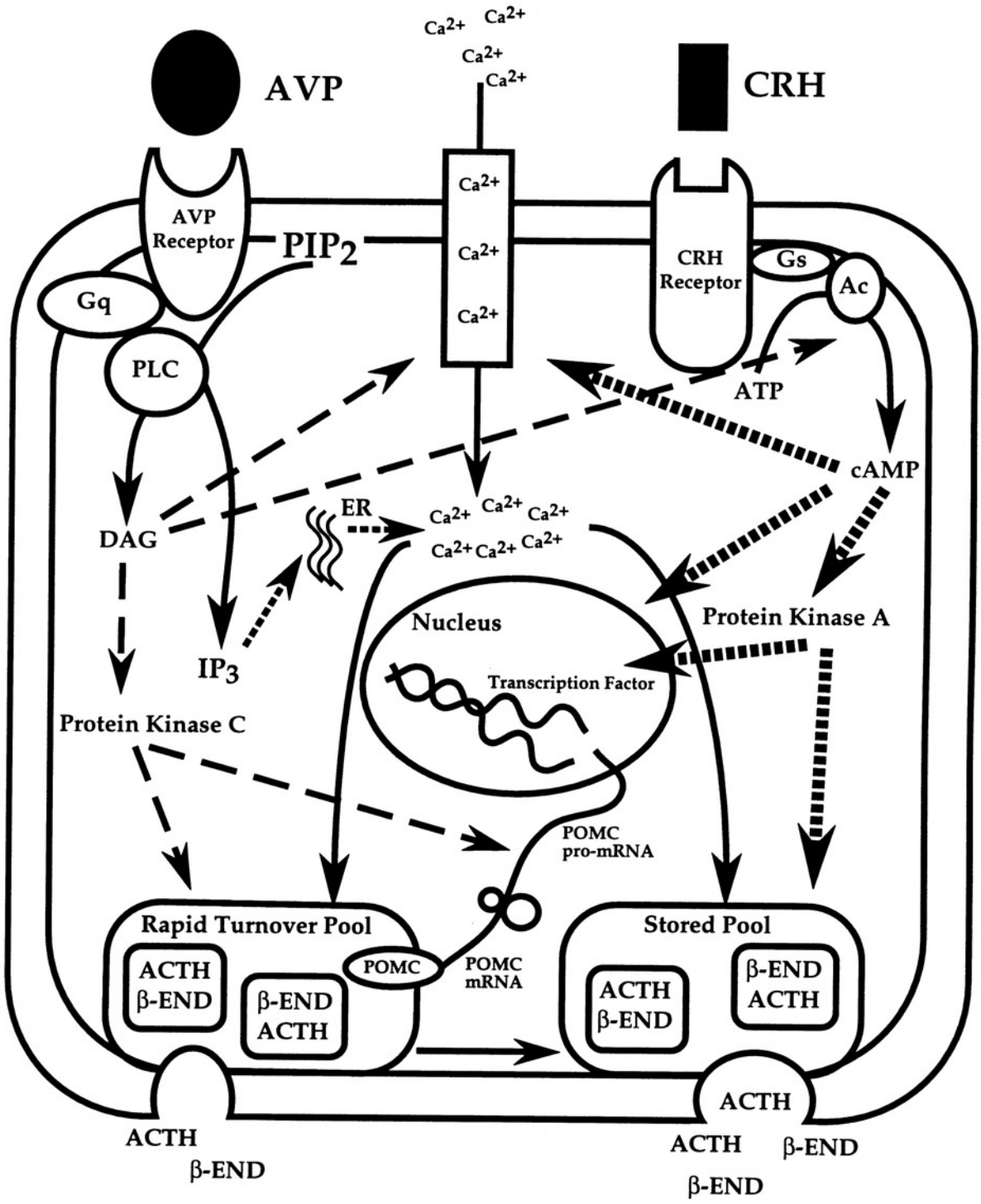
3. Glucocorticoid Release

The primary regulator of cortisol secretion by zona glomerulosa cells of the adrenal cortex is pituitary derived, peripherally circulating, ACTH (Gill, 1976; Simpson and Waterman, 1983; Simpson and Waterman, 1988). The effects of ACTH on the adrenal cortex are two-fold: it is a potent secretogogue for cortisol secretion, and it exerts a strong trophic effect. Research has shown that ACTH is responsible for maintaining adrenal mass as well as increasing the enzymatic machinery necessary for the conversion of cholesterol to cortisol.

ACTH-induced cortisol secretion is triggered by the binding of ACTH to specific, high affinity, membrane bound receptors that are linked to the adenylate cyclase second messenger system (Catalano et al., 1986; Ramachandran et al., 1987). Similar to the mechanism seen at the pituitary for CRH-induced ACTH release, increases in adenylate cyclase activity evoke an increase in intracellular cAMP concentration, which in turn activates protein kinase A. It has been suggested that protein kinase A activates cholesterol ester hydrolase

Figure 3. Mechanisms of Action for CRH and AVP Stimulated Release of ACTH and β-endorphin by Anterior Pituitary Corticotrophs.

CRH binds to its membrane receptor and activates adenylate cyclase (Ac) via guanyl nucleotide binding proteins (Gs). Increases in intracellular cAMP then can activate cAMP dependent protein kinase A, increase the influx of Ca^{2+} through membrane channels, and cause membrane depolarization, active exocytosis and an increase transcription of the ACTH precursor gene encoding POMC. AVP elicits its response through a separate second messenger system, the inositol triphosphate (IP_3) mechanism. Specifically, binding of AVP to its receptor activates phospholipase C enzyme (PLC) via a G-protein (Gq) couple. PLC then hydrolyzes phosphatidylinositol (PIP_2) into two active products: IP_3 and diacylglycerol (DAG). IP_3 and DAG in turn increase intracellular Ca^{2+} and activate protein kinase C, respectively. Both of these responses contribute to ACTH release. Both ACTH and β-endorphin are derived from the POMC gene, stored in the same secretory granule and secreted in equimolar concentrations. In addition, it has been suggested that two distinct pools are involved in ACTH release. Specifically, it has been speculated that the AVP induced second messenger pathway mobilizes a rapid turnover pool of ACTH, whereas the CRH pathway induces ACTH release from a slowly releasable stored pool. All arrows represent stimulatory actions. Figure modified from Antoni, 1993.


AVP
CRH
Ca2+
AVP Receptor
PIP2
CRH Receptor
Gs
Ac
Gq
PLC
ATP
cAMP
DAG
ER
Nucleus
Transcription Factor
Protein Kinase A
IP3
Protein Kinase C
POMC pro-mRNA
Rapid Turnover Pool
Stored Pool
ACTH
β-END
POMC
POMC mRNA
β-END ACTH

through phosphorylation and, thereby enhances the breakdown of cholesterol esters in lipid storage droplets (Gill, 1976; Sala et al., 1979). This serves to increase the pool of intracellular free cholesterol to provide mitochondria with the substrate needed for steroidogenesis. In addition, cAMP enhances the synthesis of cortisol by activating mitochondrial desmolase which catalyzes the rate-limiting conversion of cholesterol to pregnenolone as well as increasing the activity of mitochondrial cytochrome P450 enzymes involved in the conversion of pregnenolone to cortisol and other steroid products (Dubois et al., 1981; Sala et al., 1979; Simpson and Waterman, 1983; Simpson and Waterman, 1988). In addition to these rapid effects on enzyme activity, ACTH exerts long term actions on cortisol synthesis via gene expression. ACTH-induced increases in cAMP stimulate phosphorylation of cAMP response element binding protein (CREB), a transcription factor that regulates the expression of enzymes and other proteins required for steroidogenesis (Hall, 1985; Simpson and Waterman, 1983; Simpson and Waterman, 1988).

4. Negative Feedback Regulation of the HPA Axis

The adrenal steroids, cortisol (glucocorticoid) and aldosterone (mineralocorticoid), exert profound influences on physiological functions by virtue of their roles in growth, development and homeostasis (Munck et al., 1984). In fact, adrenosteroids are essential for life. In their absence normal physiological control of fluid and electrolyte homeostasis, metabolism, immune and inflammatory reactions, cardiovascular function and the body's complex

reaction to "stress" are completely disrupted and potentially fatal. Two intracellular receptor proteins, the glucocorticoid receptor (GR) (Type II) and the mineralocorticoid receptor (MR) (Type I) mediate the effects of these hormones by functioning as hormone or ligand activated transcription factors which regulate gene expression (Bradbury et al., 1991; Bradbury et al., 1994; de Kloet, 1989).

The GR (Type II) is one of the most intensely studied steroid hormone receptors, whereas the MR has been comparatively neglected. The GR is the classical glucocorticoid receptor characterized by a high affinity for cortisol and the synthetic glucocorticoid, dexamethasone (Munck et al., 1990). In the periphery, the GR is ubiquitous and mediates the actions of glucocorticoids involved in such functions as the flow of metabolic energy from lipids, muscle, and other tissues to carbohydrates as an energy source for the brain and heart during stress (Munck et al., 1990). The MR has a high affinity not only for the endogenous mineralocorticoid, aldosterone, but also for the glucocorticoid cortisol. Its expression is limited primarily to the kidney and the large intestine where it mediates the regulation of water and electrolyte balance (Munck et al., 1990). However, MR mRNA is also present in other tissues, such as the heart, pituitary gland and salivary glands, but the role of MR protein in these tissues is virtually unknown. Its affinity for cortisol is approximately 10 fold higher than for GR, thus making the MR a potential glucocorticoid receptor in the cells and tissues in which it is expressed (Beaumont and Fanestil, 1983; Krowzowski and

Funder, 1983). Thus, in the periphery, the MR and GR appear to have separate physiological roles but may share the same hormone ligands under certain conditions.

In the central nervous system, for example, the MR functions as a glucocorticoid receptor, and it appears that the MR and GR have similar, albeit, opposing physiologic roles in regulation of the activity level or set-point of the HPA axis. In the hippocampus, occupancy of the MR by the lower concentrations of cortisol present under non-stress conditions appears to determine the activity level of the HPA axis. In contrast, occupancy of the GR by the higher concentrations of cortisol that occur during stress results in feedback inhibition of the HPA axis (Bradbury et al., 1994; Dallman et al., 1992; de Kloet, 1989; de Kloet et al., 1984; Michelson et al., 1994). Thus a growing body of evidence indicates that the MR and GR have opposing roles in the regulation of the HPA axis which result in coordinated control of the axis.

As discussed above, regulation of the HPA axis by circadian signals and more abrupt physiologic stimuli is highly coordinated with cortisol as the negative feedback signal. Glucocorticoids provide negative feedback control for ACTH release by inhibiting anterior pituitary corticotrophs as well as hypothalamic neurons that secrete CRH (Beyer et al., 1988; Calogero et al., 1988a; Calogero et al., 1991; Lan et al., 1984; Lundblad and Roberts, 1988; Munck et al., 1990). The actions of cortisol on the pituitary suppress both basal and CRH-induced ACTH release (Childs and Unabia, 1990). Moreover, POMC gene

expression and the production of ACTH precursor protein are also inhibited (Bradbury et al., 1991; Keller-Wood and Dallman, 1984; Lundblad and Roberts, 1988). Similarly, within the hypothalamus, glucocorticoids appear to inhibit all steps involved in CRH secretion. For example, glucocorticoids have been shown to prevent CRH release triggered by activation of cAMP and phosphoinositide pathways in hypothalamic slice cultures (Hu et al., 1992). Glucocorticoid treatment also reduces CRH gene expression as evidenced by declines in CRH mRNA content (Adler et al., 1990; Adler et al., 1988; Beyer et al., 1988; Calogero et al., 1991; Hu et al., 1992). Finally, and relevant to the action of AVP as a stimulus for ACTH release, it has also been shown that glucocorticoids inhibit release of AVP from hypothalamic neurons (Hu et al., 1992).

E. Exercise-induced HPA Activation

1. Exercise Stimulates Metabolic and Hormonal Responses

As stated earlier, exercise can profoundly challenge the body's ability to maintain homeostasis. Numerous studies have investigated fuel mobilization during exercise: all are in agreement that glucose and free fatty acids are mobilized in response to exercise and that lactic acid accumulates in blood if the intensity of exercise exceeds one's anaerobic threshold. Moreover, it is well established that the metabolic responses to exercise are intensity and duration dependent (Deuster et al., 1989; Galbo, 1983). This fact is important to determining how activation of the HPA axis and rate of metabolism are linked during exercise.

Hormonal responses to exercise play an important role in effecting the metabolic changes necessary to sustain physical activity. Similar to the metabolic responses, endocrine responses are proportional to the intensity and duration of exercise (Convertino et al., 1981; Deuster et al., 1989; Farrell et al., 1983; Luger et al., 1987). It is well established that exercise simultaneously activates the HPA and sympathoadrenal systems, stimulates growth hormone secretion and induces pancreatic glucagon and insulin release (Convertino et al., 1981; Deuster et al., 1989; Farrell et al., 1983; Galbo, 1985; Luger et al., 1987; Viru, 1985).

2. Treadmill Exercise Activates the HPA Axis

Treadmill exercise has proven to be a good model for investigating the effects of physiologic stress on the human body. Well controlled treadmill exercise tests provide a stimulus that is both quantifiable and reproducible, as well as independent of the subject's prior experience (Deuster et al., 1989; Farrell et al., 1983; Luger et al., 1987). Important for relating the neuroendocrine response to the maintenance of metabolic homeostasis, is the observation that treadmill exercise produces both neuroendocrine and metabolic responses that are comparable in threshold, intensity and duration (Deuster et al., 1989; Galbo, 1985; Luger et al., 1987; Wade and Claybaugh, 1980). For example, treadmill exercise at a relative intensity below 60% of maximal oxygen uptake ($\dot{V}O_{2max}$) does not cause a significant rise in blood concentrations of AVP, ACTH, cortisol, catecholamines, growth hormone, glucose or lactate. However, treadmill exercise at a relative intensity greater that 60% $\dot{V}O_{2max}$ evokes proportional

increases in plasma concentrations of these hormones and metabolic substances (Deuster et al., 1989; Farrell et al., 1983; Galbo, 1985; Luger et al., 1987; Wade and Claybaugh, 1980).

Exercise studies have shown that neuroendocrine and metabolic responses to absolute treadmill exercise are inversely related to physical conditioning (Deuster et al., 1989; Luger et al., 1987). Aerobic training increases the ability of one to perform exercise at significantly greater absolute workloads with decreased pituitary-adrenal and sympathoadrenal activation. At the same time, physical conditioning facilitates the secretion of adrenomedullary hormones in response to maximal exercise. This allows one to sustain maximal output for a longer period of time as compared to an individual who is less well trained.

Importantly, however, as compared to individuals that are less trained, highly-trained individuals exhibit similar neuroendocrine and metabolic responses when evaluated at similar relative exercise intensities (Deuster et al., 1989; Luger et al., 1987). Thus, the most crucial variable in exercise studies is the measurement of maximal oxygen uptake; the critical determination for defining relative exercise intensity. Without an accurate measurement of maximal oxygen uptake, comparable assessments of relative exercise intensities and hormonal and metabolic responses cannot be achieved.

Our lab has established a treadmill exercise protocol that results in consistent and reproducible activation of the HPA axis at comparable relative exercise intensities among subjects having different degrees of physical training

(Deuster et al., 1989; Luger et al., 1987; Petrides et al., 1994; Smoak et al., 1991). Utilizing a high-intensity intermittent protocol involving 10 bouts of exercise for 30 sec at 90% $\dot{V}O_{2max}$ alternating with 30 sec of rest, consistent robust activation of the HPA axis and mobilization of fuel stores have been demonstrated. This physiological activation is evidenced by significant increases in plasma AVP, ACTH and cortisol with simultaneous mobilization of glucose and increases in anaerobic metabolism which result in marked accumulations of circulating blood lactate (Deuster et al., 1989; Luger et al., 1987; Petrides et al., 1994; Smoak et al., 1991).

F. Additional Approaches for Studying HPA Function

1. AVP Stimulation

In the present investigation, pharmacologic treatments that predictably alter the function of the HPA axis have been used in conjunction with exercise to investigate mechanisms that control CRH, ACTH, and cortisol release *in vivo*. CRH and AVP are the two main hypothalamic secretogogues for pituitary ACTH release. As noted above, AVP is a much weaker secretogogue than CRH on a molar basis, but it exerts a profound synergistic effect with CRH (Debold et al., 1985; Giguere and Labrie, 1982; Gillies et al., 1982). Gold and colleagues have shown that the ACTH response to exogenous AVP is dose and time of day dependent (unpublished data). Specifically, peripheral infusion of AVP led to a significant, dose-dependent stimulation of plasma ACTH which was more pronounced in the morning than in the evening. Similar to the pattern of ACTH

secretion, AVP infusion stimulated the secretion of cortisol in a dose-dependent fashion yet failed to produce significantly different cortisol concentrations in the morning as compared to the evening. Thus, control subjects showed a significantly greater plasma ACTH response to AVP infusion in the morning than in the evening despite higher basal plasma cortisol concentrations that naturally occur in the AM. Interestingly, these findings were opposite to their findings with pituitary-adrenal responses to CRH; ACTH responses were much greater in the evening when resting concentrations of plasma cortisol are at their lowest (Chrousos, 1992a; Gold et al., 1988d). These findings suggest that AVP-induced plasma ACTH responses may be relatively more resistant to the inhibitory actions of glucocorticoids than ACTH responses to CRH. Furthermore, CRH concentrations in hypophyseal portal blood are presumably highest in the morning and thus, could act synergistically with exogenously administered AVP, producing a greater ACTH response in the morning as compared to the evening. Therefore, in this dissertation project, an AVP infusion was administered in the morning to high- and low-responders to investigate the role of AVP in differential HPA regulation.

2. Dexamethasone Suppression

Functioning of the HPA axis can also be investigated through its suppression of the HPA axis rather than stimulation. HPA regulation is commonly assessed by the administration of dexamethasone (DEX), a synthetic glucocorticoid that suppresses the secretion of ACTH (Arana et al., 1985; Carroll

et al., 1981; Nugent et al., 1965; Sherman et al., 1984). By standard convention the dexamethasone suppression test (DST) is performed using a 1 mg dose of DEX to suppress the basal release of ACTH and cortisol in normal individuals (Arana et al., 1985; Nugent et al., 1965). The recognized criterion for normal suppression to a DST is a blood level of cortisol below 5 μg/dl which occurs in approximately 95% of all normal individuals (Carroll et al., 1981; Sherman et al., 1984). Normal male subjects participating in phase II of this project were given a standard DST to evaluate basal HPA function and to determine if differences exist for basal cortisol suppression between high- and low-responders.

3. Determination of Cellular Mechanisms

Invariably, in order to investigate the direct mechanisms involved in HPA activation, researchers must determine the mechanisms involved at the cellular level. The establishment of hypothalamic explant cultures and *in vitro* hypothalamic perfusion systems aides in determining potential activators and inhibitors of hypothalamic CRH and AVP secretion (Calogero et al., 1988a; Calogero et al., 1989a). The isolation and availability of pure CRH (Vale et al., 1981; Vale et al., 1983) and the establishment of the AtT-20 mouse corticotroph tumor cell line, which secretes ACTH, have provided important methods for elucidating physiological mechanisms regulating the HPA axis at the pituitary level (Hook et al., 1982; Sabol, 1980). In addition, dispersed primary culture of rat adenohypophysis is also useful, providing a more realistic cellular environment for studying ACTH release (Watanabe and Orth, 1988). Therefore,

this dissertation project used hypothalamic explant culture, AtT-20 rat anterior pituitary tumor cells and dispersed primary culture of rat adenohypophysis to investigate the role of lactate in mediating the cellular mechanisms involved in HPA activation.

PURPOSE

I. Regulation and Dysregulation of the Stress Response

Although glucocorticoids play important roles in the cardiovascular and metabolic responses to threatening situations, these functions are fully achieved with lower concentrations of glucocorticoids as compared to those occurring during highly stressful situations or strenuous exercise. (Munck et al., 1984) have proposed that a vital role for the enhanced secretion of cortisol is to prevent damaging over-reactions. These actions generally require high concentrations of glucocorticoids. Thus, cortisol inhibits both the secretion of hypothalamic CRH and potentially damaging inflammatory and immune responses.

Dysregulation in HPA function occurs with stress-related illnesses and contributes to the symptom complex that include, in part, fatigue, immunosuppression, weight fluctuations and behavioral changes (Chrousos, 1992a; Chrousos and Gold, 1992b; Gold et al., 1988b; Gold et al., 1988c; Griep and Boersma, 1993; Licinio et al., 1995). For example, depression can result in HPA hyperactivity that is resistant to glucocorticoid negative-feedback (Gold et al., 1988b; Gold et al., 1988c; Kathol et al., 1989; Lowy et al., 1984; Rubinow et al., 1984). The hypercortisolemia does, however, suppress immune function and thereby, facilitates disease processes (Calabrese et al., 1987). Sternberg et al. (Sternberg et al., 1989) have suggested that another form of glucocorticoid mediated dysregulation may underlie some types of autoimmune/inflammatory disease. This premise is based on the recent demonstration that cytokines

directly activate hypothalamic CRH neurons which lead to increased glucocorticoid inhibition of cytokine production (Bernardini et al., 1988; Busbridge and Grossman, 1991; Imura et al., 1991). This physiologic relationship is disturbed in the Lewis rat which exhibits a hyporesponsiveness to stressful stimuli (Calogero et al., 1992b; Sternberg et al., 1989). An inability to increase circulating cortisol during "stress" allows corresponding immune and inflammatory responses to proceed unrestrained. It has not been determined, however, whether this HPA hyporesponsiveness reflects an inability of cytokines to activate the HPA axis during stressful challenges or fundamentally different mechanisms controlling hypothalamic CRH and AVP release which leads to hypocortisolemia and an overshoot of the immune response. The long term consequence of this condition is the development of profound autoimmune and inflammatory disorders (Sternberg et al., 1992; Sternberg et al., 1990). Therefore, an intricate balance between activation and inhibition of interrelated stress mechanisms is crucial for preserving homeostasis.

II. Differential Responses to Stress

It is thought that each individual possesses a threshold or set point at which stress activates appropriate neuroendocrine and autonomic responses. Individual differences also exist in the intensities of these responses to a given stimulus, and in the capacity for counter-regulatory mechanisms to restrain the stress responses, once set into motion. Studies in experimental animals and humans have begun to document these differences and show that they result

from both differences in development and behavior. For example, Kagan el al. (Kagan et al., 1988a; Kagan et al., 1988b) have shown hyper-responsiveness of both the neuroendocrine and autonomic components of stress in children who are shy. Although the causes and the consequences of these individual differences have not been fully elucidated, it has been suggested that stress responses that turn on readily, or are resistent to counter-regulation, characterize a chronically anxious or over aroused state (Chrousos and Gold, 1992b; Dallman, 1993; de Geoij et al., 1992; Dobbs et al., 1993; Johnson et al., 1992). In contrast, the more tranquil individual exhibits a higher threshold for stress activation. The present dissertation research was undertaken to determine the basis for individual differences in the threshold for stress. This objective was based upon the interesting and unexpected finding of our preliminary study.

III. Investigating the Regulation of the HPA Axis

A. Preliminary Study

Much has been learned about the neuronal and hormonal regulation of the HPA axis under basal or resting conditions, but the roles of various blood-borne or neuronal factors coordinating HPA function during "stress", such as strenuous exercise, remains unclear. It is established that glucocorticoids strongly suppress both the basal and stimulated release of HPA hormones. Thus, one approach for examining physiologic mechanisms which coordinate the HPA axis is to enhance glucocorticoid negative feedback during exercise-induced HPA activation. The sensitivity of HPA activation to negative feedback

regulation can be examined by administering glucocorticoids prior to strenuous exercise Additionally, possible neuroendocrine and metabolic factors not affected by glucocorticoid suppression but participating in HPA activation during exercise can also be investigated.

1. Findings Providing the Basis for the Dissertation

In our preliminary study we utilized high dose glucocorticoid administration to aggressively suppress exercise-induced activation of the HPA axis in an attempt to investigate alternative neuroendocrine and metabolic mechanisms involved in HPA activation. This experiment revealed a very unexpected and interesting finding. We observed that although subjects exhibit no differences while at rest, during exercise normal male individuals can be divided into two distinct groups based upon their neuroendocrine and metabolic responses to strenuous exercise and glucocorticoid suppression (Petrides et al., 1994). Specifically, when a high dose of DEX (4 mg), a commonly used synthetic glucocorticoid, was administered 4 h prior to exercise, a differential ACTH response was observed in a group of eleven healthy male subjects. Four of the eleven individuals exhibited pronounced resistance to the negative feedback actions of DEX, whereas the remaining seven exhibited the expected suppression. Moreover, in comparison to the others, the four resistant subjects also exhibited a markedly enhanced exercise-induced ACTH response with placebo and subsequently, were designated as high-responders; the other seven were classified as low-responders. In both groups, relative exercise intensity and

plasma concentrations of DEX were equivalent throughout the protocol. Clear differences between the two groups were also evident for exercise-induced increases in plasma cortisol, AVP, lactate and glucose concentrations and heart rate. Thus, treatment with exogenous glucocorticoids reveals subgroups in normal individuals having distinctly different neuroendocrine, metabolic and cardiovascular responses to exercise at the same relative intensity.

Another important preliminary finding was the high prevalence of glucocorticoid resistance, particularly given the high dose (4 mg) of DEX used. A considerable number of studies have demonstrated that a dose as low as 0.30 mg of DEX substantially inhibits basal and stimulated cortisol release in humans (Hargreaves et al., 1987; Hohnloser et al., 1989; Kemppainen et al., 1990; Lytras et al., 1984; Von Bardeleben et al., 1985; Waltman et al., 1991). Further, the standard DST is performed using 1 mg of DEX to suppress basal release of ACTH and cortisol (Arana et al., 1985; Carroll et al., 1981; Nugent et al., 1965). In this investigation, while each subject showed normal suppression of basal cortisol concentrations with 4 mg of DEX, 36% of the subjects demonstrated resistance to DEX suppression during exercise. As factors contributing to glucocorticoid resistance appear to be different during exercise as compared to rest, it is likely that our proportion of nonsuppression during exercise would have been considerable higher following the administration of a standard 1 mg dose of DEX. Quite possibly, the frequency achieved in normal controls under conditions of 1 mg of DEX and exercise would be the same or greater than that

found in patients with psychological disorders, such as depression, obsessive compulsion and melancholia (Arana et al., 1985; Carroll et al., 1981; Lowy et al., 1984) where resting glucocorticoid resistance to 1 mg of DEX is very common (averaging 75%-95%). Thus, the determination of factors that contribute to the exercise-induced glucocorticoid resistance in normal males may aide in understanding of the mechanisms involved in glucocorticoid resistance seen at rest in patients with psychological disorders.

2. Significance of the Preliminary Findings

It is felt that even these preliminary finding have contributed to our understanding of the mechanisms which regulate the HPA axis during exercise and may also shed new light on the manifestations of certain psychological disorders. Furthermore, the present criterion defining differential responders to glucocorticoid suppression (a rise in plasma ACTH during exercise despite high dose DEX administration) may prove useful in identifying physiologically or psychologically different subgroups in the "normal" population. Therefore, the objective for extending this investigation was to identify factors contributing to differential resistance to glucocorticoid suppression seen in normal humans. Specifically, we sought to: (i) extend our preliminary findings to a larger population of subjects, (ii) determine the extent to which other physiologic responses differed, and (iii) examine the potential mechanisms involved. Overall, this research examined the roles that alternative pituitary secretogogues, metabolic products and psychological factors serve in mediating the

neuroendocrine responses to a natural physiologic stress, exercise.

B. Objectives for Extending the Preliminary Findings

1. To Investigate Glucocorticoid Receptor Differences

Differential neuroendocrine and metabolic responses to exercise with or without DEX suppression may be due, in part, to intrinsic differences in the functioning of Type I and Type II glucocorticoid receptors (discussed in the negative feedback section) (Bradbury et al., 1994; de Kloet and Reul, 1987; Reul de Kloet, 1985). To investigate this possibility we utilized equipotent doses of two glucocorticoids possessing different affinities for the glucocorticoid receptors subtypes. Specifically, we compared the effects of pretreatment with DEX, a Type II receptor ligand, to those of hydrocortisone, a combined Type I and Type II receptor ligand, on exercise-induced endocrine and metabolic responses in normal males.

2. To Investigate Hormonal Mediated Glucocorticoid Resistance

Interestingly, our previous work showed that plasma AVP responses in plasma AVP to exercise differed between high- and low-responders and were unaltered by DEX pretreatment. As such, AVP may mediate the resistance of ACTH to glucocorticoid suppression. Bilezikjian and Vale have shown *in vitro* that AVP and CRH together overcome the DEX-induced suppression of ACTH release seen with either peptide alone (Bilezikjian et al., 1987b; Bilezikjian and Vale, 1987a). Similarly, *in vivo* studies have revealed that infusions of AVP in combination with CRH elicit a marked escape from glucocorticoid suppression of

ACTH release (Von Bardeleben et al., 1985). Our preliminary studies indicate that resistance to glucocorticoid negative feedback exhibited by high-responders during high-intensity exercise may be mediated by augmented AVP release (Petrides et al., 1994). Therefore, we utilized measurements of AVP and AVP infusions to further examine the potential role of this peptide in mediating the differences that exist between low- and high-responders.

3. To Investigate Behavioral Factors Mediating Differential Physiologic Responses

The preliminary findings showed that heart rate and plasma AVP responses to exercise were both significantly greater in high-responders as compared to low-responders (Petrides et al., 1994). Because both of these responses are mediated by the CNS, this suggested that the fundamental difference between low-responders and high-responders resides within the brain. This conclusion is supported by psychological assessments which revealed that behavioral factors contribute to variable neuroendocrine and cardiovascular responses in normal subjects and animals undergoing "stress" (Chrousos and Gold, 1992b; Johnson et al., 1992; Kagan et al., 1988b; Krantz and Manuck, 1984; Manuck et al., 1989). Therefore, an extensive series of psychological profiles was administered here in an effort to better determine the extent to which low-responders and high-responders may differ psychologically. The demonstration of such differences would suggest an involvement of psychologic mechanisms in the different neuroendocrine responses to stress.

4. *In vitro* Investigations of the HPA Axis

Finally, an understanding of the role lactate serves in regulating HPA activation is currently incomplete. Our preliminary studies indicated that individuals exhibiting an exaggerated HPA activation during exercise (high-responders) also demonstrated an augmented plasma lactate concentration as compared to low-responders. There is growing evidence that lactate participates directly in CNS-mediated responses including panic and anxiety (Ahlborg et al., 1976; Carraro et al., 1989; Dager et al., 1990; Hollander et al., 1989; Luger et al., 1987; Pitts and McClure, 1967). Therefore, a likely site of action for lactate in stimulating the HPA axis is the hypothalamus. *In vitro* experiments were conducted to determine if lactate directly stimulates the release of hypothalamic CRH. This was investigated using rat hypothalamic explant culture, an established model for investigating the stimulated release of CRH, AVP and other hypothalamic hormones (Calogero et al., 1989a). Additional *in vitro* studies were performed to determine if lactate directly stimulates release or potentiates the actions of other corticotroph secretogogues. This was evaluated in both dispersed pituitary cell cultures and in the AtT-20 D-16 corticotroph tumor cell line, an established model for studying corticotroph function (Axelrod and Reisine, 1984; Hook et al., 1982; Sabol, 1980). Importantly, primary anterior pituitary cells were used to corroborate findings made in tumor cells. As such, this research involves both *in vivo* and cellular approaches to examine novel mechanisms regulating the function of the HPA axis.

EXPERIMENTAL METHODS AND DESIGN

I. Experimental Methods and Design for *In vivo* Studies in Humans

A. Phase I

1. Subjects and Protocol

We utilized treadmill exercise during enhanced glucocorticoid negative feedback, elicited by exogenous dexamethasone pretreatment, to examine physiologic mechanisms which coordinate the activation of HPA axis. All *in vivo* studies were approved by the Institutional Review Board of the Uniformed Services University of the Health Sciences and informed, written consent, was obtained from all participants. Eleven healthy, moderately trained male subjects (weekly exercise consisted of running 24 to 40 km, or 15 to 25 miles/week) not on any medications were recruited. The subjects reported to the laboratory on three occasions: a screening visit and two test visits. During the screening visit, a medical history, a physical examination and a resting 12-lead electrocardiogram were obtained and each subject underwent a progressive maximal treadmill test to exhaustion to determine maximal oxygen uptake $\dot{V}O_{2max}$ (Kyle et al., 1989). The test was conducted on a motorized treadmill and began with a 10 minute warm-up walk at 3.5 mph up a 10 % grade. Treadmill speed was then increased to 7 mph at a 0 % grade for 2 min after which the treadmill grade increased by 2.5 % increments every 2 min. Exercise continued to volitional exhaustion. Oxygen uptake and CO_2 production during this test and subsequent visits were

determined with a Metabolic Measurement Cart 2900c (Sensor Medics, Yorba Linda, CA). Electrocardiograms and heart rates were monitored continuously throughout all exercise protocols.

2. Exercise Tests

All test visits were conducted in the early evening beginning at approximately 1700 h. For each individual, test visits were separated by at least one week to allow for drug metabolism and washout. All subjects abstained from caffeine and alcohol consumption and refrained from running or other strenuous activity 24 h prior to testing. Also, they fasted for 6 h before each test. The administration of DEX (Pathway Pharmacy) (4 mg, oral) or placebo (Pathway Pharmacy) (150 mg lactose) occurred 4 h prior to exercise in a randomized, double-blinded manner; each subject received both treatments. After arriving at the lab, an intravenous catheter for blood sampling was placed in one forearm vein 30 min prior to the first blood draw. Blood was drawn 5 min before the start of exercise for baseline measurements, at the end of high-intensity exercise (time 15 min), at the end of cool-down (time 25 min), and every 10 min after exercise completion for a total of 40 min. Heart rate (HR) was also monitored before each blood draw.

Each subject exercised for 25 minutes. The initial 5 minutes served as a warm up, during which each subject jogged at an intensity equivalent to 50% of their $\dot{V}O_{2max}$. Treadmill grade was 5% and speed was adjusted to produce the desired relative workload. Immediately following the warm up, the grade was

adjusted to 10% and a high-intensity intermittent run was performed. This consisted of 10 bouts of 30 seconds of exercise at 90% $\dot{V}O_{2max}$ alternated with 30 seconds of rest. A 10 minute cool-down consisting of jogging/walking at 3.3 mph on a 5% grade followed the run (Figure 4). The speeds and grades of the treadmill were identical under each experimental condition for a given subject.

3. Psychological Questionnaires

To assess changes in mood that may occur as a consequence of drug treatment and/or exercise testing, the Bi-polar Profile of Mood States (POMS) (Lorr and McNair, 1988) questionnaire was administered for all tests at the following times: before taking the drug 4 h prior to exercise, immediately before exercise, and immediately after exercise.

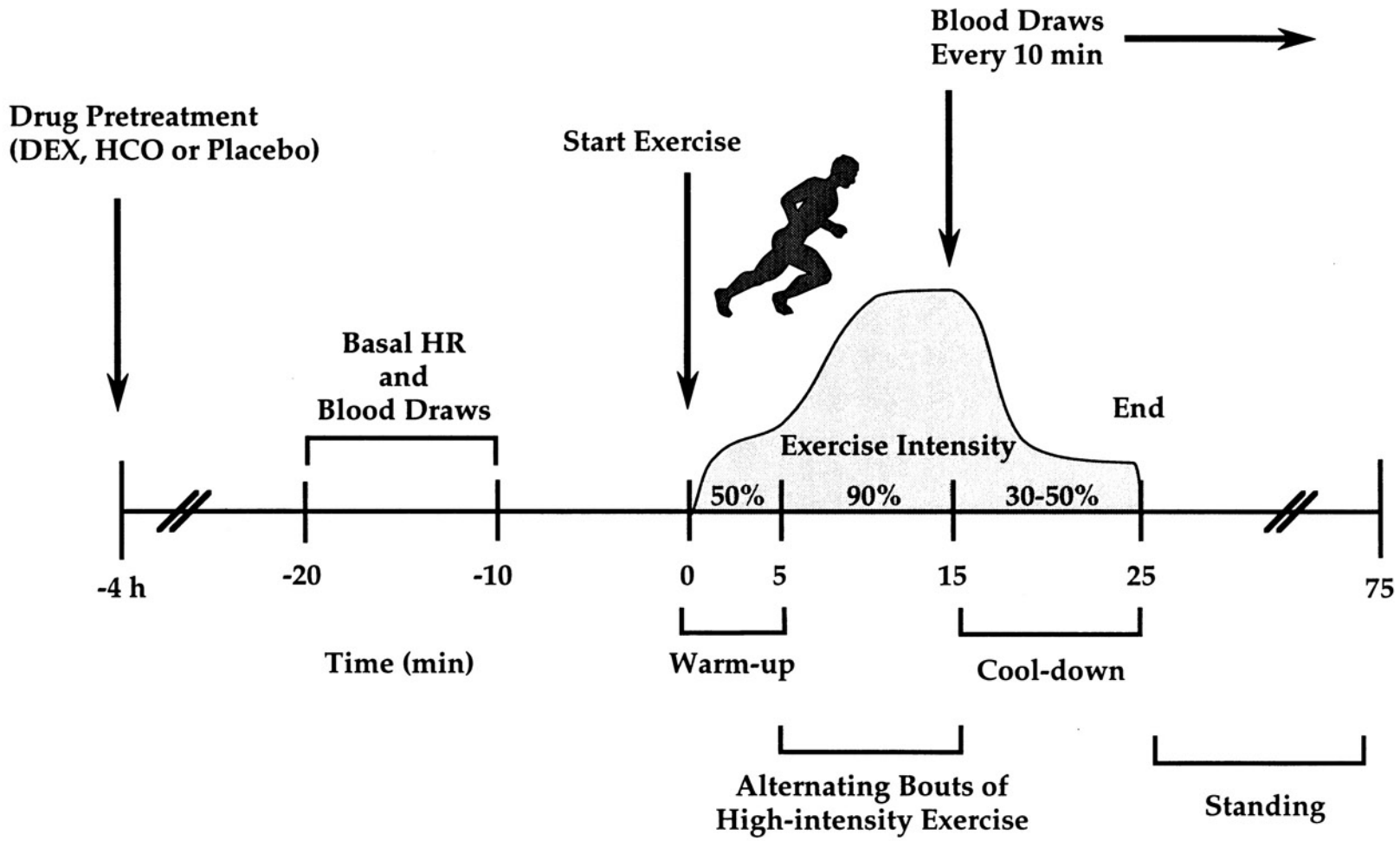
B. Phase II

1. Subjects and Protocol

Phase II of the human studies sought to determine the factors underlying the altered resistance to glucocorticoid suppression during exercise. Specifically, we extended the preliminary findings to a larger population of subjects, determined the extent to which subjects exhibit differential responses in other body systems and examined potential mechanisms involved. Nineteen healthy, moderately trained male subjects (weekly exercise consisted of running 24 to 40 km, or 15 to 25 miles/week) not on any medications were recruited. The subjects reported to the laboratory on seven occasions: a prescreening visit, one visit to determine maximal oxygen uptake, three test visits, an AVP stimulation test visit

Figure 4. Exercise Test Protocol

The administration of placebo, DEX or hydrocortisone occurred 4 h prior to exercise; each subject received each drug treatment. Blood was drawn 20 and 10 min before the start of the exercise for baseline measurements, at the end of high-intensity exercise (time 15 min), at the end of cool-down (time 25 min), and every 10 min after exercise completion for a total of 40 min. Heart rate (HR) was also monitored before each blood draw. Each subject exercised for 25 minutes at a given intensity denoted by the shaded gray area. The initial 5 minutes served as a warm up, during which each subject jogged at an intensity equivalent to 50% of their $\dot{V}O_{2max}$. Immediately following the warm up, a high-intensity intermittent run was performed. This consisted of 10 bouts of 30 seconds of exercise at 90% $\dot{V}O_{2max}$ alternated with 30 seconds of rest; treadmill grade was 10%. A 10 minute cool-down of jogging/walking (3.3 mph at 5% grade) followed the run. The subject remained standing until the end of the protocol.


Blood Draws
Every 10 min
Drug Pretreatment
(DEX, HCO or Placebo)
Start Exercise
Basal HR
and
Blood Draws
End
Exercise Intensity
50%
90%
30-50%
-4 h
-20
-10
0
5
15
25
75
Time (min)
Warm-up
Cool-down
Alternating Bouts of
High-intensity Exercise
Standing

and a visit for a Dexamethasone Suppression Test. During the prescreening visit, a medical history, a physical examination and a resting 12-lead electrocardiogram were obtained. In addition, to identify high- and low-responders prior to their enrollment into the comprehensive test protocol, each volunteer was prescreened by undergoing high-intensity exercise 4 hr after taking 4 mg of DEX. Each subject performed the same exercise protocol as described in phase I at an estimated 90% of maximal oxygen uptake ($\dot{V}O_{2max}$) based on average lab values for populations of moderately trained individuals (N>100). Utilizing the criterion of an exercise-induced plasma ACTH response during DEX suppression (ACTH concentrations > 5 pg/ml above baseline), 15 normal volunteers were separated into the two subgroups; high-responders (n=7) and low-responders (n=8). During the visit that followed the screen test, each subject underwent a progressive maximal treadmill test to exhaustion, as described in phase I, to determine actual $\dot{V}O_{2max}$ (Kyle et al., 1989).

2. Anaerobic Threshold

In the present project, each individual's anaerobic threshold was determined from gas exchange values measured during the maximal oxygen uptake test. We utilized a specific gas exchange method as described by Davis et al, (1985) to determine anaerobic threshold. Briefly, it consisted of determining the point at which an individual had a systematic increase in ventilatory equivalents of oxygen without a concomitant increase in ventilatory equivalents for CO_2 from a plot of gas exchange values versus oxygen uptake.

3. Exercise Tests

All test run visits were conducted in an identical manner as described in phase I. Test runs were performed at approximately 1700 h and in addition to DEX (Pathway Pharmacy) (4 mg oral) and placebo (Pathway Pharmacy) (150 mg lactose) each subject was tested following pretreatment with hydrocortisone (Pathway Pharmacy) (100 mg oral). Each subject received all treatments; no subject experienced any adverse reactions to either drug. The dose of hydrocortisone chosen matched the potency of 4 mg of DEX (Meikle and Tyler, 1977). Each subject exercised for 25 minutes following the same procedure as described in Phase I. At the end of each run subjects were shown the Borg Scale and asked to rate their perceived exertion (Borg, 1982).

4. AVP Stimulation Test

A resting AVP stimulation test was performed on each subject to determine pituitary responsiveness to exogenous AVP stimulation. The total infusion time was 180 minutes. An intravenous catheter was placed in one forearm vein for blood sampling and another for infusion. Normal saline (0.9% NaCl) was infused for the first 60 min at a rate of 40 cc/hr for baseline measurements. AVP infusion (Park-Davis Company) began at approximately 0800 h at a rate of 1 mIU/kg/min for 60 min and was followed by saline infusion at 40 cc/min for the last 60 min. Blood samples (10 ml) were collected every 15 min throughout the entire infusion protocol and processed as described above. Plasma concentrations of ACTH, cortisol, AVP, lactate and glucose were

determined.

5. Dexamethasone Suppression Test

A standard 1 mg dexamethasone suppression test was administered to each subject at least one week after completion of the exercise protocol to determine whether the individual was resistant to exogenous glucocorticoid suppression while at rest. Following convention, an oral dose of 1 mg DEX was taken by each subject at 2300 h and the following morning at 0800 h, a 2 ml blood sample was drawn and assayed for serum cortisol concentration (Nugent et al., 1965). Serum cortisol values < 138 μmol/L indicate that normal suppression of the HPA axis had occurred (Carroll et al., 1981; Sherman et al., 1984).

6. Psychological Profile

During the prescreening visit each subject completed a series of psychological questionnaires designed to identify overall differences in sense of well being, anxiety and or depression that may exist among the subjects. The SCL-90-R was used as a general psychological screening tool and specifically nine psychological factors were evaluated: somatization, obsessive-compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid idealism, and psychoticism (Derogatis and Cleary, 1977; Payne, 1978). The Spielberger Trait Anxiety Scale was administered to measure anxiety levels of each subject (Spielberger et al., 1983). Possible behavioral characteristics of depression were more extensively evaluated by The Beck Depression Inventory (Beck et al., 1961). In addition, the POMS test was also utilized as described in

phase I.

II. *In vitro* Experimental Procedures and Design

A. Animals

Adult male Sprague-Dawley rats weighing 200-300 gm were housed and acclimated (19°-21°C; 12:12 hour light-dark cycle) to the USUHS animal facility for at least five days before the start of experiments. Normal rat hypothalami and pituitary tissues were also periodically obtained through the Experimental Diabetes Lab at the National Institute of Health for use in studies described here.

B. Hypothalami Explant Incubations

Explanted rat hypothalami were utilized to determine the direct effects of lactate on the neuronal secretion of CRH and AVP. Following the methods of Calogero et al. (1989a), hypothalami were carefully dissected and placed into wells (48 multiwell plates, Costar, Cambridge, MA) containing medium 199 with modified Earl's salt (Gibco, Grand Island, NY), 0.1% bovine serum albumin (BSA) (fraction V, Sigma, St. Louis, MO) and 20 μM bacitracin (zinc salt, Aldrich, Milwaukee, WI). Each plate was preincubated for 90 min in a water-jacketed incubator at 37°C with 5% CO_2. Experimental protocols were completed under an identical incubator environment. The hypothalami were transferred in succession through three wells containing control media and then to treatment media in the next two wells. Transfers between wells were accomplished by suspension in nylon mesh (nylon mesh monofilament 44 μ, Small Parts, Miami, FL). Finally, the hypothalami underwent a provocative stimulus by potassium

depolarization (60 Mm KCL) to demonstrate tissue viability. Immunoreactive CRH responses exceeding 90% above baseline were used as the criterion for viability. Hypothalami were incubated for 20 min per well, an optimal incubation time established by Calogero et al. (1989a). Media samples were assayed directly for CRH and AVP.

C. Pituitary Cell Cultures

To further investigate both the direct and indirect effects of lactate on the secretory responsiveness of corticotrophs, rat AtT-20 (strain D16) corticotroph tumor cells and dispersed rat anterior pituitary cells were cultured *in vitro* according to procedures established in our lab. To minimize the use of experimental animals, *in vitro* analyses were conducted using the AtT-20 cell line. Relevant findings were then verified in primary cultures of rat pituitary gland.

Monolayers of AtT-20 cells were grown in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum, 2% L-glutamine (200 mM), and 2% penicillin (5000 units/ml) - streptomycin (5000 ug/ml, GIBCO/BRL, Gaithersburg, MD); DMEM Complete (Hook et al., 1982). Approximately 450,000 cells were plated in 35 mm wells 4-6 days before treatment. Prior to an experiment, cells were washed and allowed to equilibrate for 90 min in DMEM containing 0.1% bovine serum albumin (BSA, Sigma Chemical Co, St. Louis, MO). The medium was replaced with DMEM/BSA containing experimental treatments. Following a timed treatment (0.5 to 72 hr) incubation, the medium was transferred into 1.5 ml eppendorf tubes and centrifuged for 4 min.

Supernates were stored in polypropylene tubes at -20°C until assayed. Cells were harvested in 2 N acetic acid (5 ml), sonicated and assayed for peptide (RIA) and protein (Bradford).

Primary cell cultures of rat anterior pituitary gland were established with tissue collected from normal male rats. Anterior pituitaries were finely cut and enzymatically dissociated for 5 min at 37°C by delicately mixing in a solution of DMEM containing 10 mg/ml BSA, 4 mg/ml collagenase, 1 mg/ml hyaluronidase (Type IV-S), 0.01 mg/ml Dnase (Type I) (all enzymes from Sigma Co., Ltd.). The tissue fragments were collected by centrifugation and resuspended in 3 mg/ml trypsin (300,000 USP U/gm; Sigma) in DMEM for 5 min at 37°C and dispersed by trituration through a polished pipet. Cells were rinsed once in DMEM containing 1 mg/ml lima bean trypsin inhibitor (Sigma) and washed twice with DMEM before being plated. Approximately 70,000 cells in 2 ml DMEM containing 10% horse serum and 2.5% fetal bovine serum were plated in 35 mm wells and cultured until ready for treatments with secretogogues and lactic acid 5 to 8 days later. Treatment and collection procedures were identical to those used for the AtT-20 cell culture experiments.

III. Biochemical Analyses

A. Biochemical Handling of Human Blood Samples

Biochemical analyses of all blood samples collected throughout the human studies were completed in the manner described here. Blood samples were collected in heparinized tubes (15 IU heparin/ml blood) containing sodium

fluoride (1 mg NaFl/ml blood) for lactate and glucose determinations, EDTA tubes (1.6 mg EDTA/ml blood) for ACTH, cortisol, hydrocortisone, cortisol binding globulin, AVP, and DEX determinations, and in EGTA/glutathione (20 µl/ml blood) for catecholamine determinations. Blood samples were spun in a refrigerated centrifuge maintained at 8-10°C; plasma was separated and stored at -40°C for later analyses. Blood for determination of hemoglobin and hematocrit was collected in EDTA tubes maintained at room temperature and determined in triplicate by the cyanomethemoglobin and microcapillary methods, respectively.

B. Metabolite Assays

Plasma lactate, plasma glucose and cell culture media lactate concentrations were determined in duplicate (YSI Analyzer Model 27, Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Briefly, lactate and glucose samples were injected into respective analyzers where they traveled through a specific membrane which converted metabolite concentration into a proportional signal current. The current was then converted to a calibrated voltage, standardized to known concentrations of lactate and glucose, and displayed on a digital meter (1 mV = 1 ml/dl lactate or glucose). The sensitivity of this assay is 2 mg/dl ± 2% relative precision.

C. Hormonal Assays of Human Blood Samples

Plasma ACTH concentrations were assayed by a two-site immunoradiometric assay (IRMA) (Nichols Institute Diagnostics, San Juan Capistrano, CA). Briefly, reactions occur with a polyclonal antibody coupled to

biotin, prepared so that it will only bind to the C-terminal region of the ACTH molecule, and a monoclonal antibody, which has been radiolabled with ^{125}I, that sandwiches the ACTH molecule by specifically binding to the N-terminal region. Concentrations of ACTH were measured by adding an avidin coated bead to the reaction; avidin possesses a strong affinity for biotin. The reactions incubated for 20 ± 2 hr at which time the supernatant was aspirated and the radiolabled ACTH counts were determined in a gamma counter and the counts were converted to concentration from a standard curve of known ACTH concentrations. The sensitivity of the assay was determined to be 1.0 pg/ml of ACTH. This assay is specific for ACTH (1-39) and does not cross react with ACTH (1-10), α-melanocyte stimulating hormone (α-MSH), β-MSH, β -lipotropin stimulating hormone (LSH) or β-endorphin. Intra-assay variability was 7% and samples were measured in one assay to decrease inter-assay variation.

In phase I plasma cortisol, CBG, and DEX concentrations were determined by radioimmunoassay (RIA) (Hazelton Laboratories). Phase II plasma cortisol and hydrocortisone concentrations were determined by radioimmunoassay (RIA) (Diagnostic Systems Laboratory Los Angeles, CA). Briefly, plasma samples and radiolabled ^{125}I cortisol were added to antibody coated tubes. Plasma cortisol competed with the radiolabled cortisol for binding to the antibody bound to the tube. The reaction was incubated in a water bath (37°C) for 45 min. The supernatants were then discarded, which terminated the competition and isolated the antibody-bound fraction of the radiolabled cortisol.

Tubes were counted in a gamma counter. Plasma cortisol concentrations for each sample were calculated using a standard curve. The sensitivity of the assay for plasma cortisol is 0.2 μg/dl. The antiserum bound to the tubes is very specific for cortisol and has extremely low cross reactivity with aldosterone, corticosterone, cortisone, dexamethasone, pregnenolone, estriol and progesterone. All cortisol samples were analyzed in one assay to eliminate individual interassay variations. The intra-assay variation for cortisol concentrations was 5%.

Plasma AVP concentrations were assayed by RIA, as previously described by Rittmaster et al. (Rittmaster et al., 1987). Briefly, plasma samples were extracted using a C-18 SepPak (Waters associates, Milford MA) and 0.1% trifluoroacetic acid (TFA). The AVP peptide was eluted from the SepPak with 60% acetonitrile/40% 0.1% TFA. The eluent was then dried down and reconstituted with assay buffer at 3X concentrations. The recovery for this procedure was greater than 95%. Extracted samples for AVP measurement were reacted with a specific rabbit antiserum (Arnel, New York, NY) at a final dilution of 1:90,000 for 48 hr at 3-5°C. Radiolabled ^{125}I AVP (Amersham Corp.) was then added to the reaction and incubated for 24 h. This reaction was terminated with the addition of goat antiserum. Gamma counter counting and conversion via a standard curve gave plasma concentrations of AVP. The sensitivity of the assay was 0.5 pg/ml of AVP. Intra-assay variation was 7% for AVP. The antibody is very specific for AVP and shows no cross reactivity with oxytocin, somatostatin,

thyroid-releasing hormone, met-enkephalin, leu-enkephalin, glucagon, insulin or vasoactive intestinal peptide (VIP).

Plasma catecholamines were extracted and measured by high pressure liquid chromatography (HPLC using a modification of the procedure described by Hunter et al. (1988) (Hunter et al., 1988). The sensitivity of the assay was 0.01 ng/ml of norepinephrine and epinephrine. The detection limit of the assay was two times greater than background signal. The percent recovery for the extraction of catecholamines was 65 ± 5%. The inter-assay variation between assays was 6% for norepinephrine and 12.6% for epinephrine. The intra-assay variability was 6.4% for norepinephrine and 11% for epinephrine.

D. Hormonal Assays of *In vitro* Samples

β-endorphin peptides from incubation media were measured utilizing a radioimmunoassay (RIA) (Mueller, 1980) that involves competition for C-55 rabbit antibodies (final dilution 1:10 K) by iodinated ^{125}I camel acetylate β-endorphin$_{1-27}$ and media β-endorphin for 60-72 h. The reaction was terminated with 18% polyethylene glycol (PEG). Samples were counted in a gamma counter and conversion via a standard curve gave concentrations of β-endorphin for media samples. The sensitivity of the assay is approximately 2.7 femtomoles. The intra-assay variability was 5.6%. . The C-55 rabbit antibodies, developed against camel β-endorphin$_{1-31}$, were specific for rat β-endorphin but do not cross react with α-endorphin (β-endorphin$_{1-16}$), gamma-endorphin (γ-endorphin$_{1-17}$) methionine enkephalin (β-endorphin$_{1-17}$), leucine enkephalin, α-melanocyte

stimulating hormone (α-MSH), ACTH or other non-opiate hypothalamic or pituitary hormone.

CRH peptide concentration from incubation media was determined by radioimmunoassay (RIA) as previously described (Calogero et al., 1989a). Briefly, CRH was measured in media by utilizing a specific antiRCRH serum (TS-3). Iodinated RCRH competitively binds to the TS-3 antibody (final dilution 1:18 K) with media CRH for 48 hr. Phase separation was achieved by incubating the reaction with goat anti-rabbit serum and the supernatant was aspirated. The precipitate was counted in a gamma counter and conversion via a standard curve gave media concentrations of CRH. The sensitivity of the assay was 20 pg/ml media. The intra-assay variability was 8.1%. The anti-serum is very specific for CRH and no significant cross reactivity occurs with luteinizing-, growth- or thyrotropin-hormone releasing hormones, somatostatin, substance P, porcine VIP, neuropeptide Y, ACTH, β-endorphin, vasopressin or oxytocin.

IV. Statistical Analyses

A. Human Studies

The Statistical Analysis System (SAS) software program (SAS Institute Inc, Cary, NC) was used for all data analyses in phase I. A test for normality was performed on all data: normally distributed data were analyzed in a factorial design with repeated measures; a multivariate general linear model was used. When significant effects were detected by analysis of variance (ANOVA), Duncan's multiple range test was used to identify differences across time and

treatments. Areas under the curve (AUC) data for ACTH, cortisol, AVP, lactate, and glucose) were not normally distributed and were analyzed with non-parametric techniques (Wilcoxon Signed Rank Test). Significance was set at the 0.05 level. Area under the curve was calculated by the trapezoidal method after subtracting the baseline. Data are presented as mean ± standard error. Correlations between exercise-induced peak AVP, ACTH and lactate concentrations were determined by regression analysis. Significance was set at the 0.05 level.

The Super ANOVA and Stat View software programs (Abacus Concepts Inc, Berkeley, CA) were used for all data analyses in phase II. Data were analyzed in a factorial design with repeated measures; a multivariate general linear model was used. When significant effects were detected by ANOVA, Duncan's multiple range test was used to identify differences across time and treatments. AUC data for ACTH, cortisol, AVP, lactate, glucose, and GH and catecholamines were not normally distributed and were analyzed with non-parametric techniques (Wilcoxon Signed Rank Test). Significance was set at the 0.05 level. AUC was calculated by the trapezoidal method after subtracting the baseline. Data are presented as mean ± standard error. In addition, AVP data from phase I and II were combined for multiple regression analysis by SAS. This procedure utilized both a "dummy variable", representing the two different groups (high- and low-responders) and an interaction (%$\dot{V}O_2$ x dummy) to determine if peak and AUC AVP responses were dependent on the two groups

when controlling for relative oxygen uptake. Significance was set at the 0.05 level.

B. *In vitro* Studies

Results from *in vitro* experiments were analyzed by one-way (dose response studies) and two-way (multiple treatment studies) ANOVA followed by Duncan's new multiple range test for comparison of group means (Duncan, 1955). Data are presented as mean ± standard error with significance set at the 0.05 level.

RESULTS

I. Human Study Phase I

A. Dexamethasone Administration and Exercise

The oral administration of 4 mg DEX was well tolerated and none of the subjects experienced any adverse effects from the treatment. The mean grade and speed of the treadmill during the high-intensity run were 10.1 ± 0.1% and 9.5 ± 0.6 miles/h, respectively. Upon inspection and collective array and analysis of the ACTH data, we found that two subgroups could be clearly identified with respect to their responses to exercise after DEX pretreatment (Figure 5). The data were analyzed as one group to examine differences between DEX and placebo, and these differences were maintained when analyses of subgroups were performed. Moreover, additional significant effects were noted between the two subgroups, despite having an "n" of only 4 in one of them. Thus, the data presented here are for low-responders (n=7) and high-responders (n=4) under the two treatment conditions.

Importantly, all individuals exhibited similar plasma concentrations of DEX both prior to (low-responders: 11.0 ± 2.7 ng/ml and high-responders: 12.3 ± 2.3 ng/ml) and after exercise (low-responders: 9.6 ± 1.5 ng/ml and high-responders: 14.2 ± 0.2 ng/ml); this allowed us to discount differences in absorption and metabolism of the drug in the two groups. Furthermore, inhibition of basal release of cortisol occurred in most subjects; 9 of the 11

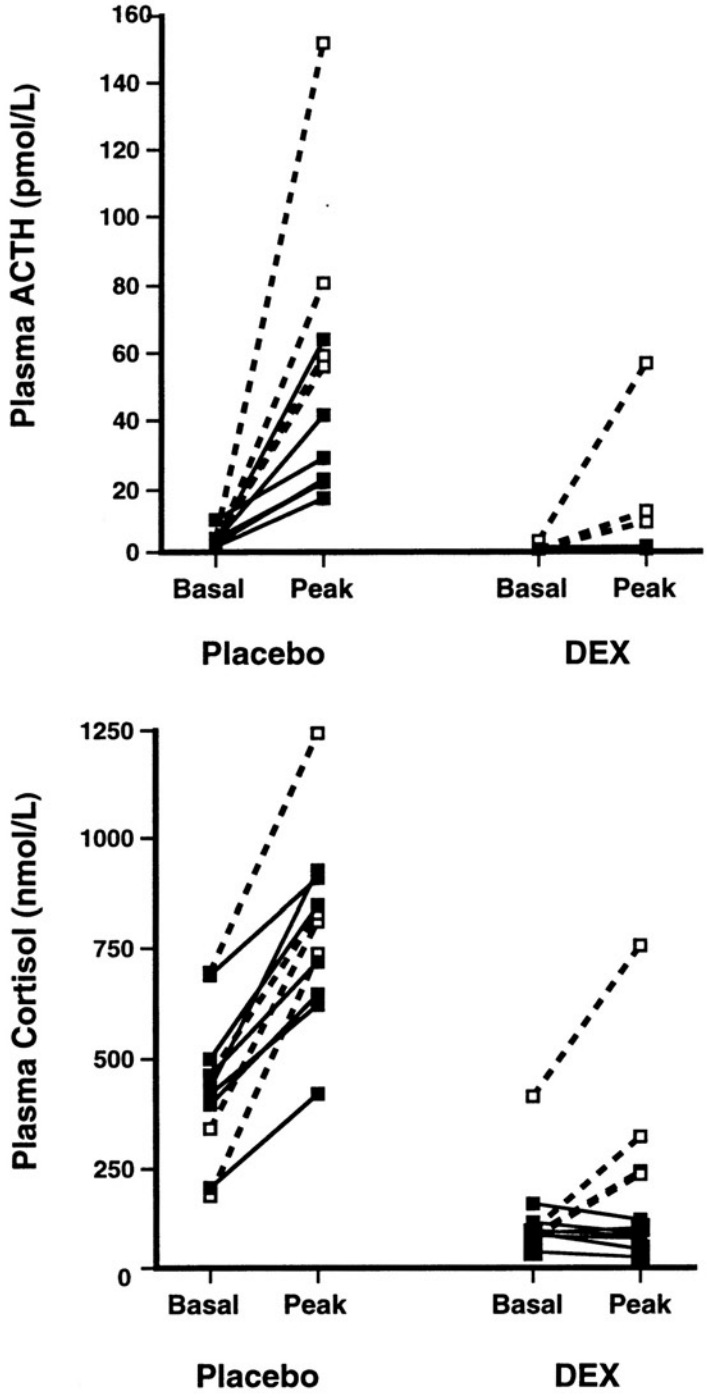


Figure 5: Basal and peak exercise plasma concentrations of ACTH and cortisol in low-responders (closed squares with solid lines; n = 7) and high-responders (open squares with dashed lines; n = 4). Individual basal and peak values are connected by a corresponding line for both the placebo and DEX pretreatments.

individuals had plasma cortisol concentrations < 138 nmol/L. Table 1 presents characteristics for the total subject population and the two subsets. No significant differences in age, weight (wt), height (ht), or $\dot{V}O_{2max}$ were noted between the low-responders or high-responders. Table 2 presents heart rate, absolute $\dot{V}O_2$, relative $\dot{V}O_2$, and respiratory exchange ratio values for the two groups averaged over the last four intermittent bouts of high- intensity exercise; all values were unaffected by DEX pretreatment. However, mean heart rate was significantly higher for the high-responder as compared to the low-responder group ($p < 0.05$) (Table 2).

B. ACTH Response to Exercise and DEX

Pretreatment with DEX significantly ($p<0.05$) reduced mean basal concentrations of ACTH in both the low-responders [5.4 ± 0.9 pmol/L versus 2.5 ± 0.1 pmol/L] and the high-responders [4.3 ± 0.4 pmol/L versus 3.0 ± 0.6 pmol/L]. Plasma concentrations of ACTH in all subjects receiving placebo were highest at 15 min into the exercise regimen (Figure 6, upper left panel). The plasma ACTH response of the high-responder group was, however, twice that observed in the low-responder group. With DEX, no exercise-induced increase in ACTH was observed in the low-responders, whereas an ACTH response was evident in high-responders. In fact, peak plasma concentrations of ACTH at 15 min in the high-responder group with DEX were equivalent to those of the low-responder group receiving placebo. The area underneath the curve (AUC)

Table 1. General Characteristics of Subjects

Subject	Age (yr)	Wt (Kg)	Ht (Cm)	Max $\dot{V}O_2$ (L/min)
		LOW RESPONDERS		
1	42	73.5	173	4.85
2	26	74.8	175	4.02
3	20	70.0	168	4.37
4	19	60.6	170	4.07
5	23	68.0	178	4.26
6	24	96.5	193	5.15
7	29	79.5	191	4.15
Mean ± SE	**26.1 ± 3.0**	**74.7 ± 4.3**	**178.3 ± 3.8**	**4.41 ± .162**
		HIGH RESPONDERS		
8	25	69.5	165	4.89
9	37	79.5	183	4.53
10	35	67.8	183	3.88
11	24	80.0	173	4.43
Mean ± SE	**30.3 ± 3.3**	**74.2 ± 3.2**	**176.0 ± 4.3**	**4.43 ± .209**
		TOTAL		
Mean ± SE	**27.6 ± 2.2**	**74.5 ± 2.8**	**177 ± 2.7**	**4.42 ± .122**

Table 2. Physiological parameters during high intensity intermittent running with Dexamethasone (DEX) or placebo pretreatment.

	TOTAL POPULATION		LOW RESPONDERS		HIGH RESPONDERS	
	DEX	PLACEBO	DEX	PLACEBO	DEX	PLACEBO
Heart rate (bpm)	176 ± 3*	178 ± 3	172 ± 2	173 ± 2	184 ± 3[a]	186 ± 3[a]
VO_2 (L/min)	4.02 ± 0.12	3.98 ± 0.12	3.93 ± 0.17	3.96 ± 0.18	4.17 ± 0.10	4.02 ± 0.08
Relative VO_2 (%)	89.9 ± 1.4	89.7 ± 1.3	89.1 ± 1.4	89.7 ± 1.6	89.9 ± 1.4	89.7 ± 2.5
RER[1]	0.99 ± 0.02	1.01 ± 0.01	1.01 ± 0.03	0.99 ± 0.01	0.99 ± 0.02	1.01 ± 0.01

*Values are the mean ± S.E.
[1]Respiratory exchange ratio
[a]$P<0.05$ as compared to total population and suppressors

was calculated to provide additional statistical analysis for the ACTH response to exercise and drug pretreatment over time. The AUC for ACTH under placebo conditions was significantly higher ($p<0.05$) in the high-responder as compared to the low-responder group (Figure 6, upper right panel). Under DEX, the AUC for ACTH was attenuated by 75% in high-responders and 99% in low-responders.

C. Cortisol Response to Exercise and DEX

Changes in circulating concentrations of cortisol closely resembled those observed for ACTH in the two groups (Figure 6; lower left panel). DEX pretreatment significantly reduced basal concentrations of cortisol in both groups (high-responders: 177 ± 77 nmol/L and low-responders: 108 ± 13 nmol/L; $p<0.05$), with the decrease in the low-responder group being greater than in the high-responder group. All individuals receiving placebo exhibited peak plasma cortisol concentrations by 20 min after the termination of exercise (experimental time = 45 min). The maximal plasma cortisol response observed in high-responders receiving placebo was approximately 35% greater than that of the low-responder group (905 ± 113 nmol/L vs 690 ± 61 nmol/L; $p<0.05$). Pretreatment with DEX completely blocked the exercise-induced release of cortisol in low-responders, but only partial blockade was noted in high-responders. This difference between the two groups was also clearly evident for AUC (Figure 6, lower right panel; $p<0.05$). Moreover, the AUC for the high-responder group receiving DEX was essentially identical to the

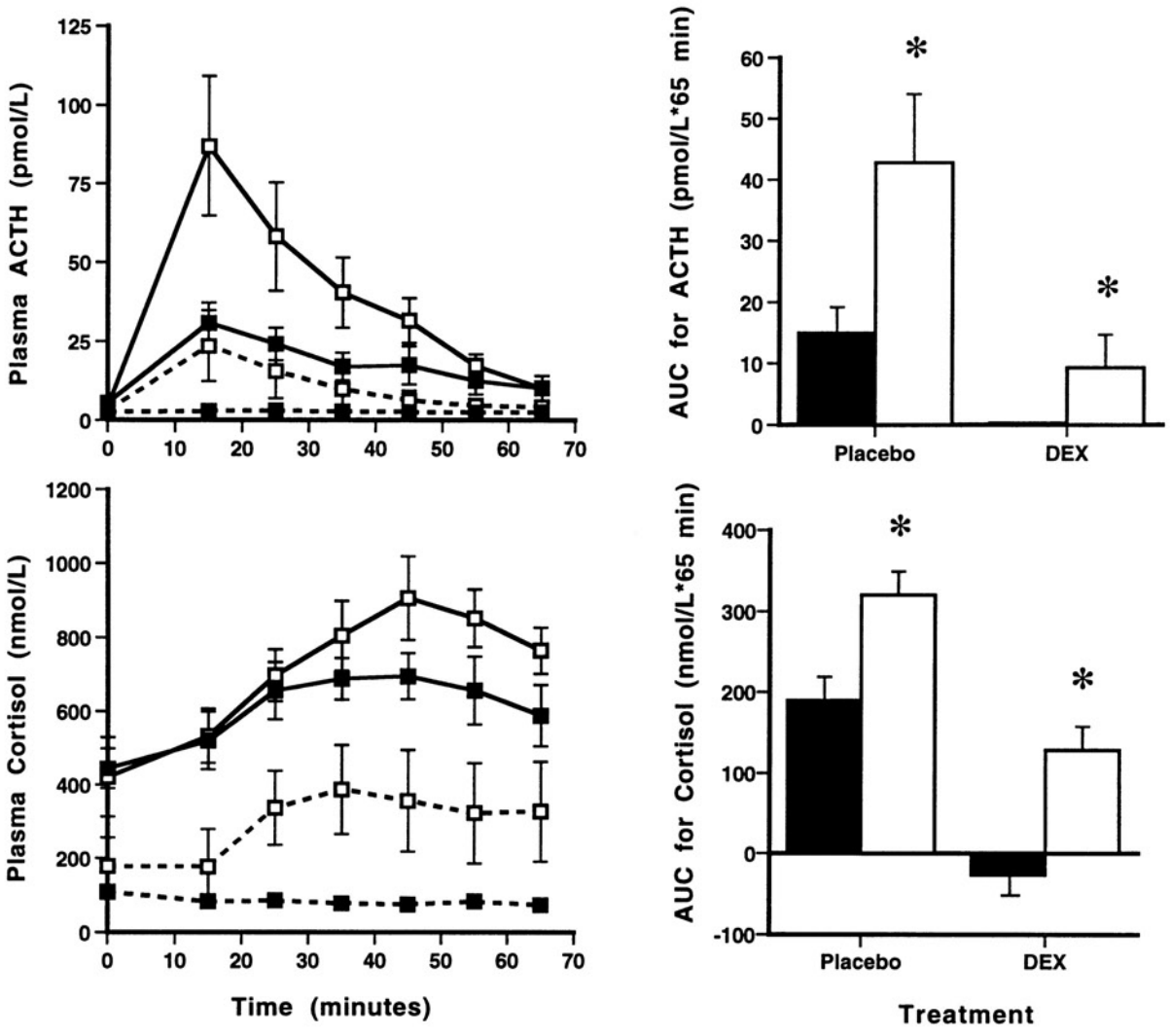


Figure 6. Exercise-induced changes in plasma ACTH (upper panel) and cortisol (lower panel) in low- (closed sqares) and high- (open squares) responders following placebo (solid line) or DEX (dashed line) pretreatments. Integrated areas under curve (AUC) for plasma ACTH (upper right) and cortisol (lower right) over the entire time for low- responders (solid bars) and high-responders (open bars) after placebo and DEX. * equals $p < 0.05$.

low-responder group receiving placebo.

D. AVP Response to Exercise and DEX

In contrast to the ACTH and cortisol responses, pretreatment with DEX did not significantly alter basal plasma AVP concentrations in either group (Figure 7, left panel). For both DEX and placebo, high-intensity exercise caused a significant increase in plasma concentrations of AVP ($p<0.05$) that was maximal by time 15 min of exercise. The plasma AVP response in the high-responder group, however, was six fold that observed in the low-responder group (28.1 ± 3.7 pmol/ml vs. 5.1 ± 1.3 pmol/L, respectively). Statistical analysis of AUC demonstrated that DEX pretreatment had no affect on exercise-induced AVP release for either group ($p<0.05$) (Figure 7, right panel). Furthermore, the AVP response for the high-responders was markedly enhanced ($p<0.05$) as compared to that of the low-responders for both treatments.

E. Lactate Response to Exercise and DEX

Induction of anaerobic metabolism by exercise was clearly evidenced by marked increases in plasma lactate concentrations for all subjects by the end of the high- intensity run (time = 15 min) (Figure 8, upper left panel). Analysis of AUC for the two subgroups demonstrated that plasma lactate responses were greater in the high-responder as compared to the low-responder group following both placebo as well as DEX pretreatment ($p<0.05$) (Figure 8; upper right panel). DEX pretreatment did not appreciably alter lactate production during exercise as compared to placebo in either group.

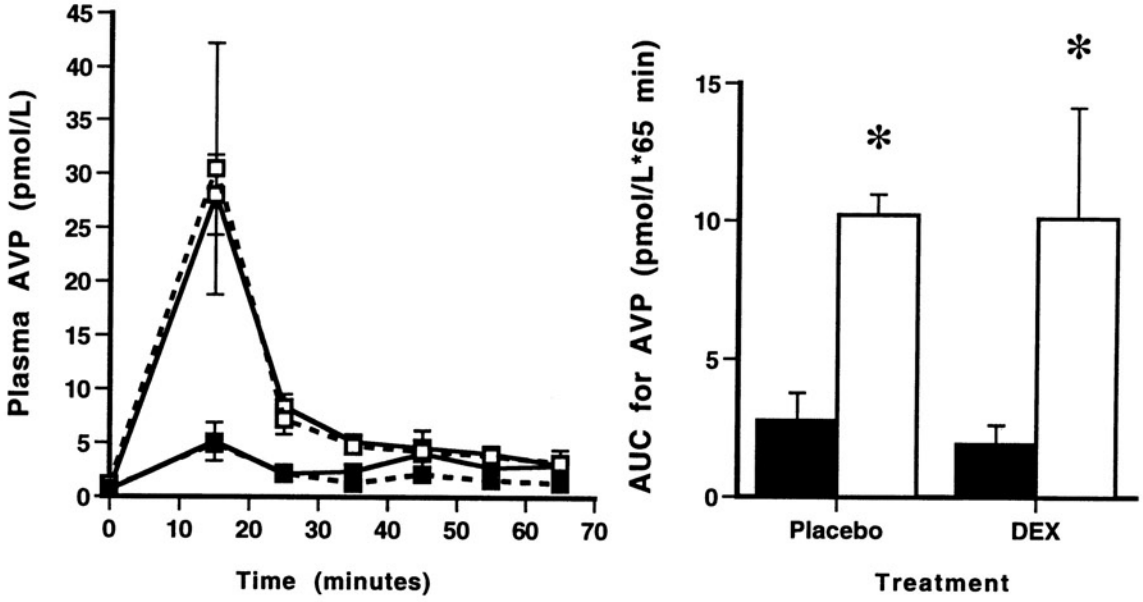


Figure 7. Exercise-induced changes in plasma AVP over time in low-responders (closed squares) and high-responders (open squares) after taking placebo (solid line) or DEX (dashed line). Integrated areas under curve (AUC) for plasma AVP for low-responders (solid bars) and high-responders (open bars) after placebo and DEX. * equals $p < 0.05$.

F. Glucose Response to Exercise and DEX

Basal and exercise-induced plasma concentrations of plasma glucose tended to be elevated in both groups after pretreatment with DEX, as compared to placebo, but the differences were not significant (Figure 8, lower left panel). Plasma glucose concentrations peaked in all individuals after termination of exercise (time = 25 min). Analysis of the AUC for plasma glucose responses revealed that high-responders had a significantly greater increase in plasma glucose during exercise as compared to low-responders for both drug treatments (Figure 8, lower right panel) ($p<0.05$).

G. Heart Rate Response to Exercise and DEX

Basal and exercise-induced increases in heart rates were unaltered by DEX pretreatment in either group (Figure 9). Heart rates rose continuously through the exercise protocol until they plateaued at the highest rate by the seventh intermittent bout of high-intensity exercise. Interestingly, heart rates during the high-intensity exercise in the high-responder group were significantly higher than those of the low-responder group (185 ± 3 bpm versus 173 ± 2 bpm; $p<0.05$) with either treatment, whereas resting heart rates were similar.

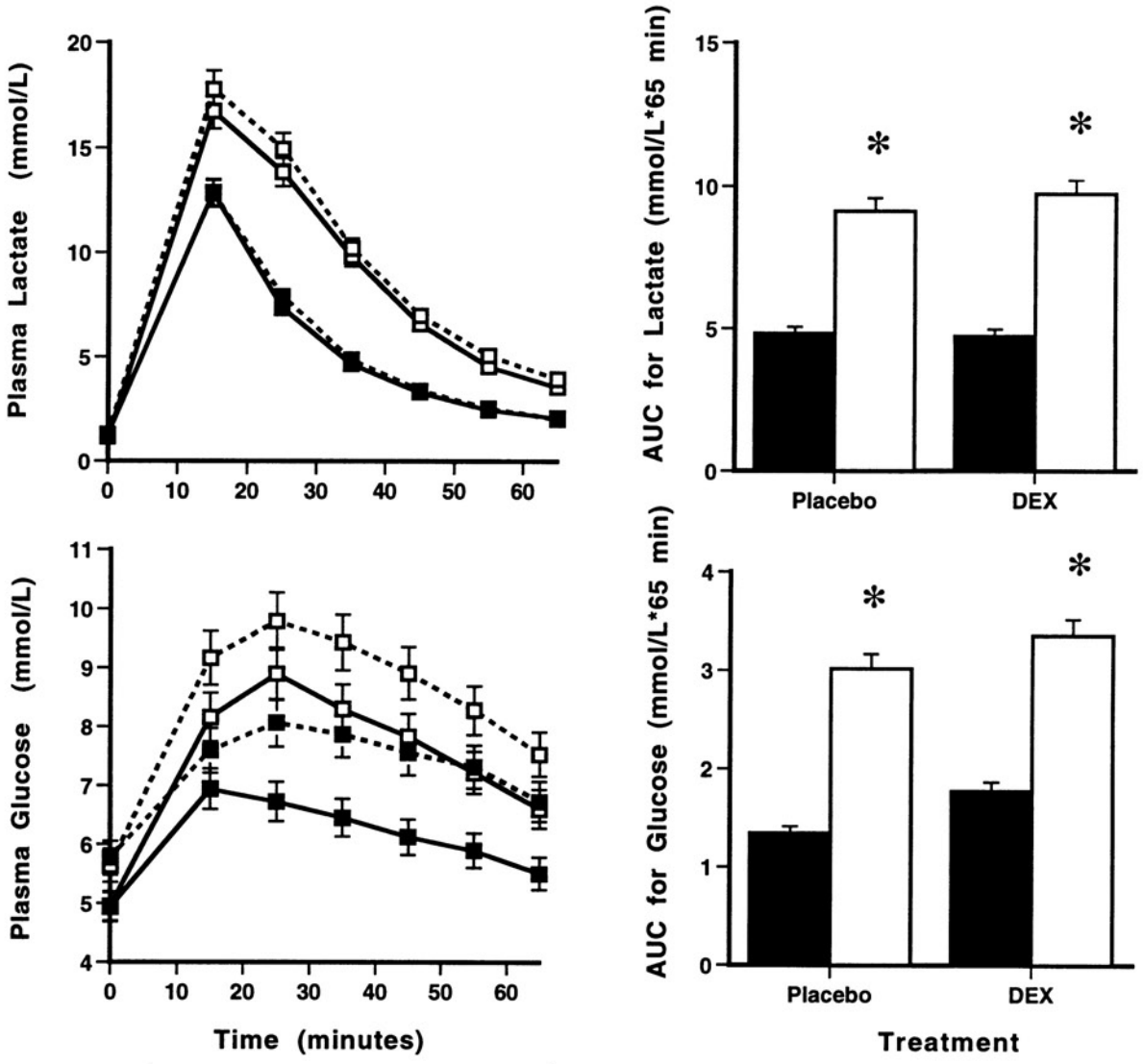


Figure 8. Exercise-induced changes in plasma lactate (upper panel) and glucose (lower panel) over time in low-responders (closed squares) and high-responders (open squares) after taking placebo (solid line) or DEX (dashed line). Integrated areas under curve (AUC) for plasma lactate (upper right) and glucose (lower right) for low-responders (solid bars) and high-responders (open bars) after placebo and DEX. * equals $p < 0.05$.

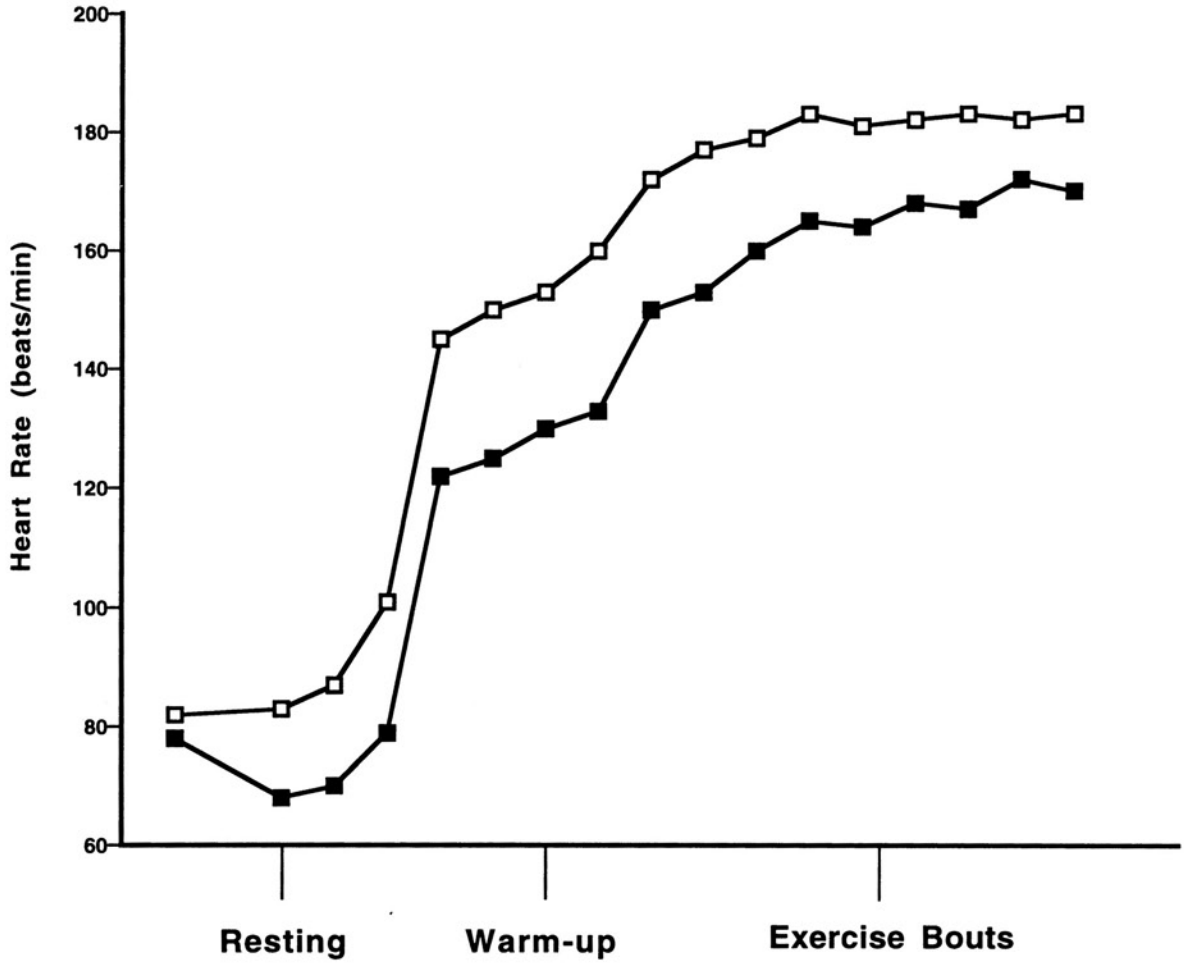


Figure 9. Exercise-induced changes in heart rates over time averaged for both treatments in low-responders (open squares, solid line) and high-responders (closed squares, solid line).

H. Catecholamine Response to Exercise and DEX

Resting concentrations of norepinephrine and epinephrine did not differ between low-responders and high-responders and were unaltered by pretreatment with DEX (Figure 10). By time 15 min of exercise plasma norepinephrine responses were three-fold greater and plasma epinephrine responses were seven-fold greater in high-responders as compared to low-responders. Interestingly, pretreatment with DEX tended to decrease plasma catecholamine responses (norepinephrine and epinephrine) in high-responders ($p< 0.05$) but not low-responders. These differences for drug pretreatment, between the two groups, are seen more clearly for the AUC of plasma norepinephrine and epinephrine responses (Figure 10, lower panel; $p<0.05$).

I. Lactate Correlation to ACTH and AVP

To elucidate mechanisms for ACTH release during exercise, linear regression revealed significant correlations ($p< 0.05$) between the exercise-induced peak response to lactic acid and those for ACTH and AVP following placebo pretreatment ($r=0.78$). In addition, during the placebo condition, the exercise-induced peak response for AVP was significantly correlated with that of ACTH in all subjects ($r=0.82$).

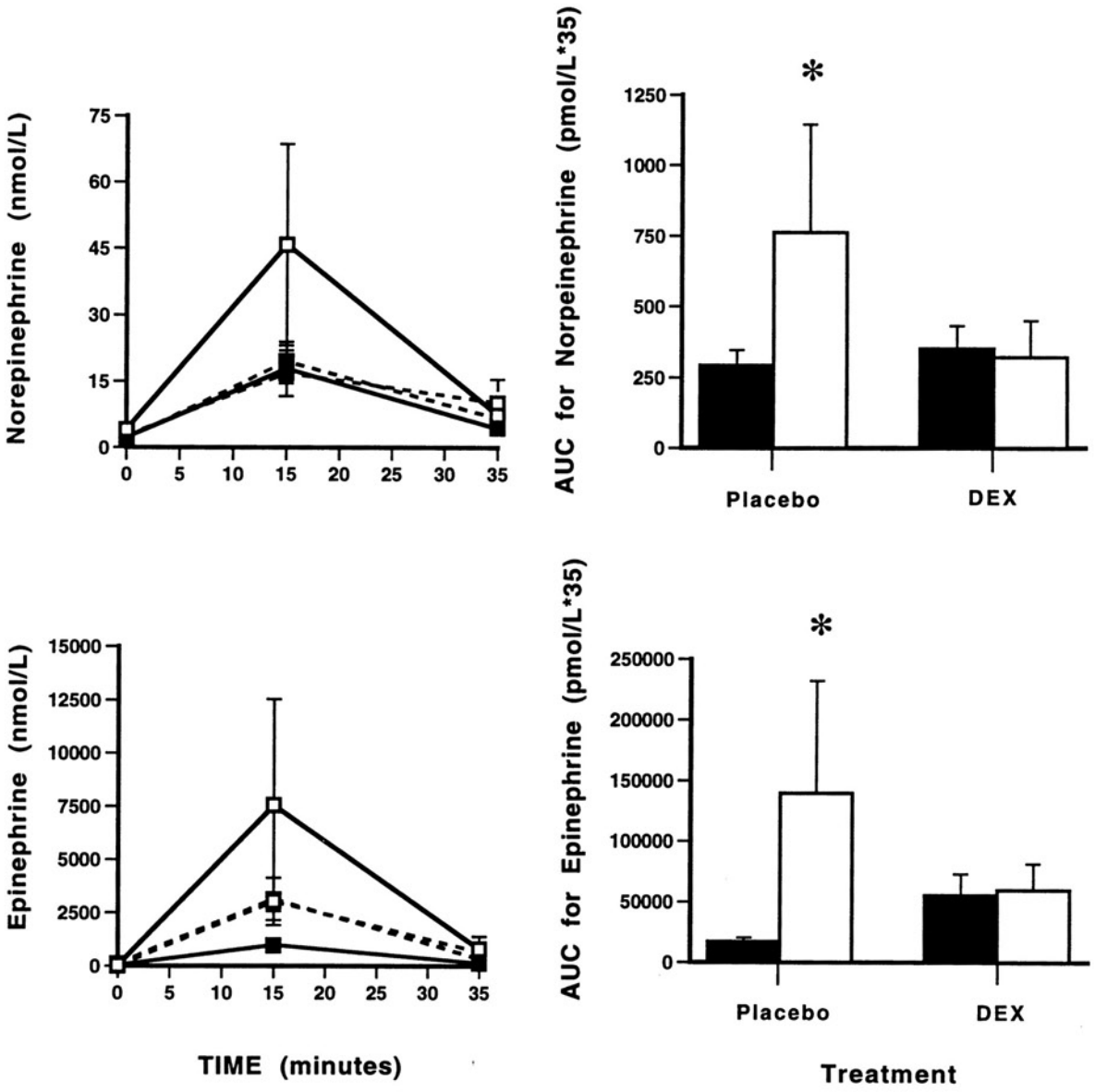


Figure 10. Exercise-induced changes in plasma norepinephrine (upper panel) and epinephrine (lower panel) over time in low-responders (closed squares) and high- responders (open squares) after taking placebo (solid line) or DEX (dashed line). Integrated areas under curve (AUC) for plasma norepinephrine (upper right) and epinephrine (lower right) for low-responders (solid bars) and high-responders (open bars) after placebo and DEX. * equals $p < 0.05$.

II. Human Study Phase II

A. Exercise Prescreen Test to Identify High- and Low-responders

1. DEX Administration and Exercise

To identify high- and low-responders prior to their enrollment into a comprehensive test protocol nineteen healthy males were prescreened with 4 mg of DEX and high-intensity exercise. Each subject performed the same exercise protocol as utilized in phase I at an estimated 90% of $\dot{V}O_{2max}$ based on average lab values for populations of moderately trained individuals (N>100). The mean grade and speed of the treadmill during the high-intensity run were 10.1 ± .1% and 8.5 ± .2 miles/h, respectively. Utilizing the criterion of an exercise-induced ACTH plasma response during DEX suppression (ACTH concentrations > 5 pmol/L above baseline), healthy volunteers were separated into the two sub-groups: high- responders (n=7) and low-responders (n=12). Table 3 presents age, weight (wt) and height (ht) characteristics for the total subject population and its two subsets; no significant differences between high- and low-responders were noted. In addition, no differences were found between the subgroups for heart rate during the screen exercise test (Figure 11). However, relative $\dot{V}O_{2max}$ or percent of maximal oxygen uptake for the screen run was significantly greater for the high- responders as compared to the low-responders ($p<0.05$) (Figure 12). It was also found that the groups differed with respect to anaerobic threshold (low-responders: 83 ± 1% $\dot{V}O_{2max}$ vs high-responders: 89 ± 1% $\dot{V}O_{2max}$); (Figure 12). Figure 12 represents data gathered from high- (n=7) and low-responders

Table 3. General Characteristics of Subjects

Subject	Age (yr)	Wt (Kg)	Ht (Cm)
		LOW RESPONDERS	
1	38	84.5	178
2	23	80.0	185
3	23	87.4	180
4	28	79.5	180
5	30	85.8	180
6	30	73.5	175
7	28	67.0	191
8	24	82.0	170
9	29	79.5	180
10	23	72.0	173
11	27	77.5	175
12	33	74.5	173
Mean ±SE	**28 ± 1**	**79.0 ± 2.0**	**177 ± 1**
		HIGH RESPONDERS	
13	37	79.5	183
14	35	67.8	183
15	24	80.0	173
16	26	76.6	183
17	28	73.4	185
18	29	76.5	188
19	28	89.0	178
Mean ± SE	**30 ± 1**	**82.0 ± 3.0**	**182 ± 2**
		TOTAL	
Mean ± SE	**28 ± 1**	**81.0 ± 2.0**	**180 ± 1**

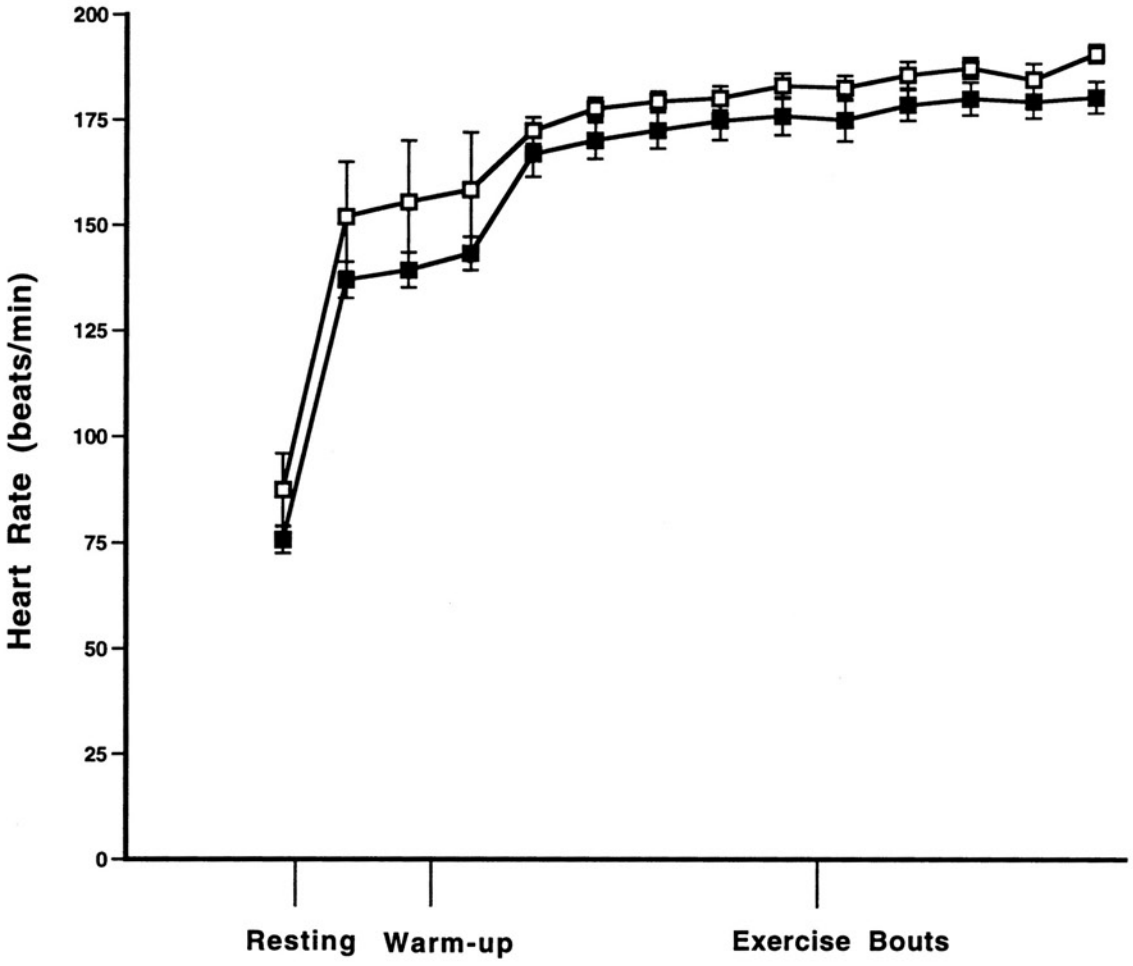


Figure 11. Screen run patterns of change in heart rates over time averaged for DEX treatment in low-responders (closed squares) and high-responders (open squares).

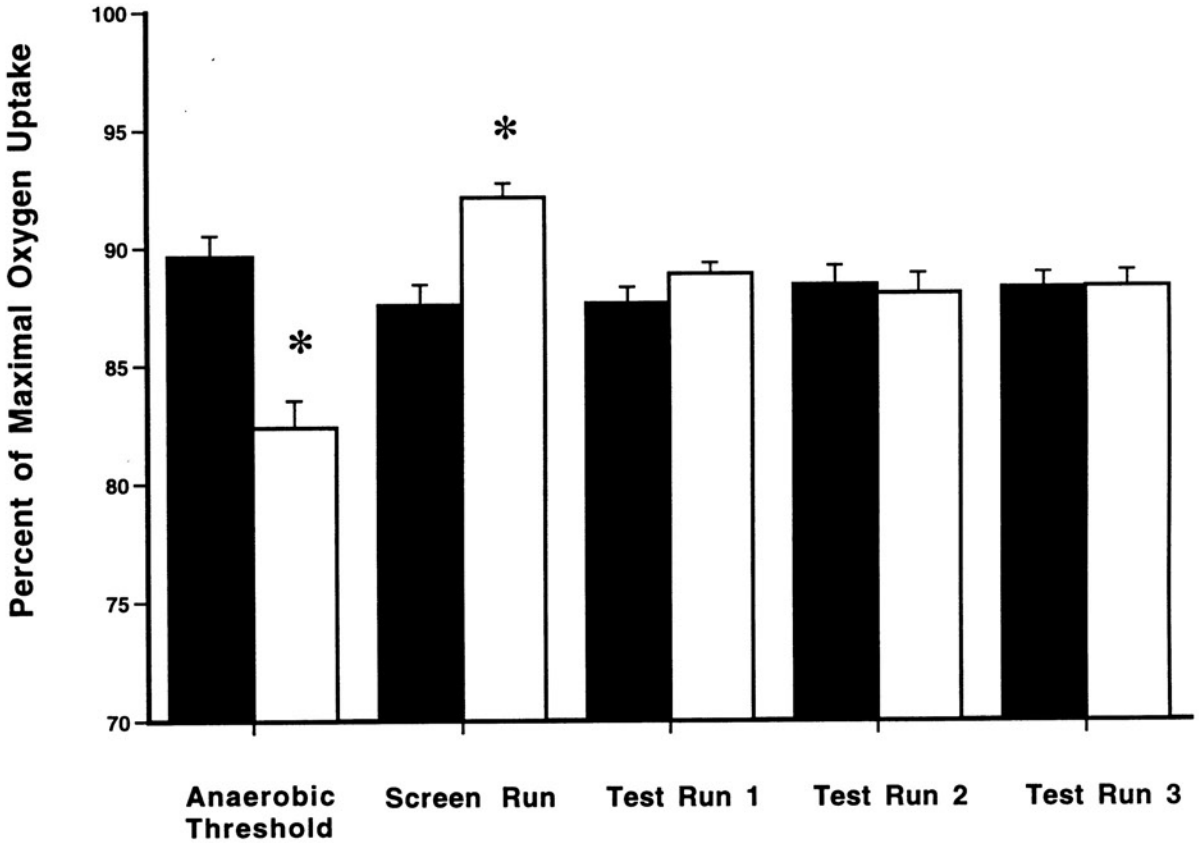


Figure 12. Percent of maximal oxygen consumption determined for each subject's anaerobic threshold and exercise test runs. Averaged values for low-responders are represented by solid bars and high-responders by open bars. * equals $p < 0.05$.

(n=8) that completed the entire comprehensive test protocol.

2. Screen Test ACTH Response to Exercise and DEX

Basal plasma concentrations of ACTH did not differ between low- and high-responders (Figure 13). However, while DEX administration completely abolished the exercise-induced rise in plasma ACTH in low-responders, plasma ACTH significantly increased four-fold in high-responders receiving DEX (Figure 13). Even though the exercise-induced increases in heart rate values were similar for the two groups, the high-responders scored significantly higher on the Borg perceived exertion rating scale as compared to the low-responders ($19.5 \pm .4$ versus $17.0 \pm .5$, $p<0.05$) (Figure 14). It was determined through linear regression analysis that a strong correlation existed between the peak ACTH response and Borg scale rating to the screen exercise test for each subject ($r=.78$) ($p < 0.05$).

B. Exercise Test Runs for High- and Low-responders

1. Dexamethasone and Hydrocortisone Pretreatment with Exercise

The subjects were separated into two subgroups based upon each individual's ACTH response to the screen run; high-responders (n=7) and low-responders (n=8; four low-responders declined participation in the remaining protocol) and all subsequent results were analyzed according to this grouping.

To further investigate the possibility that differential sensitivity to glucocorticoid suppression during exercise results from intrinsic differences in the functioning of glucocorticoid Type I and Type II receptors, and mechanisms, hydrocortisone and DEX were used to preferentially elicit Type I and Type II

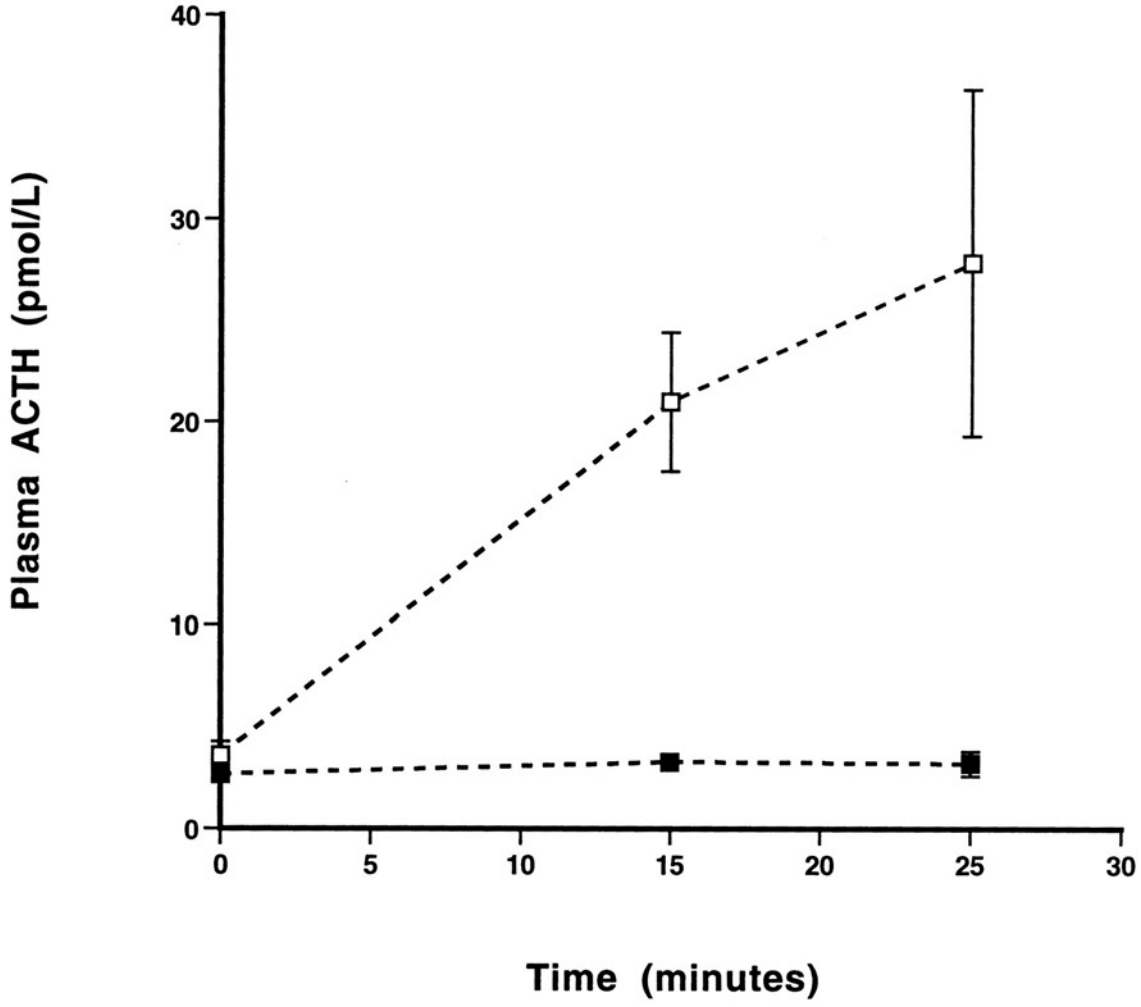


Figure 13. Exercise-induced changes in plasma concentration of ACTH in low- (closed squares) and high-responders (open squares) after receiving DEX.

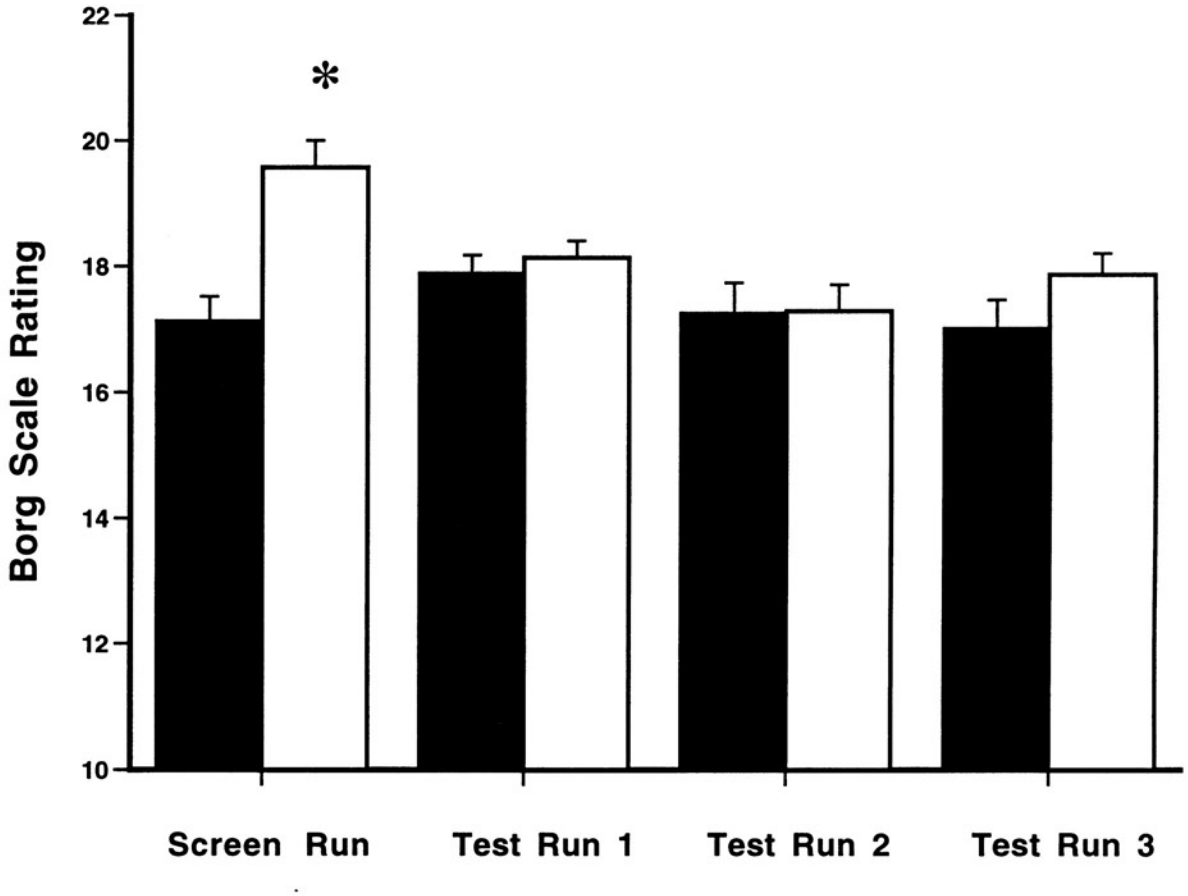


Figure 14. Borg scale rating for perceived exertion reported by each subject immediately following high-intensity exercise for the screen test run and three test runs. Low-responders are represented by solid bars and high-responders by open bars. * equals $p < 0.05$.

receptors, respectively. Hydrocortisone was given orally in a dose of 100 mg to match the effectiveness of an oral administration of 4 mg of DEX (Meikle and Tyler, 1977). None of the subjects experienced any adverse reactions to either of the drugs. The total mean grade and speed of the treadmill during the high-intensity exercise were 10.2 ± .1% and 8.4 ± .2 miles/h, respectively.

2. Glucocorticoid Suppression of Basal Plasma Cortisol and Subject Characteristics of High- and Low-responders

Importantly, all individuals exhibited a similar suppression of basal cortisol release in a standard 1 mg DST (Table 4). Each subject received 1 mg of DEX at 2300 h and blood samples collected at 0800 h the next day. All subjects met the criterion established by (Sherman et al., 1984) for a normal DST which requires suppression of basal cortisol release to concentrations below 138 nmol/L. Since plasma concentrations of DEX and hydrocortisone were unaffected by exercise in both groups, potential differences in the absorption and metabolism of either drug were discounted. Table 4 also presents characteristics for the total subject population (n=15) and the two subsets for the tests runs. No significant differences in age, weight (wt), height (ht), or $\dot{V}O_{2max}$ were noted between the low-responders or high-responders. Within each subset, heart rate, absolute $\dot{V}O_2$, relative $\dot{V}O_2$, and respiratory exchange ratio values averaged over the last four intermittent bouts of high-intensity exercise were found to be unaffected by DEX or hydrocortisone pretreatment (Table 5). In contrast to Phase I, mean heart rates were not significantly elevated for the high-responder as compared to the low-responder group.

Table 4. General Characteristics of Subjects

Subject	Age (yr)	Weight (Kg)	Height (Cm)	Max $\dot{V}O_2$ (L/min)	Plasma Cortisol* (nmol/L)
TOTAL POPULATION					
Mean ± SE	**29 ± 1**	**81.0 ± 2.0**	**180 ± 1**	**3.93 ± 0.10**	**45.3 ± 5.4**
LOW RESPONDERS					
1	38	84.5	178	4.38	33.1
2	23	80.0	185	4.25	66.2
3	23	87.4	180	4.56	33.1
4	28	79.5	180	3.31	33.1
5	30	85.8	180	3.86	44.2
6	30	73.5	175	4.61	33.1
7	28	67.0	191	3.73	46.9
8	24	82.0	170	3.69	57.9
Mean ± SE	**28 ± 1**	**80.0 ± 3.0**	**178 ± 2**	**4.05 ± 0.17**	**43.5 ± 4.6**
HIGH RESPONDERS					
9	37	79.5	183	4.53	33.1
10	35	67.8	183	3.88	110.4
11	24	80.0	173	4.43	35.9
12	26	76.6	183	3.96	41.4
13	28	73.4	185	3.64	33.1
14	29	76.5	188	3.39	44.1
15	28	89.0	178	3.87	33.1
Mean ± SE	**30 ± 1**	**82.0 ± 3.0**	**182 ± 2**	**3.81 ± 0.10**	**47.3 ± 10.7**

*Plasma Cortisol for Standard Dexamethasone Suppression Test

Table 5. Physiological parameters during high intensity intermittent running with Dexamethasone (DEX), Hydrocortisone (HCO) or placebo pretreatment.

	TOTAL POPULATION			LOW RESPONDERS			HIGH RESPONDERS		
	PLACEBO	DEX	HCO	PLACEBO	DEX	HCO	PLACEBO	DEX	HCO
Heart rate (bpm)[1]	181 ± 2	181 ± 3	182 ± 3	179 ± 4	180 ± 4	181 ± 5	183 ± 3	183 ± 3	184 ± 4
VO_2 (L/min)[2]	3.6 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.6 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.7 ± 0.1	3.6 ± 0.1	3.7 ± 0.1
Relative VO_2 (%)[3]	88 ± 1	89 ± 1	88 ± 1	88 ± 1	88 ± 1	88 ± 1	88 ± 1	89 ± 1	88 ± 1
RER[4]	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1

*Values are the mean ± SE
[1]Heart rate in beats per minute
[2]Oxygen uptake
[3]% of maximal oxygen uptake achieved
[4]Respiratory exchange ratio

3. ACTH Response to Exercise with DEX and Hydrocortisone

Resting concentrations of circulating ACTH did not differ between low- and high- responders, whereas both DEX and hydrocortisone pretreatments decreased these levels by approximately 75% ($p<0.05$). Plasma concentrations of ACTH in all subjects receiving placebo were highest at 15 min into the exercise regimen (Figure 15, upper left panel). The exercise-induced plasma ACTH response of the high-responder group was, however, twice that observed in the low-responder group. With DEX, no significant exercise-induced increase in ACTH was observed in the low-responders, whereas an ACTH response was evident in high-responders. Interestingly, as compared to DEX pretreatment, hydrocortisone produced a greater inhibition of stimulated ACTH release in high-responders (Figure 15, lower left panel) ($p<0.05$). Peak plasma concentrations of ACTH at 15 min in the high-responder group receiving hydrocortisone were equivalent to those of the low-responder group receiving either DEX or hydrocortisone. The AUC for ACTH under placebo conditions was significantly greater ($p<0.05$) in the high-responder as compared to the low-responder group (Figure 15, upper right panel). With DEX pretreatment, the AUC for ACTH was attenuated by 80% in high-responders and 99% in low-responders (Figure 15, lower right panel), whereas hydrocortisone, in both subgroups, decreased the AUC for ACTH by 99%.

4. Cortisol Response to Exercise with DEX and Hydrocortisone

Changes in circulating levels of cortisol following placebo pretreatment,

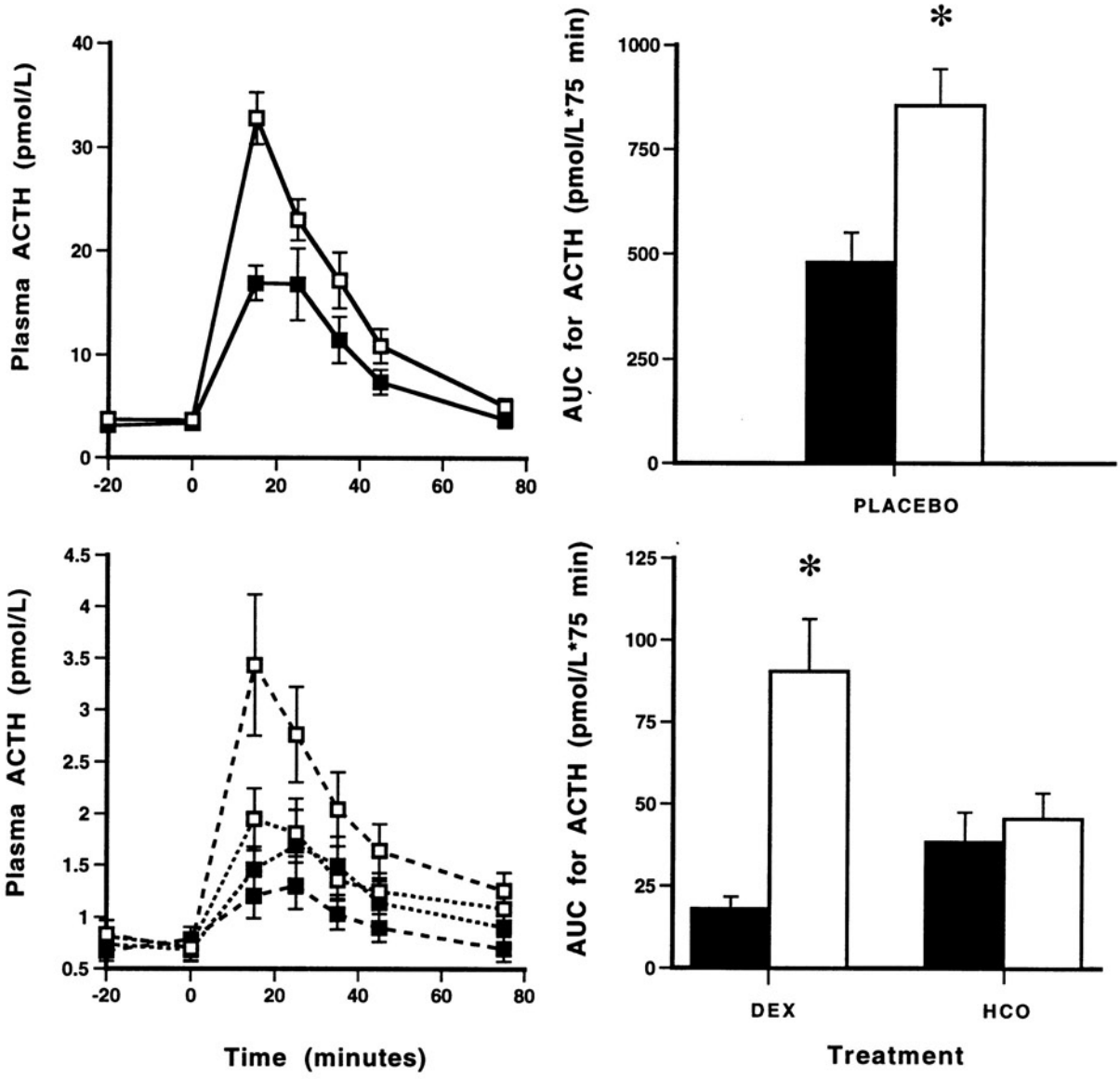


Figure 15. Exercise-induced changes in plasma concentration of ACTH in low- responders (closed squares) and high-responders (open squares) following placebo (upper panel) or either DEX (long-dashed lines) or HCO (short-dashed line) preatreatments (lower panel). Integrated areas under curve (AUC) for plasma ACTH (upper and lower right) over the entire time for low-responders (solid bars) and high- responders (open bars) after placebo, DEX and HCO. * equals $p < 0.05$

closely resembled those observed for ACTH in the two groups (Figure 16, left panel). DEX pretreatment significantly reduced basal concentrations of cortisol in both groups equally by 65%, whereas hydrocortisone pretreatment increased basal concentrations of plasma cortisol seven-fold as expected. All individuals receiving placebo exhibited peak plasma cortisol concentrations by 20 min after the termination of exercise (experimental time = 45 min). The maximal plasma cortisol response observed in high-responders receiving placebo was approximately 50% greater than that of the low-responder group (740 ± 60 nmol/L vs 510 ± 40 nmol/L; $p<0.05$). Pretreatment with DEX completely blocked the exercise-induced release of cortisol in both the high- and low-responders. The difference between the two groups for the placebo condition was also clearly evident for AUC (Figure 16, right panel; $p<0.05$). The AUC for both groups receiving either DEX and hydrocortisone pretreatments was negative due to the metabolism of plasma cortisol during the exercise protocol.

5. AVP Response to Exercise with DEX and Hydrocortisone

In contrast to the ACTH and cortisol, pretreatment with DEX or hydrocortisone did not significantly alter basal plasma AVP concentrations in either high- or low-responders. High-intensity exercise caused a significant increase in plasma concentrations of AVP ($p<0.05$) which were maximal by time 15 min of exercise for all pretreatments (Figure 17, left panel). A significantly greater ($p<0.05$) increase in release of AVP occurred in high-responders as

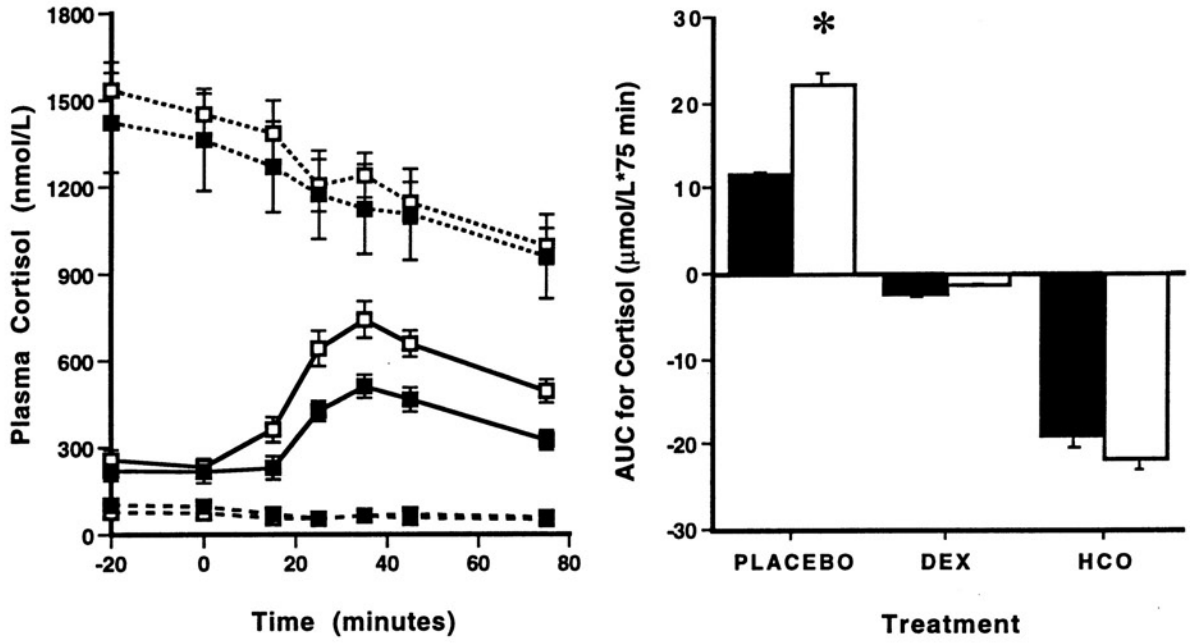


Figure 16. Exercise-induced changes in plasma concentrations of cortisol over time in low-responders (closed squares) and high-responders (open squares) after taking placebo (solid line), DEX (long-dashed line), or HCO (short-dashed line). Integrated areas under curve (AUC) for plasma CO for low-responders (solid bars) and high-responders (open bars) after placebo, DEX and HCO. * equals $p < 0.05$.

compared to low-responders. Interestingly, as compared to placebo, the exercise-induced release of AVP was potentiated by pretreatment with both DEX and hydrocortisone in both groups. This pattern of response was most dramatic in high-responders where plasma AVP responses were six-fold those observed in low-responder ($p<0.05$). Statistical analysis of AUC further demonstrated that exercise-induced AVP release was markedly enhanced ($p<0.05$) in the high-responder as compared to the low-responder group for all treatments (Figure 17, right panel).

In addition, post-placebo exercise-induced AVP responses were combined from both phase I and II and analyzed using multiple regression analysis (data not shown). Specifically, it was observed that the relationship between changes in relative oxygen uptake and both exercise-induced peak and AUC AVP responses were dependent on group (high- and low-responders) ($p < 0.05$). The regression equation for peak AVP of high-responders was determined to be peak AVP = 7.56 x $\%\dot{V}O_2$ - 654.9 and peak AVP = 0.74 x $\%\dot{V}O_2$ - 62.98 for low-responders. The regression equation for AUC AVP was determined to be AUC AVP = 91.85 x $\%\dot{V}O_2$ - 7801.52 for high-responders and AUC AVP = 18.17 x $\%\dot{V}O_2$ - 1557.3 for low-responders. Multiple regression analysis revealed that the slopes of the regression equations for both peak and AUC AVP responses were significantly different for high- as compared to low-responders ($p < 0.05$) when controlling for differences in relative exercise intensity.

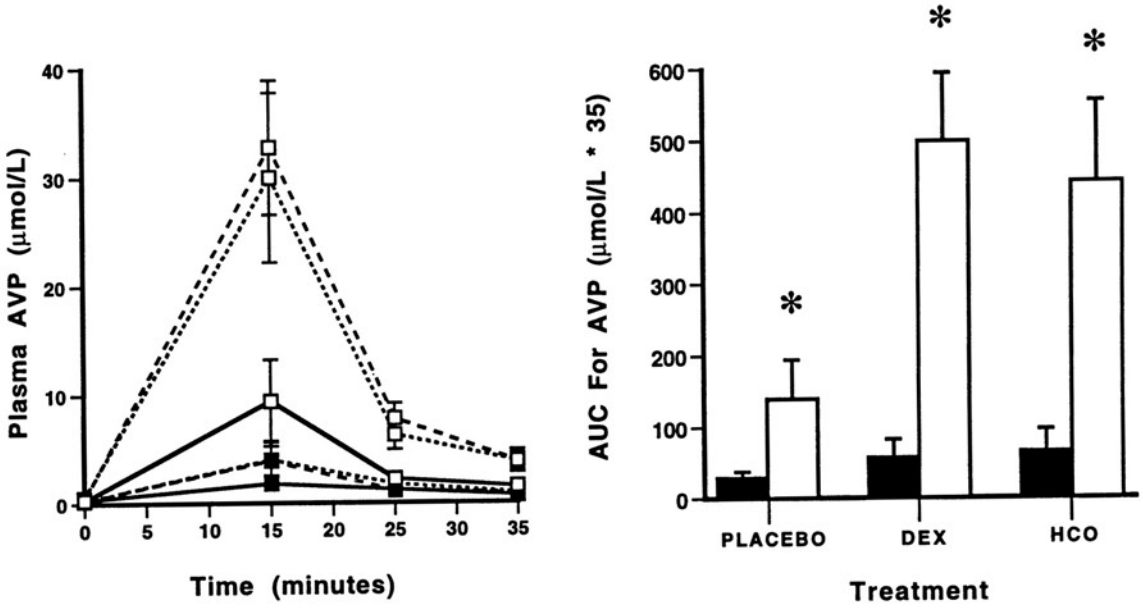


Figure 17. Exercise-induced changes in plasma concentrations of AVP in low- responders (closed squares) and high-responders (open squares) pretreated with either placebo (solid line), DEX (long-dashed line), or HCO (short-dashed line). Integrated areas under curve (AUC) for plasma AVP for low-responders (solid bars) and high-responders (open bars) after placebo, DEX and HCO. * equals $p < 0.05$.

6. GH Responses to Exercise with DEX and Hydrocortisone

Basal concentrations of GH were significantly lower in high-responders as compared to those of low-responders ($p<0.05$). DEX and hydrocortisone pretreatments did not alter basal GH release in high-responders. In contrast, low-responders showed significantly elevated basal GH concentrations after DEX pretreatment, however, hydrocortisone did not significantly alter basal GH concentrations in these individuals. High-intensity exercise caused a significant increase in plasma concentrations of GH ($p<0.05$) which were maximal by time 25 min of exercise for all pretreatments (Figure 18, left panel). A significantly greater ($p<0.05$) increase in GH release occurred in low-responders as compared to high-responders. Interestingly, as compared to placebo, the exercise-induced release of GH was potentiated by pretreatment with DEX in low-responders ($p<0.05$) but not in high-responders. Hydrocortisone pretreatment did not significantly alter the exercise-induced GH response in either group. Statistical analysis of the AUC for plasma GH responses further demonstrated that exercise-induced GH release was markedly enhanced ($p<0.05$) in low-responders as compared to high-responders during DEX pretreatment (Figure 18, right panel).

7. Lactate Response to Exercise with DEX and Hydrocortisone

Similar to Phase I, exercise-induced anaerobic metabolism was clearly evident by the marked increases in plasma lactate concentrations that occurred for all subjects by the end of the high-intensity run (time = 15 min) (Figure 19,

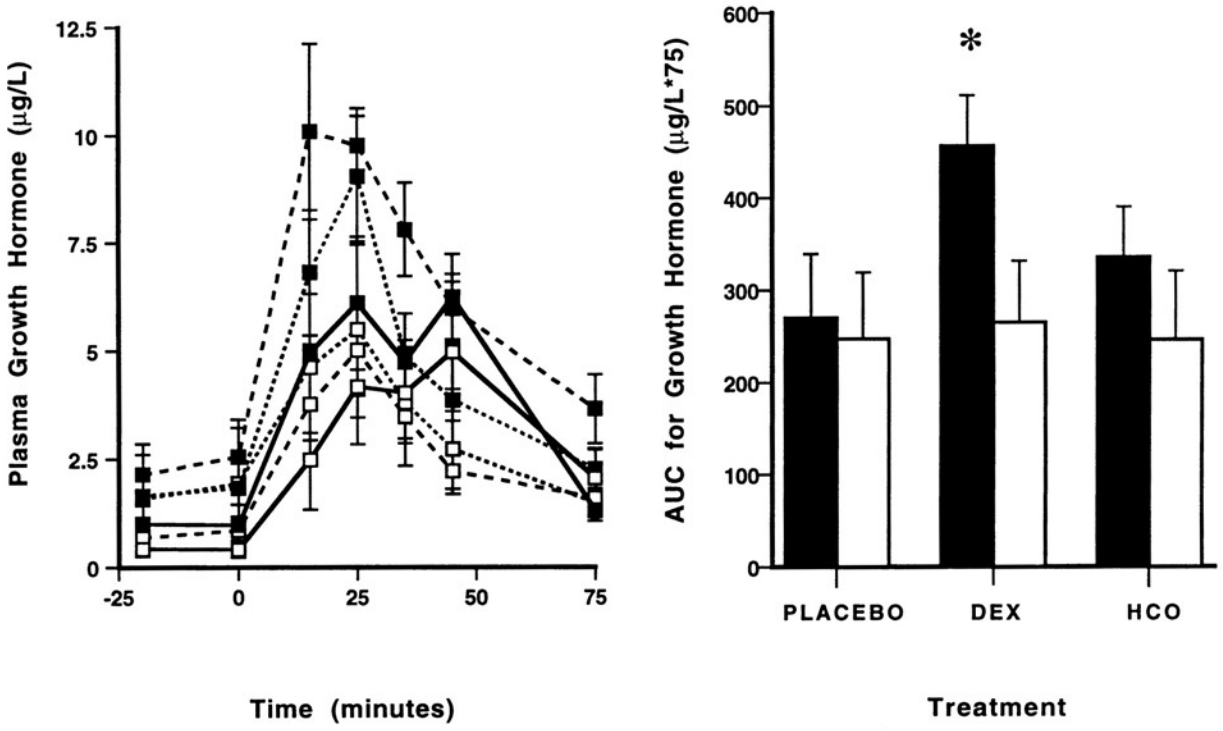


Figure 18. Exercise-induced changes in plasma concentrations of growth hormone in low- (closed squares) and high-responders (open squares) following placebo or either dexamethasone (DEX) (long-dashed line) or hydrocortisone (HCO) (short-dashed line) pretreatments. Integrated areas under curve (AUC) for plasma growth hormone for low- responders (solid bars) and high-responders (open bars) after placebo, DEX and HCO.

* equals $p < 0.05$

upper left panel). Analysis of AUC for the two subsets demonstrated that plasma lactate responses were greater in high-responders as compared to low-responders following placebo, as well as DEX and hydrocortisone pretreatment ($p<0.05$) (Figure 19, upper right panel). Neither DEX nor hydrocortisone pretreatment appreciably altered lactate production during exercise as compared to placebo in either group.

8. Glucose Response to Exercise with DEX and Hydrocortisone

Basal and exercise-induced plasma concentrations of plasma glucose were significantly elevated in both subgroups after pretreatment with DEX or hydrocortisone (Figure 19, lower left panel) as compared to placebo ($p < 0.05$). Plasma glucose concentrations peaked in all individuals after termination of exercise (time = 25 min). However, analysis of the AUC for plasma glucose revealed no significate differences between low- and high-responders for all pretreatments (Figure 19, lower right panel).

9. Heart Rate Response to Exercise with DEX and Hydrocortisone

Basal and exercise-induced increases in heart rates were unaltered by DEX or hydrocortisone pretreatment in either group (Figure 20). Heart rates rose continuously throughout the exercise protocol and plateauxed by the seventh intermittent bout of high-intensity exercise. Similar to the screen test, heart rates during the high-intensity exercise in the high-responder group were equivalent to those of the low-responder group with either treatment. In contrast, resting

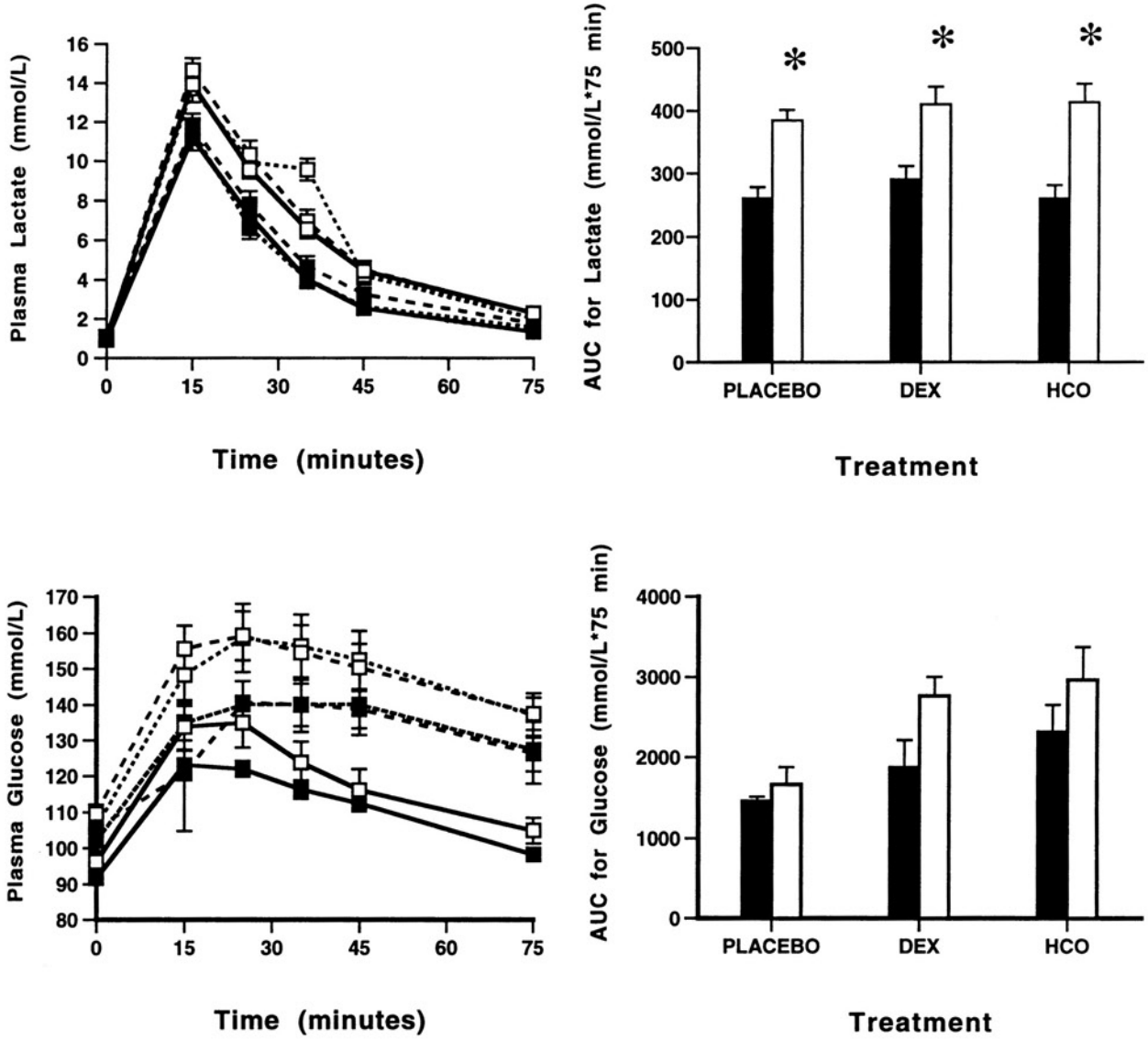


Figure 19. Exercise-induced changes in plasma concentrations of lactate (upper panel) and glucose (lower panel) in low-responders (closed squares) and high-responders (open squares) pretreated with either placebo (solid line), DEX (long-dashed line), HCO (short-dashed line). Integrated areas under curve (AUC) for plasma lactate (upper right) and glucose (lower right) for low-responders (solid bars) and high-responders (open bars) after placebo, DEX and HCO. * equals $p < 0.05$.

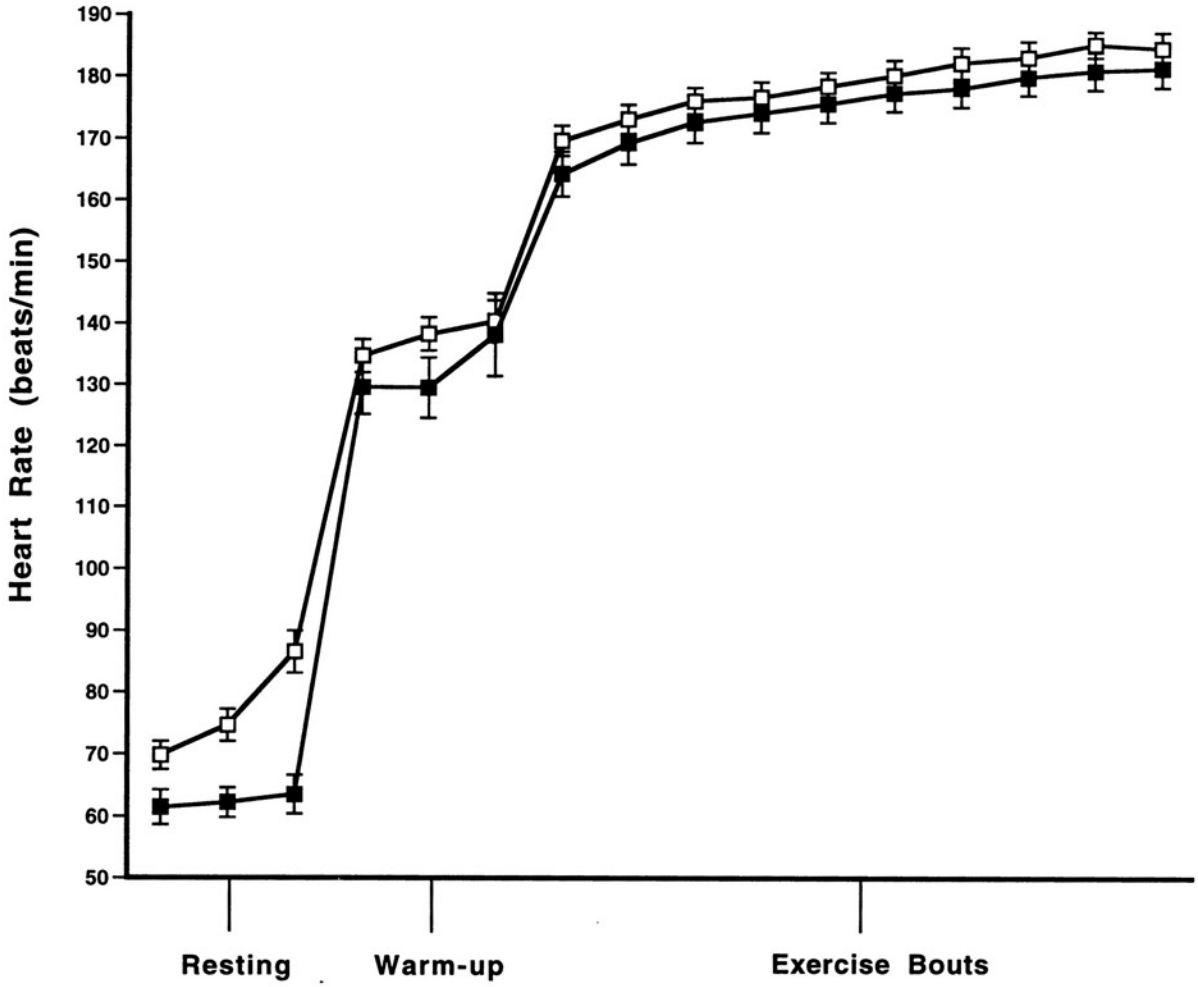


Figure 20. Patterns of change in heart rates over time averaged for all treatments in low-responders (open squares, solid line) and high-responders (closed squares, solid line).

heart rates were significantly ($p<0.05$) higher in the high-responders as compared to the low-responders.

10. Catecholamine Response to Exercise with DEX and Hydrocortisone

Resting concentrations of norepinephrine and epinephrine did not differ between low-responders and high-responders and were unaltered by pretreatment with DEX (Figure 21). The highest plasma catecholamine concentrations were observed at time 15 in both high-responders and low-responders, but no significant differences were noted. In addition, no differences for drug pretreatment between the two groups were observed for the AUC of plasma norepinephrine and epinephrine responses (Figure 21, lower panel).

C. Psychological Tests

1. POMS

To assess changes in mood that may occur as a consequence of drug treatment and/or exercise testing, the Bi-polar Profile of Mood States questionnaire was given each test day before drug treatment, after drug administration and after exercise. No significant differences were found before drug, before run, or after run between subject groups or drug pretreatment.

2. Beck Inventory

Individual tendencies toward depression were evaluated by the Beck Depression Inventory. There was no difference in the determined score for low- and high-responders. Each subject scored low on this questionnaire indicating

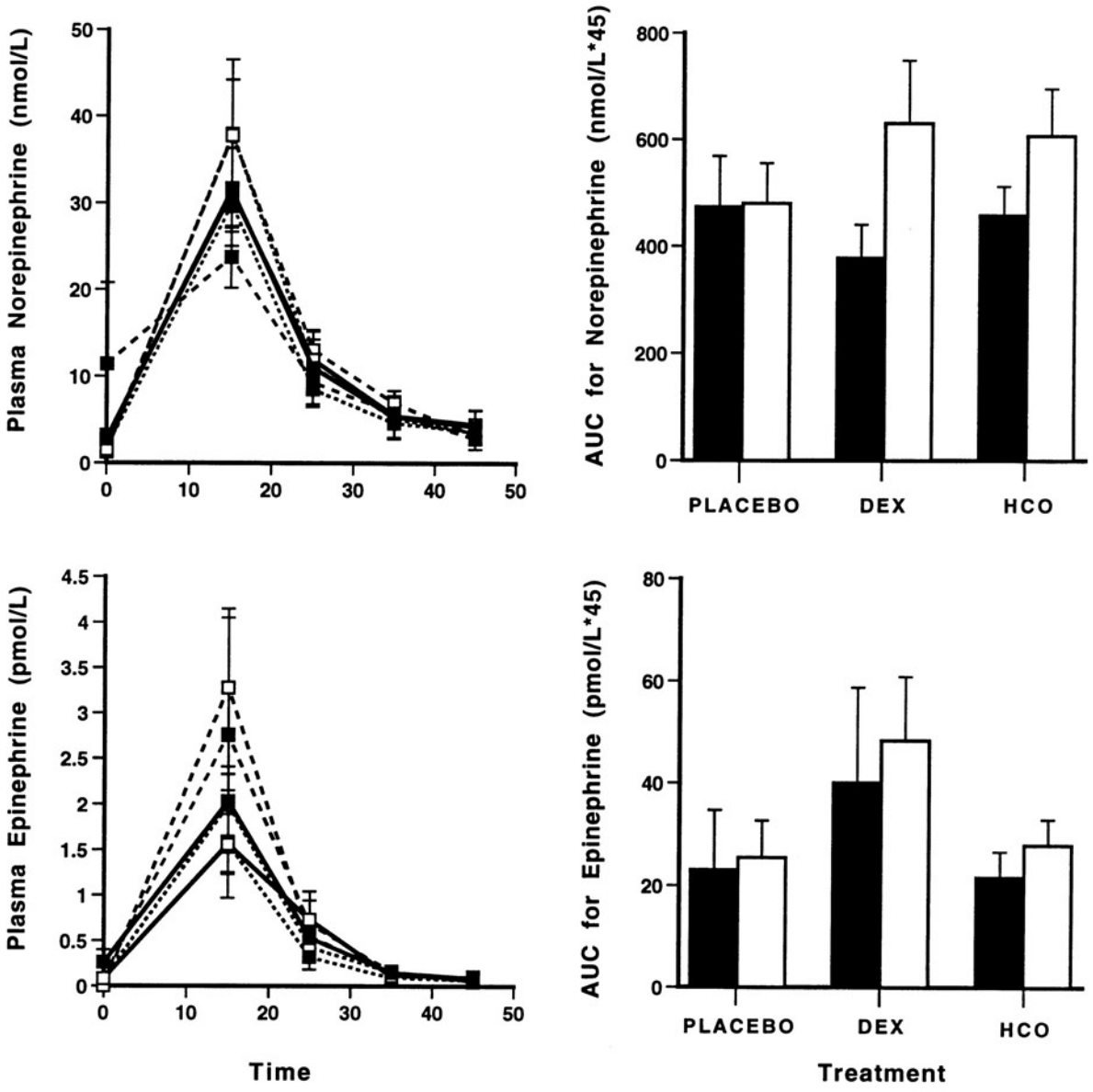


Figure 21. Exercise-induced changes in plasma concentrations of norepinephrine (upper panel) and epinephrine (lower panel) in low- (closed squares) and high-responders (open squares) following placebo or dexamethasone (DEX) (long-dashed line) or hydrocortisone (HCO) (short-dashed line) pretreatments. Integrated areas under curve (AUC) for plasma catecholamines for low- (solid bars) and high-responders (open bars) after placebo, DEX and HCO. * equals $p < 0.05$.

no signs of depression.

3. Spielberger Trait Anxiety Scale (STAI Form Y-2)

The Spielberger Trait Anxiety scale is a self-report scale measuring trait anxiety. It consists of twenty statements that assess how people generally feel, including evaluations of each individual's feelings of overall apprehension, tension, nervousness and worry. Figure 22 shows that high-responders tended to have higher scores on trait anxiety than low-responders ($p<0.09$). As compared to normal data for adult males, ages 10-39, both the high- and low-responders scored within the normal range (35.8 ± 10.4) (Spielberger et al., 1983).

4. SCL-90-R

The SCL-90-R was used as a general psychological screening tool to evaluate nine psychological factors; Somatization, Obsessive-Compulsion, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Idealism, and Psychoticism. It appears that the high-responders tended to score higher in phobic anxiety and lower in interpersonal sensitivity as compared to low-responders. However, these differences were determined to be insignificant (Figure 23). In addition, the global severity index, which is an overall score normalized to the number of questions on the SCL-90-R, was similar for high- (0.27 ± 0.07) and low-responders (0.35 ± 0.07) and both groups scored within the range for a normal population (0.31 ± 0.01) (Derogatis and Cleary, 1977).

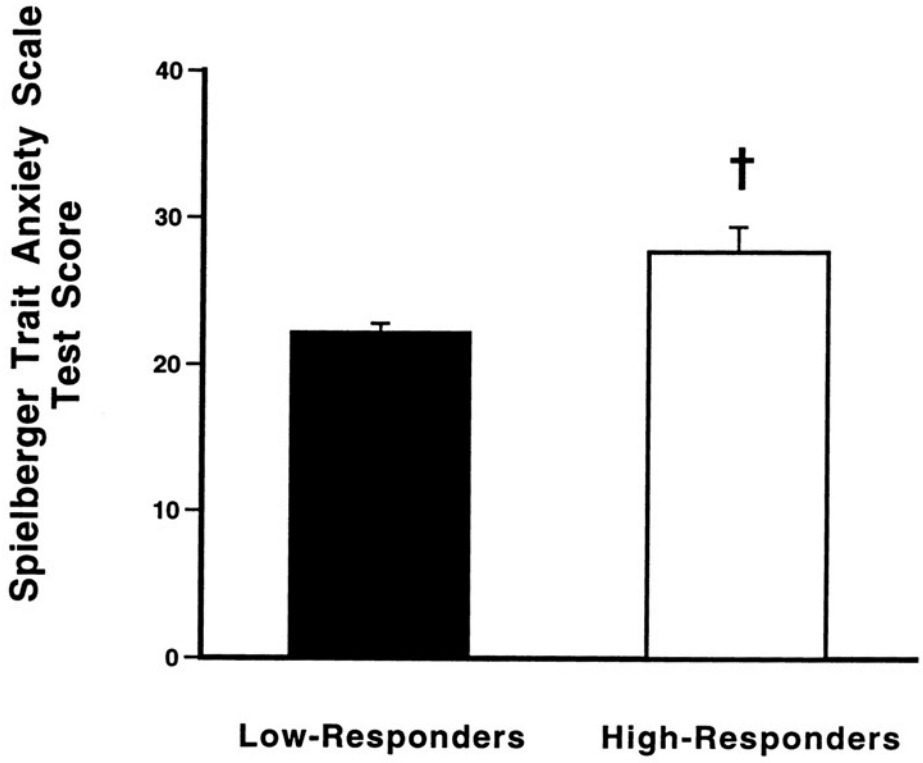


Figure 22. Averaged Test scores for the Speilberger Trait Anxiety Scale for low- and high-responders. Low-responders are represented by solid bars and high-responders by open bars. † equals p = 0.09.

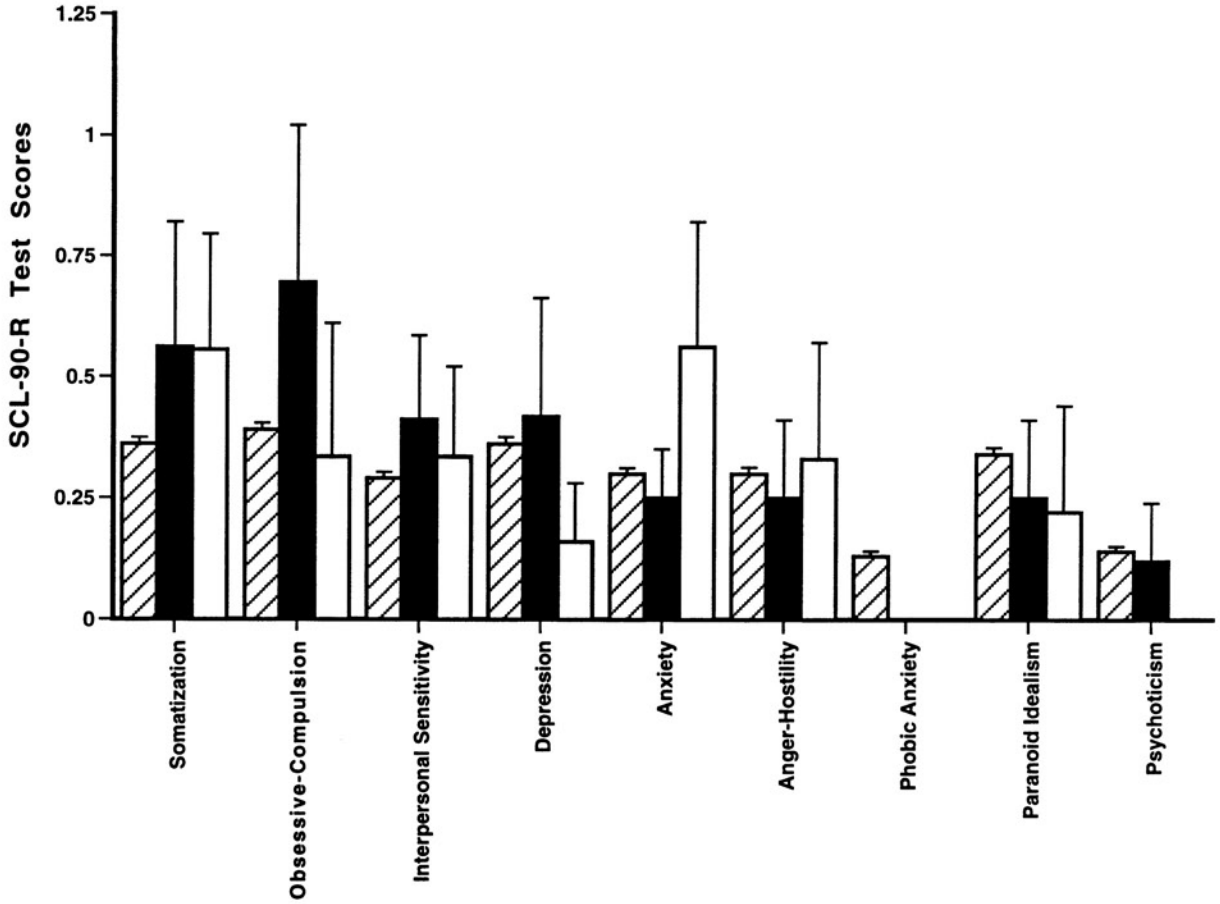


Figure 23. Averaged Test scores for the SCL-90-R questionnaire for low- and high- responders. Normative controls are represented by hatched bars, low-responders by solid bars and high-responders by open bars.

D. AVP Stimulation Test

1. Intravenous AVP Infusion

Intravenous infusion of 1 mIU/kg/min AVP was generally tolerated, although most subjects (80%) experienced a brief and transient period of slight nausea at the onset of the AVP infusion. Also, by the end of the drug infusion all subjects reported mild abdominal cramping which ceased immediately upon termination of the drug infusion.

2. ACTH Response to AVP Stimulation

The 60 min intravenous AVP infusion significantly ($p<0.05$) increased plasma ACTH concentrations above baseline values in both the low- and high-responders (Figure 24, upper left panel). Plasma concentrations of ACTH in all subjects receiving the infusion were highest at the end of the drug infusion period. Although both subgroups began the infusion with equivalent basal ACTH concentrations, the AVP-induced plasma ACTH response for the high-responder group was significantly ($p<0.05$) greater than that observed in the low-responder group. Analysis of the AUC for the ACTH response to an AVP infusion was 40% greater ($p<0.05$) in the high-responder as compared to that of the low-responder group (Figure 24, upper right panel).

3. Cortisol Response to AVP Stimulation

Changes in circulating levels of cortisol resembled those observed for ACTH in the two groups. Intravenous infusion of AVP evoked a significant increase in basal cortisol concentrations in both the low- and high-responders

(Figure 24, lower left panel). All individuals receiving AVP exhibited the maximal concentrations of plasma cortisol by the end of the drug infusion period. (experimental time = 60 min). The maximal plasma cortisol response observed in high-responders tended to be greater than that of the low-responder group. This difference between the AUC of cortisol for the two groups was not to significant (Figure 24, lower right panel; $p<0.05$).

4. Lactate Response to AVP Stimulation

Anaerobic metabolism was unaffected by AVP treatment (Figure 25, upper left panel). Lactate concentrations tended to rise slightly during the infusion period and then declined after the termination of the drug. The AUC for plasma lactate revealed that there was no significant difference in the lactate response to AVP stimulation between the two groups (Figure 25, upper right panel).

5. Glucose Response to AVP Stimulation

In contrast to lactate, circulating glucose did increase significantly ($p<0.05$) in response to the AVP infusion in both the high- and low-responders (Figure 25, lower left panel). Similar to the ACTH and cortisol responses, all subjects exhibited peak concentrations of plasma glucose by the end of the drug infusion. Analysis of the AUC for plasma glucose determined that the responses were equivalent for both subgroups (Figure 25, lower right panel).

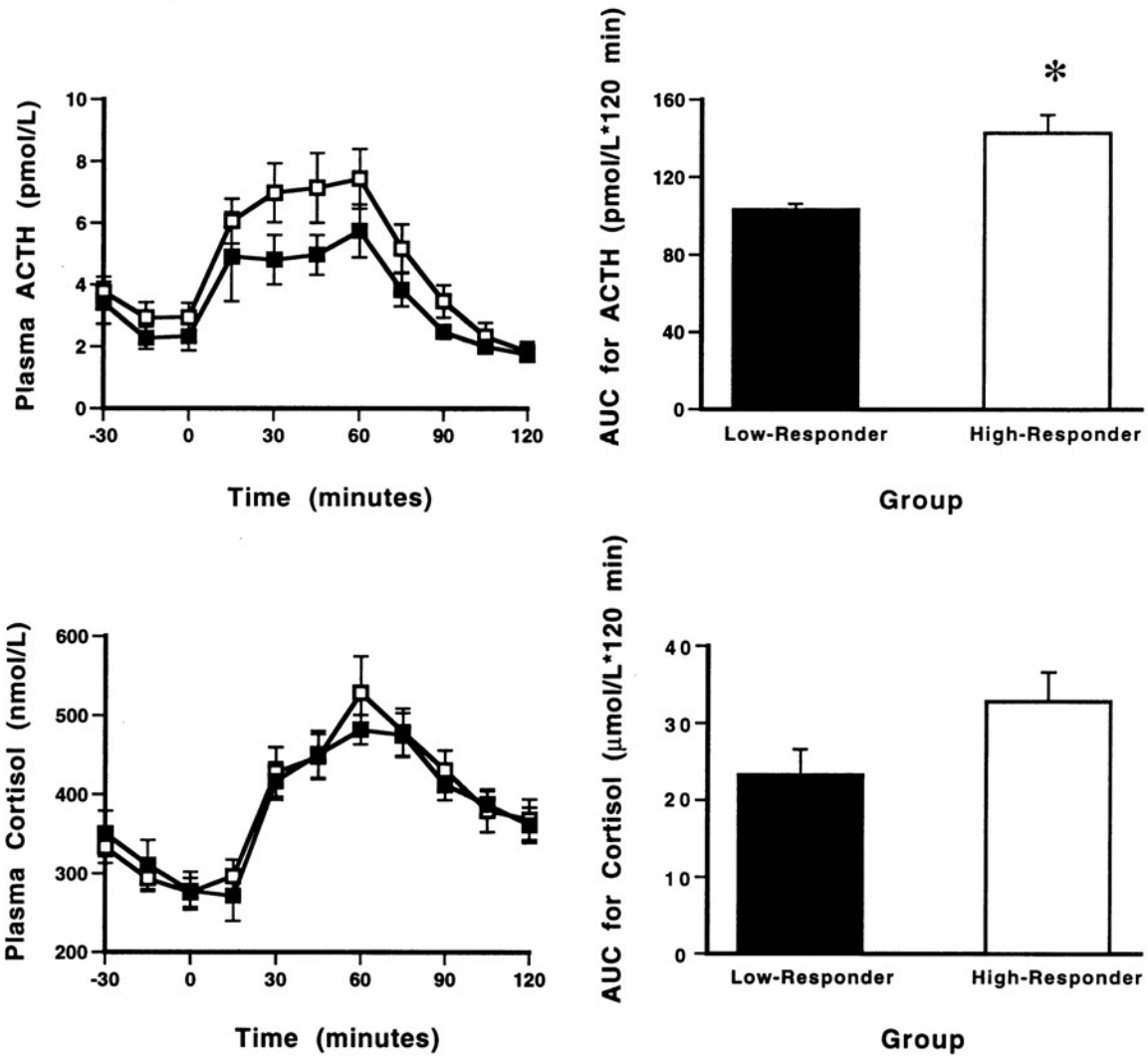


Figure 24. Patterns of change in plasma concentrations of ACTH (upper panel) and cortisol (CO-lower panel) in low responders (closed squares) and high responders (open squares) recieving 1 mIU/Kg/min AVP infusion. Integrated areas under curve (AUC) for plasma ACTH (upper right) and CO (lower right) over the entire infusion time for low responders (solid bars) and high responders (open bars). * equals $p < 0.05$.

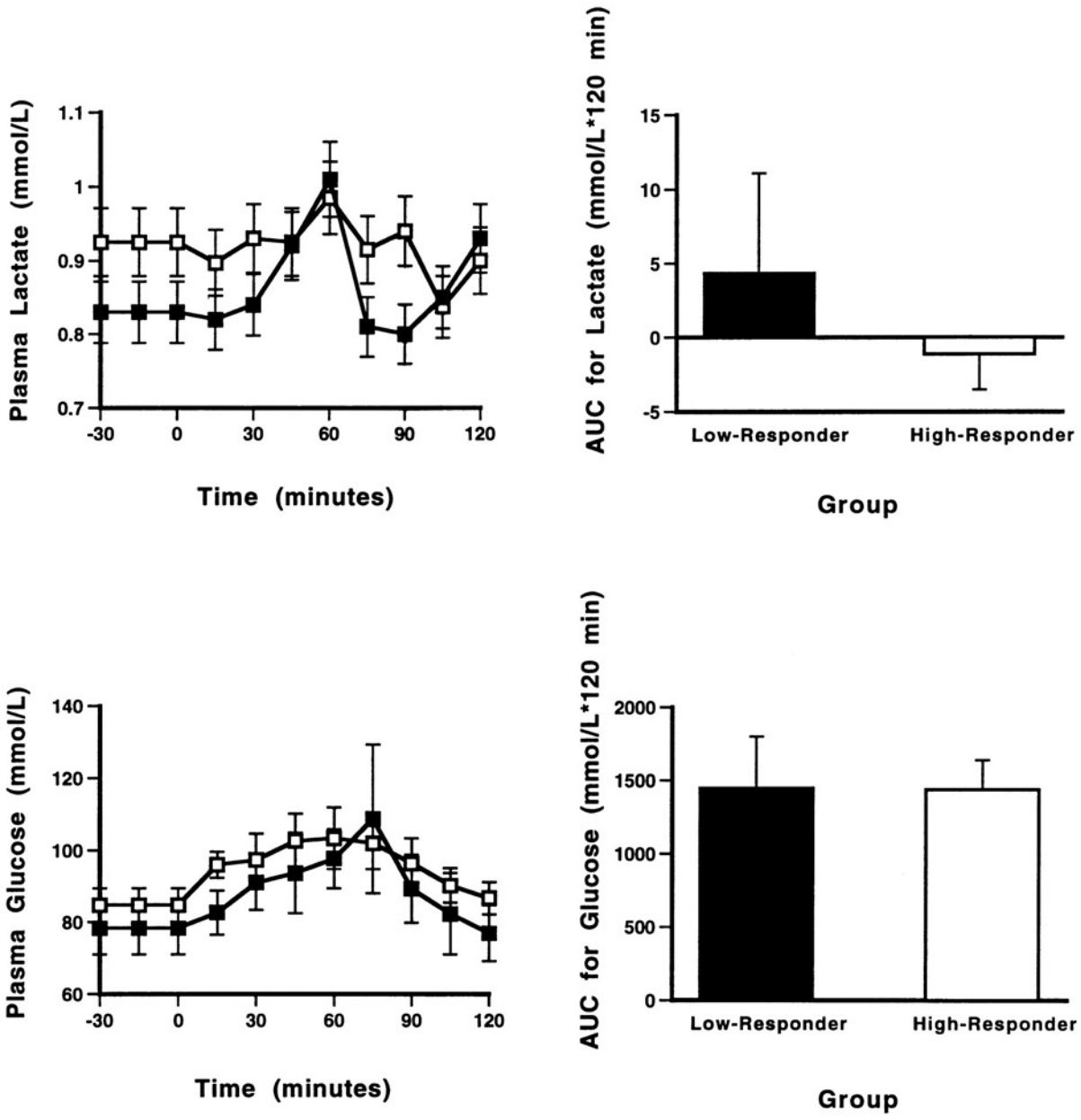


Figure 25. Exercise-induced changes in plasma concentrations of lactate (upper panel) and glucose (lower panel) in low-responders (closed squares) and high-responders (open squares) recieving 1 mIU/Kg/min AVP infusion. Integrated areas under curve (AUC) for plasma lactate (upper right) and glucose (lower right) over the entire infusion time for low-responders (solid bars) and high-responders (open bars).

III. *In vitro* Studies

A. Cell Culture

Pituitary cell culture studies were carried out to investigate whether lactate directly regulates corticotroph secretion either alone or in conjunction with established physiologic regulators. The effects of lactate alone and in combination with CRH, norepinephrine, epinephrine or AVP on the release of β-endorphin by cultured AtT-20 D16 cells and dispersed primary cultures of anterior pituitary were investigated. Numerous *in vitro* studies have shown that β-endorphin is released from AtT-20 cells as well as dispersed rat anterior pituitary cells in a 1:1 ratio with ACTH. Therefore, we utilized our lab's established β-endorphin assay to assess corticotroph responses.

1. CRH and Lactate Dose Response in AtT-20 Cells

Several studies were performed to determine the effects of lactate and CRH on basal release of β-endorphin from AtT-20 cell cultures, representative findings are shown in Figure 26. AtT-20 cells exhibited a spontaneous release of basal β-endorphin of 6.7 ± 0.4 pmol/well over the 3 h incubation period. Incubation for 3 h with lactate at physiologic concentrations did not affect the basal release of β-endorphin from the AtT-20 cells. In contrast, CRH stimulated the release of β-endorphin over 3 h from AtT-20 cells in a dose-dependent manner (Figure 26). CRH at a concentration of 10^{-8}, 10^{-7}, and 10^{-6} M increased β-endorphin release 1.5-, 3-, and 6- fold, respectively, over basal concentrations. Lower concentrations of CRH (10^{-10} and 10^{-9}) tended to increase basal release of

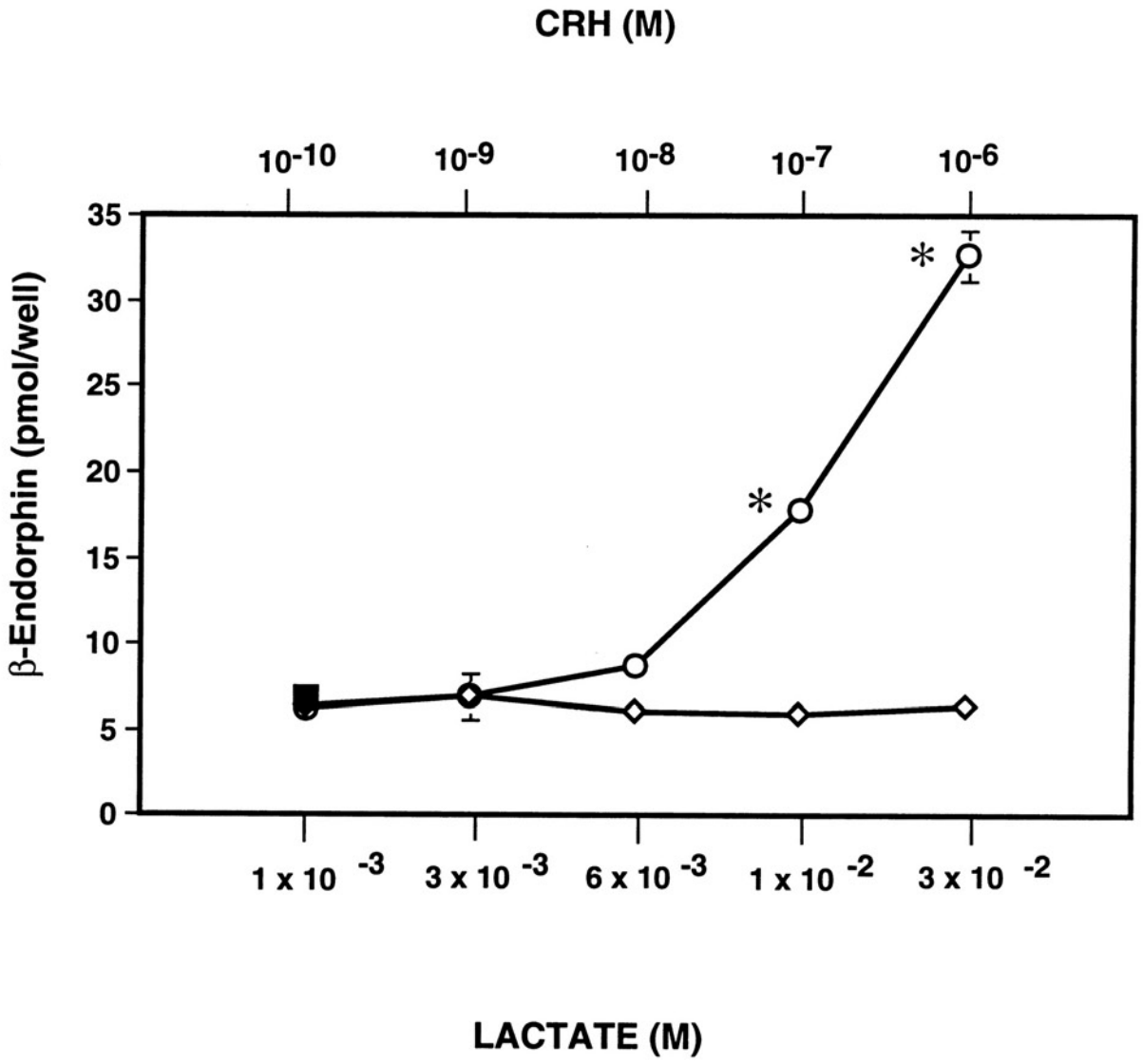


Figure 26. The effects of corticotropin-releasing hormone (CRH) and lactic acid on the spontaneous release of β-endorphin from rat AtT-20 cells. Cultured cells were incubated for 3 H with either CRH of lactate at the indicated concentrations. Basal release is represented by the closed square. CRH treatment is represented by the open circles and solid line and lactate treatment is represented by open diamonds and a solid line. * represents significant difference from basal release ($p < 0.05$).

β-endorphin but these responses were not significant.

2. Acute and Long Term Effects of Lactate on AtT-20 Cells

In addition to the dose response experiments, multiple studies were performed to determine the effects of lactate on both basal release and cellular content of β-endorphin in AtT-20 cells. Physiologic concentrations of lactate had no acute affect on the release or cellular content of β-endorphin over time. Furthermore, both basal release and cellular content of β-endorphin were unaltered by long term incubation in the presence of lactate (3×10^{-2} M) for 1, 2 and 3 days; (data not shown).

3. Secretory Effects of Lactate and Secretogogues, in Combination, on AtT-20 Cells

a. CRH and Lactate

The effects of physiologic concentrations of lactate in combination with CRH on the spontaneous release of β-endorphin by AtT-20 cells was extensively investigated. The data presented in Figure 27 summarize the overall findings of these experiments. Incubation for 3 h with D- or L-lactate (3×10^{-2} M) alone, and in combination with increasing doses of CRH (10^{-11}, 10^{-10}, and 10^{-9} M) had no affect on β-endorphin release singly and was unable to potentiate the dose-response of CRH on β-endorphin release. At the highest dose (10^{-9} M) CRH alone significantly ($p<0.05$) increased β-endorphin release form AtT-20 cells 5-fold.

b. Norepinephrine and Lactate

Similar experiments were conducted to determine the effect of physiologic

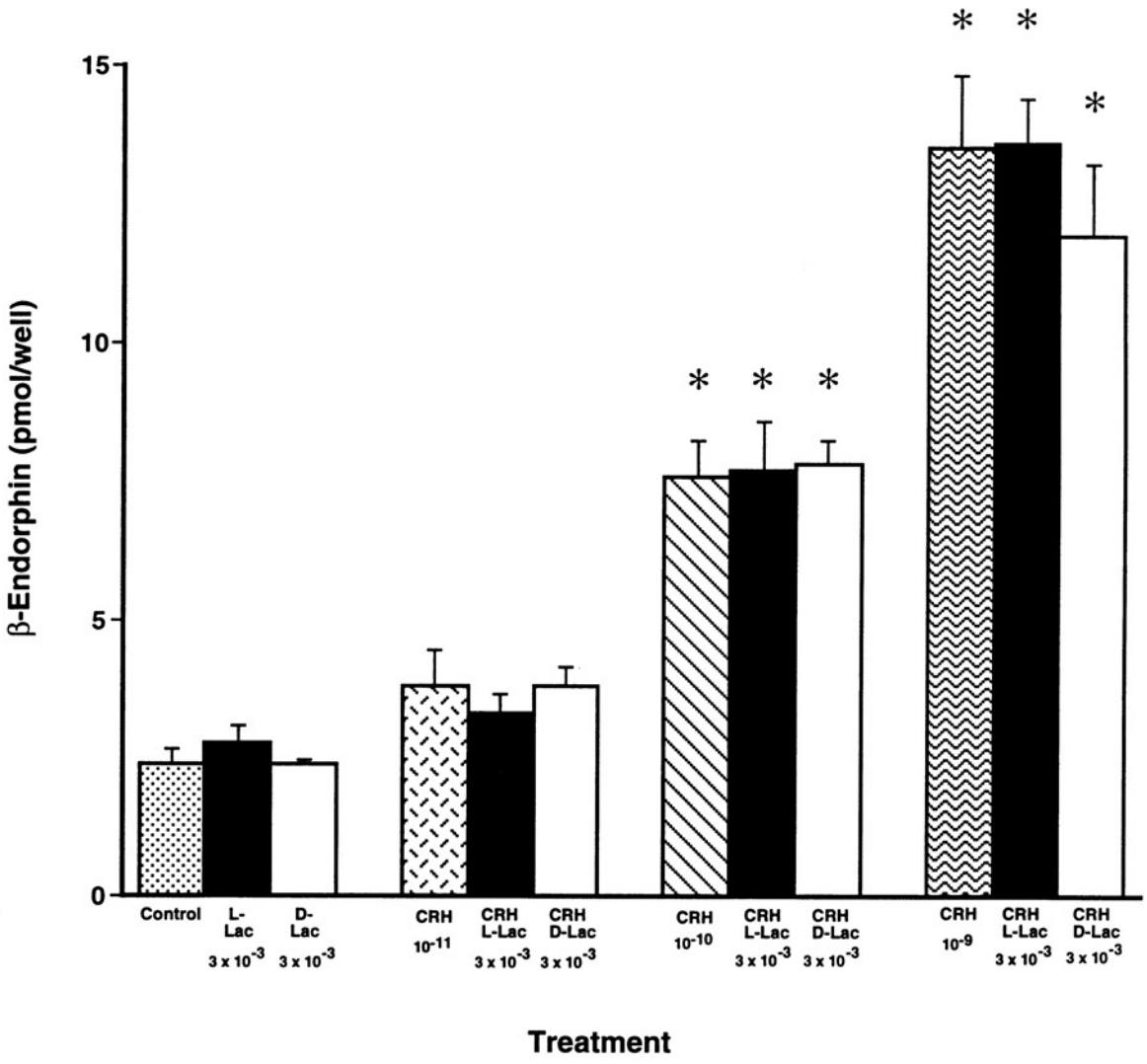


Figure 27. The effects of corticotropin-releasing hormone (CRH) and lactic acid on the spontaneous release of β-endorphin from rat AtT-20 cells. Cultured cells were incubated for 3 h with either CRH, lactate (D and L) or CRH and lactate combined at the indicated molar concentrations. Basal release is represented by the control bar. All other treatments are as indicated. * represents significant difference from control ($p < 0.05$).

concentrations of lactate in combination with norepinephrine on the spontaneous release of β-endorphin by AtT-20 cells (Figure 28). AtT-20 cells were treated with lactate (1×10^{-2} M) alone and in combination with increasing doses of norepinephrine, a known rat β-endorphin releasing factor, for 3 h. Again, lactate failed to alter basal release of β-endorphin by AtT-20 cells (7.4 ± 0.7 pmol/well basal vs $5.9 \pm .13$ pmol/well for lactate), whereas norepinephrine stimulated β-endorphin release in a dose-related manner. Norepinephrine at concentrations of 10^{-6} M and 10^{-5} M significantly ($p<0.05$) increased β-endorphin release two and three times over basal levels, respectively. Lower concentrations of norepinephrine (10^{-8} and 10^{-7} M), however, did not stimulate β-endorphin release. As seen with lactate in combination with CRH, lactate did not alter the β-endorphin response to norepinephrine. Rather, lactate treatment tended to inhibit the norepinephrine-induced release of β-endorphin by AtT-20 cells. This tendency for inhibition by lactate of norepinephrine stimulated β-endorphin release was not significant over several studies ($p<0.05$).

In addition, several related studies were conducted to determine the effects of lactate in combination with other corticotroph releasing factors on β-endorphin release by AtT-20 cells. Both epinephrine and AVP produced significant dose dependent increases in β-endorphin release by AtT-20 cells. However, lactate did not potentiate the β-endorphin response induced by either secretogogue.

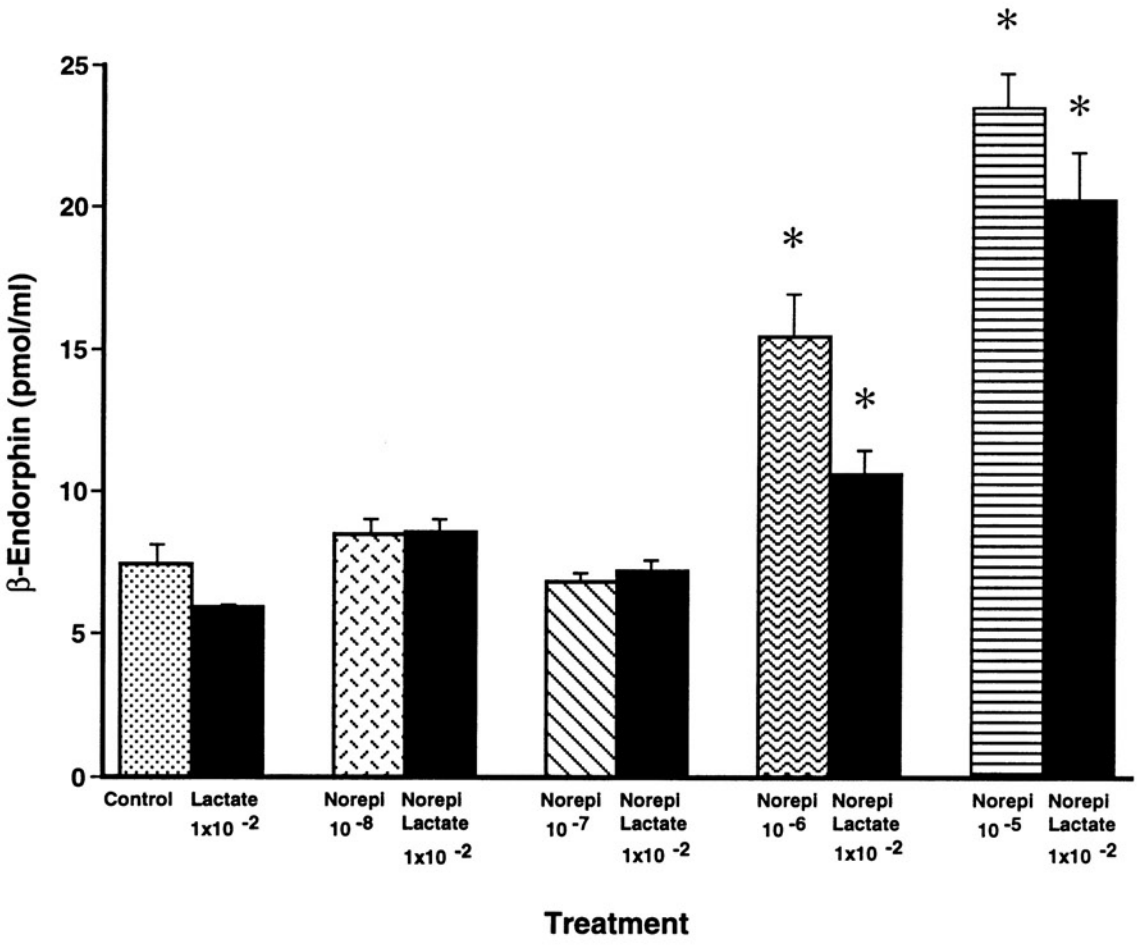


Figure 28. The effects of norepinephrine and lactic acid on the spontaneous release of β-endorphin from rat AtT-20 cells. Cultured cells were incubated for 3 h with either norepinephrine, lactate or norepinephrine and lactate combined at the indicated molar concentrations. Basal release is represented by the control bar. All other treatments are indicated. * represents significant difference from control ($p < 0.05$)

4. Secretory Effects of Lactate and Secretogogues, in Combination, on Anterior Lobe Primary Culture

The effects of physiologic concentrations of lactate alone and in combination with CRH, norepinephrine, epinephrine, or AVP on the spontaneous release of β-endorphin from anterior lobe (AL) primary cell cultures were extensively studied.

a. Norepinephrine and Lactate

Figures 29 and 30 represent the overall findings of these experiments. AL primary culture cells were treated with lactate (5×10^{-3} M) alone and in combination with increasing doses of norepinephrine for 3 h (Figure 29). Lactate did not significantly alter basal release of β-endorphin from AL cells (1.0 ± 0.1 pmol/well basal vs 0.7 ± 0.1 pmol/well for lactate), whereas norepinephrine at concentrations of 10^{-7} and 10^{-6} M significantly ($p<0.05$) increased β-endorphin release two and three times over basal levels, respectively. A lower concentration of norepinephrine (10^{-7} M), however, failed to increase β-endorphin release above basal levels. Similar to the combined lactate/CRH response on β-endorphin release in AtT-20 cells, lactate did not potentiate the release of β-endorphin when combined with increasing doses of norepinephrine. Combined treatment of lactate and norepinephrine (10^{-8} and 10^{-7} M) tended to inhibit the release of β-endorphin from AL cells as compared to the response produced by norepinephrine alone. At the highest dose of norepinephrine (10^{-6}), lactate tended to enhance the β-endorphin response from AL cells. These tendencies,

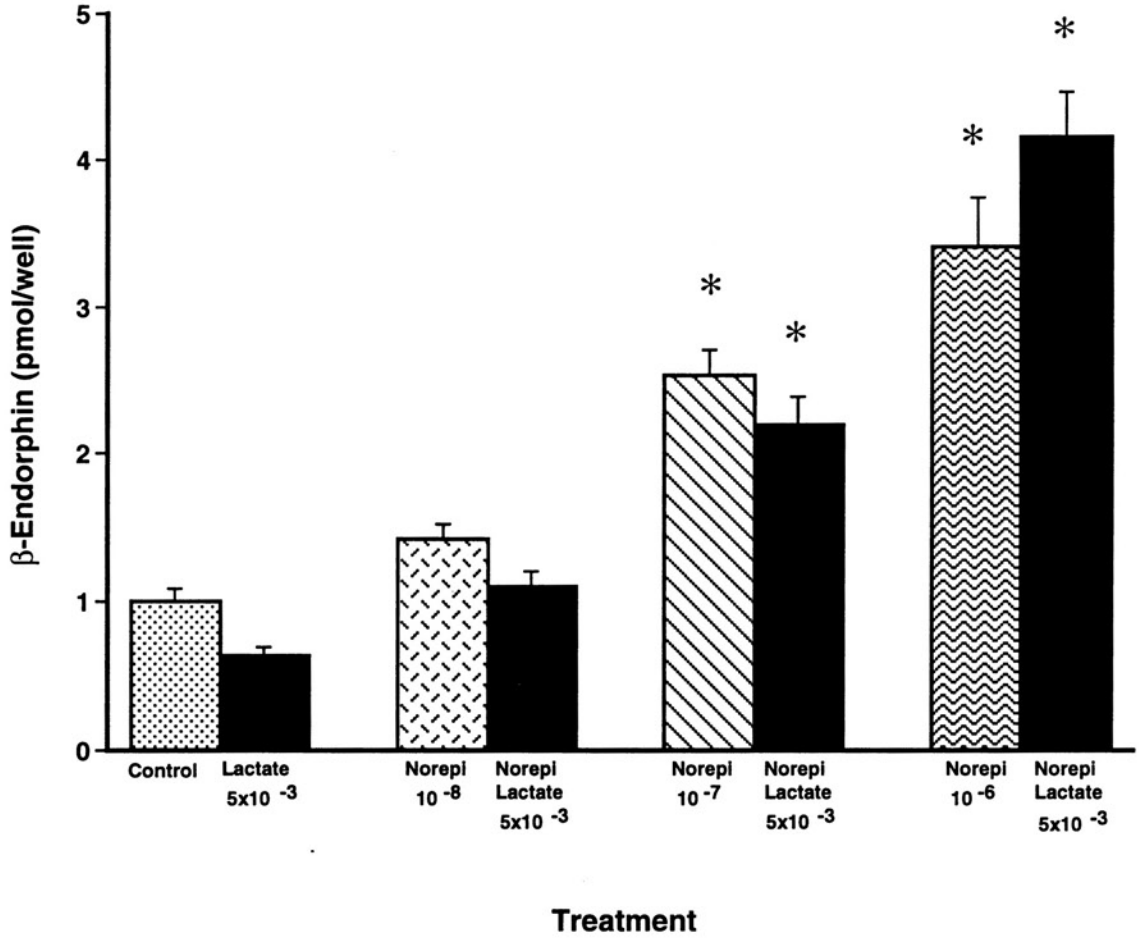


Figure 29. The effects of norepinephrine and lactic acid on the spontaneous release of β-endorphin by rat primary cultures of rat anterior lobe cells. Cultured cells were incubated for 3 h with either norepinephrine, lactate or lactate and norepinephrine combined at the indicated molar concentrations. Basal release is represented by the control bar. All other treatments are as indicated. * represents significant difference from control ($p < 0.05$).

were shown to be insignificant over several studies. Similar to the experiments involving the AtT-20 cells, lactate (3×10^{-2} M) alone or in combination with increasing doses of CRH, epinephrine or AVP failed to influence basal release or potentiate the stimulated β-endorphin release from primary cultures of AL cells (data not shown).

b. "Cocktail" and Lactate

The effect of physiologic concentrations of lactate alone and in combination with a solution or "cocktail" of CRH (10^{-10} M), norepinephrine (10^{-7} M), epinephrine (10^{-6} M), and AVP (10^{-10} M) on the spontaneous release of β-endorphin in AL primary culture cells is shown in Figure 30. Primary cultures of AL cells were treated with lactate (0.5×10^{-3} M and 5×10^{-3} M) alone and in combination with 1/2 dose of "cocktail" and full "cocktail" for 3 h. Again, lactate had no direct effect on basal release of β-endorphin by AL cells. The "cocktail" stimulated the release of β-endorphin by AL in a dose-related manner. 1/2 "cocktail" and full "cocktail" significantly ($p<0.05$) increased β-endorphin release 2 and 2.5 fold, respectively, over basal levels. However, lactate failed to significantly potentiate the release of β-endorphin when combined with increasing doses of "cocktail". Combined treatment of lactate (5×10^{-3} M) and "cocktail" tended to inhibit the release of β-endorphin by AL cells as compared to the response produced by cocktail alone or combined with lactate (0.5×10^{-3} M).

B. Rat Hypothalamic Explant

An investigation was performed to determine the possible effects of

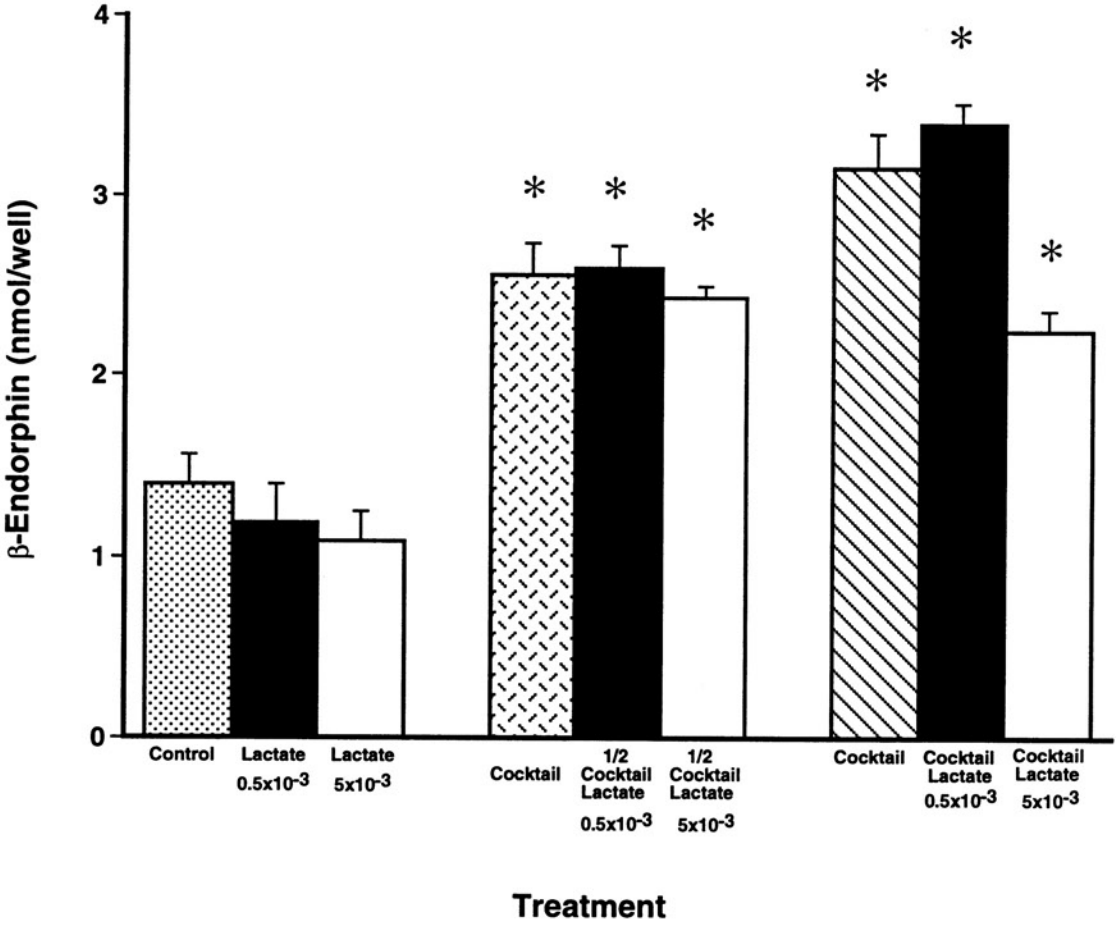


Figure 30. The effects of "cocktail", a combined treatment media (corticotropin-releasing hormone, arginine vasopressin, norepinephrine and epinephrine) and lactic acid on the spontaneous release of β-endorphine from primary cultures of rat anterior lobe cells. Cultured cells were incubated for 3 h with either "cocktail", lactate or "cocktail" and lactate combined at the indicated molar concentrations. Basal release is represented by the control bar. All other treatments are as indicated. * represents significant difference from control ($p < 0.05$).

lactate on the release of CRH by rat hypothalamic explants. As shown in Figure 31, hypothalamic explants exhibited a spontaneous release of basal CRH of 14.3 ± 0.5 pmol/100 μL over the 30 min incubation period. Incubation for 30 min with lactate at physiologic concentrations significantly decreased basal release of CRH by hypothalamic cells (Figure 31) ($p<0.05$). In contrast, cellular depolarization by KCl (60 mM) stimulated the release of CRH two-fold over basal levels ($p<0.05$).

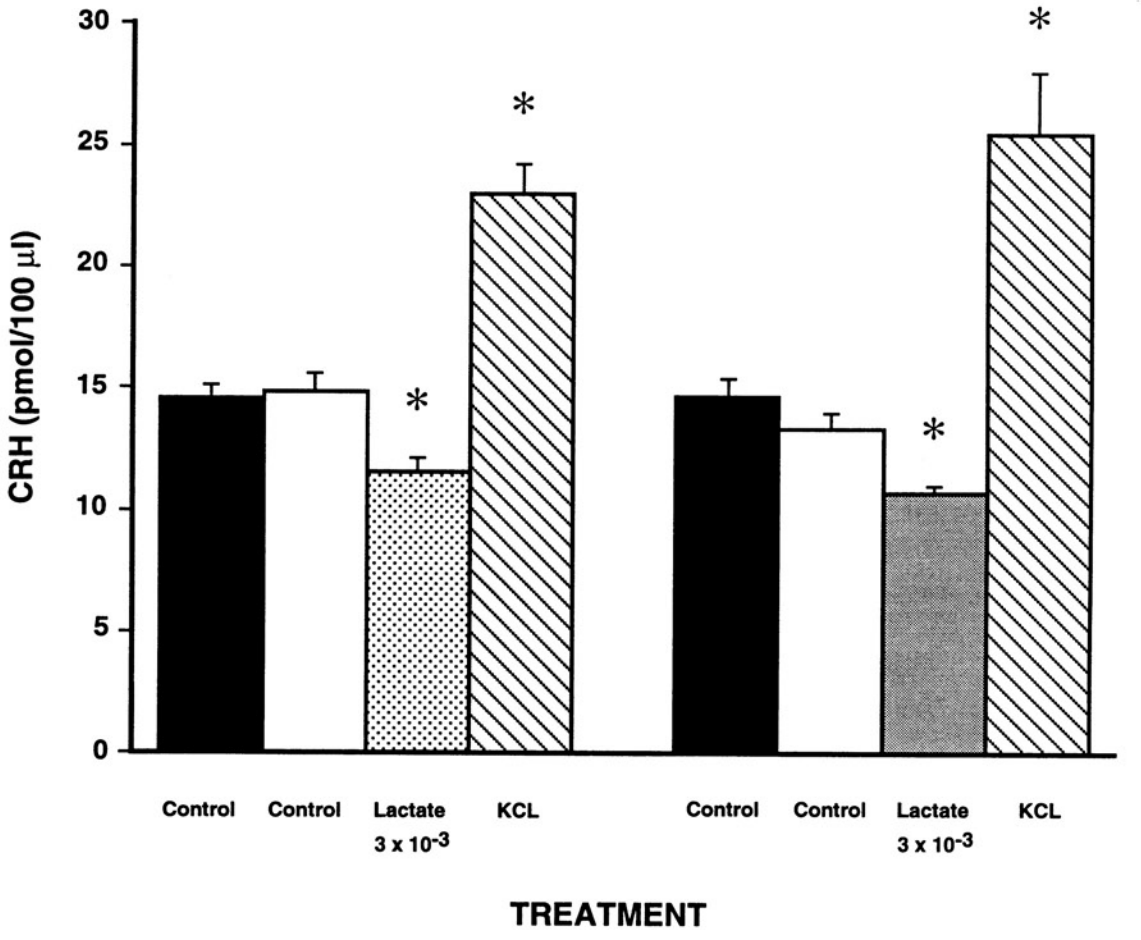


Figure 31. The effects of lactic acid and 60 mM KCL on the release of corticotropin-releasing hormone (CRH) from rat hypothalamic explants. Hypothalami were incubated for 30 min in four consecutive well passages. Both sets of hypothalami were passed through corresponding control wells, high or low lactate treatments then KCL at the indicated molar concentrations. Basal release is represented by the control bar. All other treatments are as indicated. * represents significant difference from control ($p < 0.05$).

DISCUSSION

The results of this project show that normal males can be divided into two groups, high- and low-responders, based upon the criterion of a significant ACTH response above baseline concentrations during high-intensity exercise and 4 mg DEX pretreatment. In addition to exhibiting pronounced resistance to the negative feedback actions of DEX during exercise, high-responders exhibited a markedly enhanced exercise-induced ACTH release with placebo as compared to low-responders. High-responders also exhibited clear differences in the magnitude of other endocrine and metabolic responses to exercise and DEX. These included increases in plasma cortisol, AVP, lactate and glucose, as well as increases in heart rate. The research was extended to show that, as compared to low-responders, high-responders exhibit decreased GH responses to exercise and DEX suppression, and enhanced ACTH responses to AVP stimulation. Furthermore, high-responders had lower anaerobic thresholds and tended to have higher scores on the Spielberger Trait Anxiety scale as compared to low-responders. Together, these findings are interpreted to show that the fundamental physiologic mechanisms involved in regulating HPA function during stress are different in these two groups of normal male subjects. Quite possibly, this hyperresponsive neuroendocrine, metabolic and behavioral profile exhibited by high-responders may be predictive of a predisposition toward disorders involving HPA dysregulation. If so, the findings have implications for

long term health.

Phase I Human Studies

In phase I of this project when 4 mg of DEX were administered 4 h prior to exercise, a differential ACTH response was observed in a group of 11 normal male subjects. Specifically, four of the 11 individuals exhibited pronounced resistance to the negative feedback actions of DEX, whereas the remaining seven exhibited full suppression following DEX. Moreover, the four resistant subjects exhibited a markedly enhanced exercise-induced ACTH release with placebo, as compared to the other seven, and were designated as high-responders; the others were classified as low-responders. In both groups, relative exercise intensity and plasma concentrations of DEX were equivalent throughout the protocol. Clear differences between the groups were also evident for all other endocrine and metabolic responses to exercise examined here; plasma cortisol, AVP, lactate and glucose, as well as heart rate (Petrides et al., 1994). This preliminary study showed that the range of normal endocrine and metabolic responses to exercise was wide after both placebo and DEX pretreatments and clearly reflected a differential sensitivity for HPA activation and suppression by exogenous glucocorticoids.

In the initial study, it appeared that a differential sensitivity to negative feedback by glucocorticoids was not the sole mechanism underlying the different neuroendocrine and metabolic responses observed. During DEX suppression, basal concentrations of ACTH and cortisol in the two groups were equivalent

rather than elevated in the high-responders, an indication that the resting set points for high dose glucocorticoid negative feedback were similar in both groups. During stimulated ACTH release, however, the inhibitory actions of glucocorticoids were distinctly different. Thus, these findings suggest that additional neuroendocrine and metabolic regulators released during exercise mediate the glucocorticoid resistance seen in high-responders.

The finding that AVP responses to exercise were significantly greater in high-responders as compared to low-responders under both placebo and DEX conditions is intriguing. AVP is known to directly stimulate the release of ACTH from anterior pituitary corticotrophs *in vitro* (Antoni, 1993; Carvallo and Aguilera, 1989; Childs and Unabia, 1990; Fleischer and Vale, 1968; Giguere and Labrie, 1982; Kjaer, 1993) and, AVP in combination with CRH, but not singly, can overcome DEX-induced inhibition of ACTH release (Bilezikjian et al., 1987b; Bilezikjian and Vale, 1987a; Childs and Unabia, 1990). Moreover, in humans, infusions of AVP in combination with CRH enabled escape of ACTH release during glucocorticoid suppression (Von Bardeleben et al., 1985). Thus, enhanced AVP release during exercise in the high-responders may be responsible for, at least in part, the increased resistance to glucocorticoid suppression.

The differential plasma AVP response to exercise suggests that a fundamental difference exists in the regulation of hypothalamic mechanisms that operate in the two subsets of normal individuals studied here. We believe that

the origin of these differences between low- and high-responders resides within the central nervous system (CNS). Consistent with this hypothesis, it was observed that exercise heart rates in high-responders were significantly elevated above rates measured in low-responders. Whether this difference is due to augmented sympathetic drive or attenuated vagal tone has yet to be determined.

The initial investigation also revealed a high proportion of glucocorticoid resistance during exercise, particularly given the high dose of DEX used. A considerable number of studies have demonstrated that a dose as low as 0.30 mg of DEX, less than 8% of that used here, substantially inhibits both basal and stimulated HPA function in humans (Hargreaves et al., 1987; Hohnloser et al., 1989; Kemppainen et al., 1990; Waltman et al., 1991). Glucocorticoid resistance to a standard DST using 1 mg of DEX is commonly found in patients suffering from depression, melancholia and several related psychiatric disorders (Arana et al., 1985; Carroll et al., 1981; Gold et al., 1988b; Gold et al., 1988c). Moreover, between 5-25% of all normal individuals exhibit nonsuppression to a standard DST. A plasma cortisol concentration of less than 138 nmol/L is the established criterion for normal HPA suppression by 1 mg of DEX administration (Nugent et al., 1965; Sherman et al., 1984). Thus, differential sensitivity to DEX suppression has been previously reported in normal males following the administration of 1 mg of DEX at rest. In this investigation however, 36% of normal adult males demonstrated glucocorticoid resistance when exercise-induced activation of the HPA axis was suppressed with 4 mg of DEX. Because factors contributing to

glucocorticoid resistance appear to be different during physical exercise as compared to rest, it is likely that our frequency of nonsuppression would have been higher following the administration of 1 mg of DEX.

In summary, the results from phase I indicate that differential sensitivity of the HPA axis to glucocorticoid suppression is a normal feature of human physiology and that the frequency of glucocorticoid resistance among the normal population is higher when exercise is used as a delineating criterion. In addition, it appears likely that centrally mediated AVP release contributes to glucocorticoid resistance. This preliminary study demonstrates that exercise may be useful in defining other physiologic responses (neural, endocrine, and immune) that differentiate subsets of people within the normal population.

Phase II Human Studies

Phase II of this project confirmed and extended our previous findings of differential neuroendocrine and metabolic responses to exercise with and without DEX suppression in normal males. Phase II was conducted on a new group of normal males and utilized the identical exercise protocol as in phase I. In agreement with our previous study, we found that normal male individuals differ in their neuroendocrine and metabolic responses to exercise and glucocorticoid suppression. Furthermore, differences were also evident in the ACTH response to exogenous AVP administered during resting conditions. Interestingly, high-responders appeared more sensitive to hydrocortisone negative feedback inhibition during exercise as compared to DEX while the same

individuals scored higher when evaluated for trait anxiety.

Specifically, in phase II of this project, healthy male controls were divided into two groups based on post-DEX plasma ACTH responses to an estimated exercise intensity of 90% $\dot{V}O_{2max}$; those showing a significant plasma ACTH response during exercise despite pretreatment with DEX (high-responders), and those whose plasma ACTH responses to exercise were fully suppressed by DEX (low-responders). The segregation of healthy controls into high and low ACTH responders during post-DEX exercise at an estimated 90% $\dot{V}O_{2max}$ was associated with the following profile of significant differences in physiological and behavioral parameters: (1) high-responders showed a significantly higher plasma ACTH response to an exercise intensity of 90% $\dot{V}O_{2max}$ following placebo and DEX pretreatments; (2) high-responders showed significantly higher plasma cortisol and AVP responses during exercise following placebo administration; (3) high-responders showed a much greater DEX- and hydrocortisone-induced augmentation of plasma AVP responses during exercise; (4) high-responders failed to show the expected significant DEX or hydrocortisone-induced augmentation of both basal and stimulated growth hormone secretion; (5) high-responders showed significantly higher plasma ACTH responses during the sustained infusion of AVP; (6) high-responders showed a significantly lower anaerobic threshold; and (7) high-responders tended to have higher ratings on the Spielberger Trait Anxiety scale.

The segregation of healthy controls into high- and low-responders after

the screen exercise test, on the basis of post-DEX plasma ACTH responses to estimated 90% $\dot{V}O_{2max}$, does not appear to be the result of differential responsiveness to acute novel stress. The plasma ACTH responses of both high- and low-responders to the screen test and DEX pretreatment were not significantly different from their response when to the experimental 90% $\dot{V}O_{2max}$ test under DEX. Specifically, six of the original seven high-responders showed a significant plasma ACTH response to the experimental 90% $\dot{V}O_{2max}$ test, while only one of eight of the original low-responders showed a plasma ACTH to the experimental 90% $\dot{V}O_{2max}$ test after DEX pretreatment. Furthermore, a significantly higher plasma ACTH response to both exercise and AVP administration was seen in the high-responders despite the fact that these two procedures were carried out in each subject at least six months apart. In addition, these differences between high- and low-responders were not due to differences in drug metabolism since plasma concentrations of hydrocortisone were equivalent for all subjects.

It is well known that exercise produces an intensity-dependent activation of the HPA axis (Deuster et al., 1989; Farrell et al., 1983; Luger et al., 1988; Wade and Claybaugh, 1980). Although the significantly different exercise-intensities of the screen test could possibly explain the differential ACTH response, both groups exercised at similar exercise-intensities during the experimental test sessions. These 90% $\dot{V}O_{2max}$ tests continued to elicit differential neuroendocrine and metabolic responses. In addition, multiple regression analysis that

controlled for differences in relative exercise intensities (% of maximal oxygen uptake) revealed that exercise-induced peak and AUC AVP responses were significantly different for high-responders as compared to low-responders ($p < 0.05$). This result supports the hypothesis that high-responders have inherently exaggerated neuroendocrine responses to exercise, rather than their simply having reached a higher relative exercise intensity, which could be capable of triggering a greater neuroendocrine response.

In contrast to significant differences between high- and low-responders in the post-DEX plasma ACTH responses to exercise, high- and low-responders showed similar post-hydrocortisone plasma ACTH responses to 90% $\dot{V}O_{2max}$. Similarly, while 6/7 high-responders escaped DEX suppression during experimental 90% $\dot{V}O_{2max}$, only 3/7 high-responders escaped suppression by hydrocortisone. Moreover, the three high-responders who escaped suppression by hydrocortisone showed considerably lower peak ACTH responses after hydrocortisone pretreatment than after DEX. This finding suggests that *differential* receptor mechanisms may be controlling negative feedback during stress within this subset of individuals. It is possible that the mineralocorticoid receptor (MR) is upregulated in this group whereas the glucocorticoid receptor (GR) could be down-regulated or otherwise functionally impaired.

Despite the apparent specific resistance of the HPA axis in high-responders to type II glucocorticoid agonists, high-responders, however, showed exaggerated plasma AVP and deficient GH responses after both DEX and

hydrocortisone pretreatment. In addition, there were no differences in basal ACTH and cortisol plasma concentrations between high- and low-responders after DEX pretreatment. Furthermore, all high- and low-responders showed normal suppression of plasma cortisol concentrations following a standard 1 mg DST. Hence, the present differentiation of high- and low-responders on the basis of post-DEX exercise-induced cortisol responses would not have emerged on the basis of standard endocrine screening. This suggests that the dosage of DEX used in this project to determine basal cortisol suppression may have not been adequate to detect subtle basal differences in glucocorticoid negative feedback regulation. Quite possibly a smaller dose of DEX (e.g. 0.5 mg), such as that used to differentiate glucocorticoid suppression of basal cortisol concentrations between post traumatic stress disorder patients and normal control (Yehuda et al., 1993) may reveal differential glucocorticoid resistance in low- and high-responders under resting conditions. Or simply, differences in basal glucocorticoid suppression may not exist between high- and low-responders. In summary, the results from phase II of this project further suggest that multiple factors contribute to the differential neuroendocrine and metabolic responses elicited by low- and high-responders.

In vitro Studies

The role which metabolic products serve in regulating HPA activation remains unclear. Several recent reports indicate that lactic acid, a principal product of anaerobic metabolism, may modulate the magnitude of HPA

activation (Hollander et al., 1989; Pitts and McClure, 1967). Our lab has shown that the rise in plasma lactate during exercise is strongly correlated with the rise in plasma ACTH and cortisol. Results of this dissertation project have shown that exercise-induced increases in plasma lactate also are strongly correlated to exercise-induced increases in plasma AVP. Therefore, in vitro studies were performed to determine if lactate directly stimulates release or potentiates the actions of other corticotroph secretogogues. Additionally, experiments were conducted to determine if lactate directly stimulates the release of hypothalamic CRH. The overall findings of these in vitro experiments showed that physiologic concentrations of lactic acid did not directly stimulate pituitary function in either the rat AtT-20 anterior pituitary cell line or rat anterior lobe primary cell culture. Secondly, lactate did not potentiate the effects of CRH, norepinephrine, epinephrine or AVP and did not alter DEX- induced inhibition of pituitary function. Lastly, lactate did not stimulate the release of CRH from rat hypothalamic explant culture. Thus, these in vitro findings demonstrated convincingly that lactate's actions on HPA function are not at or above the level of pituitary at least in rats, the species we studied. Such regulation may involve activation of higher brain centers. Moreover, it is a possible that this particular model is not appropriate for the question given. In other words, in vitro conditions are not comparable or do not mimic exercise-induced activation of the HPA axis. Whether similar findings would have been observed in tissues derived from animals exercised prior to sacrifice in unknown.

Global Discussion of Dissertation Findings

In phase I of this project, we showed that 4/11 healthy controls escaped high dose DEX suppression of plasma ACTH and cortisol responses to exercise at 90% $V0_{2max}$. Similarly, we also noted that each of these high-responders showed a significant plasma AVP response during exercise. Utilizing the identical exercise paradigm, in phase II, we replicated these findings in a larger number of subjects and extended them to include pituitary-adrenal responses to continuous AVP infusion, indices of basal and exercise-induced GH secretion and assessment of trait anxiety.

It is of interest that high-intensity exercise induced a significantly greater plasma AVP response in high- as compared to low-responders ($p < 0.05$). As noted in the discussion of phase I of this project, it is well established that AVP, while a relatively weak ACTH secretogogue alone, produces a profound enhancement of CRH-induced ACTH release which is resistant to inhibition by DEX (Antoni et al., 1983; Debold et al., 1985; Giguere and Labrie, 1982; Gillies et al., 1982; Kjaer, 1993). Therefore, it appears likely that the enhanced AVP response exhibited by high-responders, in part, mediates their differential exercise-induced HPA activation. However, the extent to which a heightened AVP response during exercise contributes to enhanced, DEX-resistant pituitary-adrenal responses remains undetermined. We believe that an important aspect to better understand this mechanism involves defining potential roles of parvocellular and magnocellular AVP during exercise and DEX

suppression.

It is widely held that pituitary-adrenal function is regulated, in part, by paraventricular parvocellular neurons that secrete AVP into hypophyseal portal blood, whereas magnocellular neurons primarily contribute to peripherally circulating AVP (Kiss, 1988a; Whitnall, 1988; Whitnall, 1990). Thus, one would not expect a significantly greater exercise-induced plasma AVP concentration, such as seen in high-responders, to be functionally involved in affecting pituitary ACTH secretion. However, anatomical data have suggested that AVP released from magnocellular neurons also may play a role in ACTH secretion. Specifically, while fibers originating from paraventricular magnocellular neurons project to the neurohypophysis and release AVP into the systemic circulation, these same fibers pass through the median eminence and hypophyseal stalk and send collaterals to the hypophyseal portal circulation. This additional neuronal pathway thus provides a magnocellular AVP transport mechanism to ACTH secreting corticotrophs (Baertschi, 1980a; Baertschi et al., 1980b; Holmes et al., 1986; Raff et al., 1988; Vandesande and Dierickx, 1975; Whitnall, 1988). Recent studies have shown that rat neurohypophysectomy attenuates stress-induced ACTH release and that electrical stimulation of the rat neural lobe enhances stress-mediated ACTH release (Fagin et al., 1985). Thus, these findings further support a role for magnocellular AVP in regulating corticotroph ACTH secretion.

Moreover, in vitro studies have shown that parvocellular CRH and AVP secretion is highly glucocorticoid suppressible, while, in contrast, the actions of

glucocorticoids on magnocellular AVP release remain unclear. Therefore, our findings of DEX-resistant exercise-induced AVP responses strongly indicate that plasma AVP concentrations serve as a marker for AVP released by magnocellular neurons during exercise. Furthermore, the finding of an augmented AVP response accompanying enhanced pituitary-adrenal activation during exercise also supports the hypothesis that magnocellular AVP contributes to hypothalamic-pituitary function, possibly by way of the hypophyseal portal system.

In phase II of this project, glucocorticoids actually enhanced rather than suppressed plasma AVP responses to exercise at 90% VO2max. This phenomena occurred in both low- and high-responders, but was much more pronounced in the latter group. A similar trend was observed in phase I, although the AVP differences were not found to be statistically significant. In contrast, glucocorticoid administration to normal subjects has been shown to decrease osmotically mediated plasma AVP secretion (Aubry et al., 1965). Thus, the present observation of a DEX facilitation of exercise-induced plasma AVP secretion appears to be mechanistically distinct from the effect of glucocorticoid administration on AVP release in response to osmotic stimuli. Moreover, the present finding of glucocorticoid-mediated facilitation of pituitary-adrenal responsiveness during stress is counter to the generally accepted notion, as described by Munck, et al., (1984); that is, one of the main roles of glucocorticoid secretion during stress is to restrain or counter-regulate the principal effectors of

the stress response. However, there appear to be no other studies investigating the impact of glucocorticoids on plasma AVP secretion in the context of an acute stressor, such as treadmill exercise. The results of this dissertation project suggest that DEX administration exerts a positive rather than a negative effect on exercise-induced AVP release. Therefore, it appears that in the context of an intense physical stressor, a feedback mechanism that ordinarily serves to signal attenuation of HPA function may actually facilitate it.

It should be noted that while DEX pretreatment did not suppress the plasma AVP response to 90% VO2max in both high- and low-responders, DEX nevertheless caused a significant attenuation in the plasma ACTH response. In the low-responder group DEX pretreatment abolished the plasma ACTH response to experimental 90% VO2max, while in high-responders it was attenuated by an average of 75%. Therefore, the present findings do not challenge the accepted notion that parvocellular CRH and AVP play dominant roles in regulating plasma ACTH secretion and are highly glucocorticoid suppressible (Adler et al., 1988; Calogero et al., 1991; Kovacs and Mezey, 1987). Importantly however, while studies in experimental animals show that glucocorticoids exert a potent suppressive effect on paraventricular parvocellular AVP and CRH release (Antoni, 1993; Calogero et al., 1991), results are less clear regarding the impact of glucocorticoid administration on magnocellular AVP secretion (Kiss et al., 1988b). The results of this dissertation project suggest that magnocellular AVP secretion is not suppressible by DEX administration in the

context of high-intensity exercise. Furthermore, it appears that in high-responders, exercise-induced magnocellular AVP secretion is actually enhanced by exogenous glucocorticoids and provides an important mechanism in sustaining ACTH secretion in the face of potent glucocorticoid suppression.

By utilizing an AVP stimulation test, a new and very interesting finding in phase II of this project was revealed. High-responders not only showed enhanced AVP secretion, but also showed significantly enhanced pituitary responses to exogenous administration of AVP. While no differences in plasma AVP concentrations were noted between subjects during the resting infusion of AVP, high-responders exhibited an increased sensitivity to exogenous AVP stimulation. This was evidenced by higher ACTH plasma concentrations during the AVP infusion of AVP in high-responders as compared to those of low-responders. In fact, ACTH responses to a resting AVP stimulation test could be used to distinguish these two subsets of individuals.

The finding of an enhanced ACTH response to AVP infusion coupled with exaggerated ACTH, cortisol and AVP responses to exercise is of interest, and further suggests that AVP mediates stress-induced glucocorticoid resistance. However, it is unclear whether the observed differential AVP regulation mediates or is a product of glucocorticoid resistance. The results of this dissertation project imply that this resistance may be brought about not only by higher concentrations of circulating AVP, but also by an enhanced pituitary sensitivity to the stimulatory actions of AVP on ACTH secretion. This could be

due, in part, to increased CRH secretion or effect, both of which could have resulted from glucocorticoid resistance of the hypothalamus or the pituitary. Interestingly, enhanced AVP stimulation of pituitary ACTH release has been seen in depressed patients presumably due to increased pituitary AVP receptor responsiveness or to hypersecretion of hypothalamic CRH (Gispen-de Weid et al., 1992).

Furthermore, it has been previously postulated that plasma ACTH responses to AVP are in part a reflection of the ambient concentrations of CRH in hypophyseal portal blood (Gold, unpublished data). Specifically, AVP-induced plasma ACTH responses in healthy controls were shown to be higher in the morning as compared to the evening. It has been suggested that basal CRH secretion is highest in the morning and therefore, contributes to this diurnal AVP-induced ACTH response. Paradoxically, the increased ACTH response observed in the morning also occurs in the presence of higher concentrations of plasma glucocorticoids that would be expected to suppress plasma ACTH release. Thus, an elevated plasma ACTH response to AVP in the morning despite higher plasma cortisol concentrations could also reflect the relative insensitivity of AVP-induced ACTH release to glucocorticoid negative feedback.

Experimental animals subjected to chronic or repeated stress have been shown to exhibit increases in the expression and secretion of both AVP and CRH (Bartanusz et al., 1993; de Geoij et al., 1992; de Goeij et al., 1991; Makino et al., 1995b; Makino et al., 1995c). In addition, rats subjected to the stress of repeated

immobilizations or fluid deprivation have demonstrated increases in circulating AVP and an upregulation in expression of the AVP receptor (Aguilera et al., 1994; Kiss and Aguilera, 1993). Furthermore, after repeated stress, rats showed enhanced HPA activation when exposed to a novel stress (Aguilera et al., 1994; Kiss and Aguilera, 1993; Mamalaki et al., 1992). Thus, it is possible that AVP receptors are upregulated in high-responders and that chronic stress may contribute to the enhanced HPA activation exhibited by these individuals during exercise.

Results from other animal studies also suggest an effect of chronic stress on HPA activation. Specifically, investigations have demonstrated maintenance, or even enhancement of stress-induced pituitary-adrenal activation during chronic or repeated stress, despite the presence of hypercortisolism (Dallman, 1993; Makino et al., 1995a; Makino et al., 1995b). Makino et al. (1995a, 1995b) have suggested that repeated glucocorticoid-mediated negative feedback elicits a downregulation of CNS glucocorticoid receptors. Therefore, with a decrease in potential for glucocorticoid negative-feedback, HPA function would be sustained, or even enhanced, during stress. Additionally, they also have postulated that glucocorticoid-mediated downregulation of paraventricular hypothalamic CRH receptors leads to an attenuation of CRH-mediated ultra short-loop negative feedback of CRH release (Makino et al., 1995b; Makino et al., 1995c). Therefore, these findings from investigations in chronic stress suggest a higher basal "stress" level may result in increases in both CRH and AVP tone,

AVP receptor upregulation and glucocorticoid-mediated receptor down-regulation. Together these adaptations may contribute to the mechanism that potentiates HPA function to stress. Ultimately, all of these factors may contribute to the enhanced AVP function as well as the potential AVP-mediated glucocorticoid resistance observed in high-responders.

Previous studies in healthy controls have shown that glucocorticoid administration acutely increases both basal and stimulated growth hormone release (Casanueva et al., 1990; Casanueva et al., 1988; Thakore and Dinan, 1994). The present project documented this phenomena with exercise-induced GH secretion and further showed that GH concentrations were significantly different both basally and during exercise in high-responders as compared to low-responders. Specifically, the high-responders receiving DEX had lower plasma GH concentrations as compared to those of the DEX-treated low-responders. These differences between high- and low-responders were only found with DEX pretreatment. Whether the failure of high-responders to exhibit increased basal and exercise-induced GH secretion after DEX results from a glucocorticoid resistance (type II GR), a subtle suppression of GH release by increased glucocorticoid secretion in the context of chronic stress, or other undefined factors, remains to be determined. In conjunction with the AVP and HR findings presented here, the GH data support the hypothesis that the origin for the differential neuroendocrine responses seen in high- and low-responders resides within the CNS. Furthermore, the GH findings provide additional

support for our proposal that fundamental hypothalamic mechanisms governing the exercise stress response are different in these two subgroups.

There is a substantial body of evidence that shows that the responsiveness of the HPA axis to stimuli is directly proportional to one's level of perceived anxiety (Cash et al., 1992; Dunn and Berridge, 1990; Gerra et al., 1992; Jones and Mellersh, 1946). Consistent with this hypothesis, we observed that high-responders scored higher on the Spielberger Trait Anxiety Scale as compared to the low-responders ($p < 0.1$), although the means for both groups were within the normal range. This finding is potentially important in that these differences may reflect conscious perceptions measured under basal conditions. Thus, trait anxiety may be linked to augmented hypothalamic drive accompanied by exercise-induced elevated secretions of CRH and AVP. The combined synergistic actions of these two pituitary secretogogues during exercise may contribute to the glucocorticoid resistance seen in the high-responders during exercise.

In addition, this trend of higher scores obtained by high-responders on the Spielberger Trait Anxiety Scale suggests a concordance between hyper-responsiveness of the HPA axis during the stress of exercise and a bias towards basal behavioral arousal. If borne out, these data suggest that although the pathways mediating pituitary-adrenal responses to physical and emotional stressors differ in several respects, there is sufficient overlap to demonstrate concordance between a physical stressor and an index of behavioral arousal.

These data also suggest that graded treadmill exercise may be a useful stimulus to assess the functional integrity of stress-responsive neurohormonal systems in patients whose illnesses are postulated to be associated with abnormalities of the stress response, such as major depression.

An additional finding in phase II of this project was that high-responders had lower anaerobic thresholds compared to those of the low-responders. This finding may provide an additional mechanism for the differential neuroendocrine and metabolic responses observed between high- and low-responders. Individuals tested at identical relative exercise intensities may physiologically or psychologically perceive that exercise intensity as easier or harder depending upon where that workload is in relation to their anaerobic threshold. It is possible that high-responders perceived the exercise intensity as being harder as compared to low-responders. This, in effect, would produce a larger stimulus for HPA activation even though the subjects were exercising at the same relative intensity with respect to maximal oxygen uptake.

The causes or consequences of a lower anaerobic threshold in high-responders are not known. While poorly trained individuals show lower anaerobic thresholds than well trained individuals, each subject in this study fell into the category of moderately trained. Moreover, the level of training achieved by high- and low-responders was virtually identical as evidenced by similar values for maximal oxygen uptake. Genetic differences in muscle fiber type may differentially influence the anaerobic threshold. Individuals with a relatively

larger proportion of type II muscle fibers (glycolytic) than type I muscle fibers (oxidative), would have a lower anaerobic threshold at a similar level of conditioning (Vrbova, 1979). It is also possible that a chronically stressed individual (high basal cortisol and low basal growth hormone) would be metabolically catabolic more frequently than an individual who is consistently relaxed or calm which may also lower that individual's anaerobic threshold.

The method utilized to determine anaerobic threshold during the maximal oxygen uptake test in this dissertation project involved a specific gas exchange method (Davis, 1985). It consisted of determining the point at which an individual had a systematic increase in ventilatory equivalents of oxygen without a concomitant increase in ventilatory equivalents for CO_2 from a plot of gas exchange values versus oxygen uptake. This method may not be as precise as interval determinations of plasma lactate concentrations, in part because of inter-individual variability in gas exchange during the $\dot{V}O_{2max}$ test and the occurrence of rapid and large workload increases. Our determinations for anaerobic threshold were consistently higher compared to those previously reported in the literature (Farrell et al., 1979). However, since there was virtually no overlap in the determined anaerobic thresholds between high- and low-responders utilizing this gas exchange methodology, we feel our findings for anaerobic threshold are at a minimum, accurate representations of the relative difference that exists between these two groups. Therefore, testing an individual at exercise-intensities that are relative to both anaerobic threshold and maximal

oxygen uptake should be given further consideration in future studies.

The results from phase II of this project further support our conclusion from phase I that the origin of these differences between high- and low-responders resides within the CNS. Two metabolic signals that may coordinate centrally driven neuroendocrine responses to exercise are changes in blood glucose concentrations and the accumulation of circulating lactic acid. AVP release is stimulated by hypoglycemia, perhaps through a direct effect of neuroglycopenia in the hypothalamus (Ellis et al., 1990). Also, infusion of AVP has been shown to increase blood glucose, perhaps through neural mechanisms that promote hepatic glycogenolysis (Spruce et al., 1985). Thus, glucose levels may both regulate and be regulated by AVP. These glucose/AVP interactions may explain why high-responders had significantly higher concentrations of blood glucose and AVP during exercise as compared to low-responders.

Plasma lactic acid, a principal metabolic product of anaerobic metabolism was greatly elevated during exercise in both high- and low-responders. As expected, high-responders, whose anaerobic thresholds were lower, showed higher plasma lactate responses at a given exercise intensity as compared to low-responders. Several investigators have suggested that lactate plays a role in HPA activation (Ahlborg et al., 1976; Carraro et al., 1989; Dager et al., 1990; Hollander et al., 1989; Inao et al., 1988; Luger et al., 1987). We have previously reported that plasma lactate responses during exercise correlate positively with plasma ACTH responses in untrained, moderately trained, and highly trained healthy controls.

Through extensive *in vitro* investigations, we have determined that lactate does not act directly on the pituitary to alter basal or stimulated ACTH release (Petrides, unpublished data). It is possible, therefore, that lactate may act centrally to increase the drive for CRH, AVP, and catecholamine release. Importantly, in primates, elevations in blood lactate concentrations are accompanied by parallel increases in CNS lactate concentrations; these findings are consistent with a central role for lactate in neuroendocrine control (Dager et al., 1990). A CNS action for lactate has also been postulated based on studies of anxiety and panic attacks (Hollander et al., 1989). It is well recognized that lactate elicits panic attacks in normal subjects (Pitts and McClure, 1967) and that humans most susceptible to panic attacks exhibit higher concentrations of serum lactate during exercise as compared to those who are less susceptible (Jones and Mellersh, 1946). Thus, the enhanced neuroendocrine responses seen in the high-responder group may have been affected by increased plasma lactic acid acting as a stimulus for the release of CRH, AVP, and sympathetic discharge. Moreover, Gold and colleagues (unpublished data) have shown that rat hypothalamic organ cultures show a dose dependent increase in CRH release when incubated with concentrations of lactate that correspond to the levels seen prior to exercise and during exercise in healthy controls. In the present project, however, physiologic concentrations of lactate failed to stimulate CRH release from hypothalamic explant culture. At present, therefore, we feel that the action of lactate upon the hypothalamus remains to be defined. Quite possibly, the

central actions of lactate primarily involve the activation of extrahypothalamic brain centers. Thus, further studies are needed to determine how lactate is involved in regulating the HPA axis.

CONCLUSION

This dissertation project shows that exercise can be used to define physiologic differences that distinguish subsets of people within the normal population. Specifically, this project shows that normal males exhibit differential neuroendocrine and metabolic responses to exercise with or without exogenous glucocorticoid suppression. These differential responses to exercise and glucocorticoid suppression show concordance with HPA responses to exogenous AVP stimulation. Table 6 summarizes key neuroendocrine and metabolic responses to exercise and AVP stimulation observed between high- and low-responders. Furthermore, the subset of individuals that exhibits an enhanced HPA activation to exercise shows a tendency for increased trait anxiety. Ultimately, it may be shown that these response patterns are predictive of one's ability to adapt to physiologic and/or psychologic challenges.

Additionally, an evaluation of neuroendocrine and metabolic responsiveness to exercise may have important implications for long term health. The subgroup that showed significantly greater HPA activation during exercise and AVP infusion also showed significantly decreased basal and exercise-induced GH release that was most apparent during DEX pretreatment. In light of the fact that patients with major depression show exaggerated ACTH and cortisol responses to AVP infusions as well as basal hypercortisolism and resistance to DEX-induced growth hormone secretion, the present findings raise the possibility that this response pattern indicates a predisposition for the

Table 6. Differential Neuroendocrine and Metabolic Responses to Exercise and AVP Stimulation

	Low-responders			High-responders		
	Placebo	DEX	HCO	Placebo	DEX	HCO
ACTH	+++	—	+	+++++	++	+
Cortisol	+++	—	—	+++++	—	—
AVP	+++	+++	+++	+++++	++++++	++++++
Lactate	+++	+++	+++	+++++	+++++	+++++
ACTH Response to AVP Infusion	+			++		

+++ represents average response for low-responders

— represents no response

development of depressive illness or stress-related disorders.

The differential responses to exercise discussed here may be explained on the basis of resistance to type II glucocorticoid agonists. If so, this mechanism would only be operative under conditions of stimulated release since no differences between high- and low-responders were noted in basal concentrations of cortisol or their suppression by exogenous glucocorticoids. Thus, the exact physiological basis for the present findings is more complex than simple negative-feedback control by cortisol. Apparently, this form of glucocorticoid resistance is mediated by additional factors that contribute to differential activation of the HPA axis during strenuous exercise. The present findings strongly indicate that two important factors contributing to glucocorticoid resistant exercise-induced ACTH release are augmented AVP release and enhanced sensitivity to AVP action. It is unclear whether this differential AVP regulation mediates or is a product of glucocorticoid resistance. Therefore, the basis for these phenomena remains speculative.

We believe that the origin for the differential neuroendocrine and metabolic responses observed between high- and low- responders resides within the CNS and is mediated by several factors. In addition to possible differences in glucocorticoid receptor function and alterations in AVP release and actions, differences in behavioral characteristics may also play a role. High-responders showed tendencies for increased trait anxiety as compared to low-responders. This finding suggests the possibility that a greater arousibility leads to enhanced

activation of the stress response during exercise. Interestingly, it has been shown that experimental animals faced with repeated stress show exaggerated HPA responses to a subsequent external stimulus. Since indices of chronic stress were not evaluated in this investigation, the possibility that high-responders develop enhanced HPA activation through the effects of some type of chronic stress should be considered in future studies.

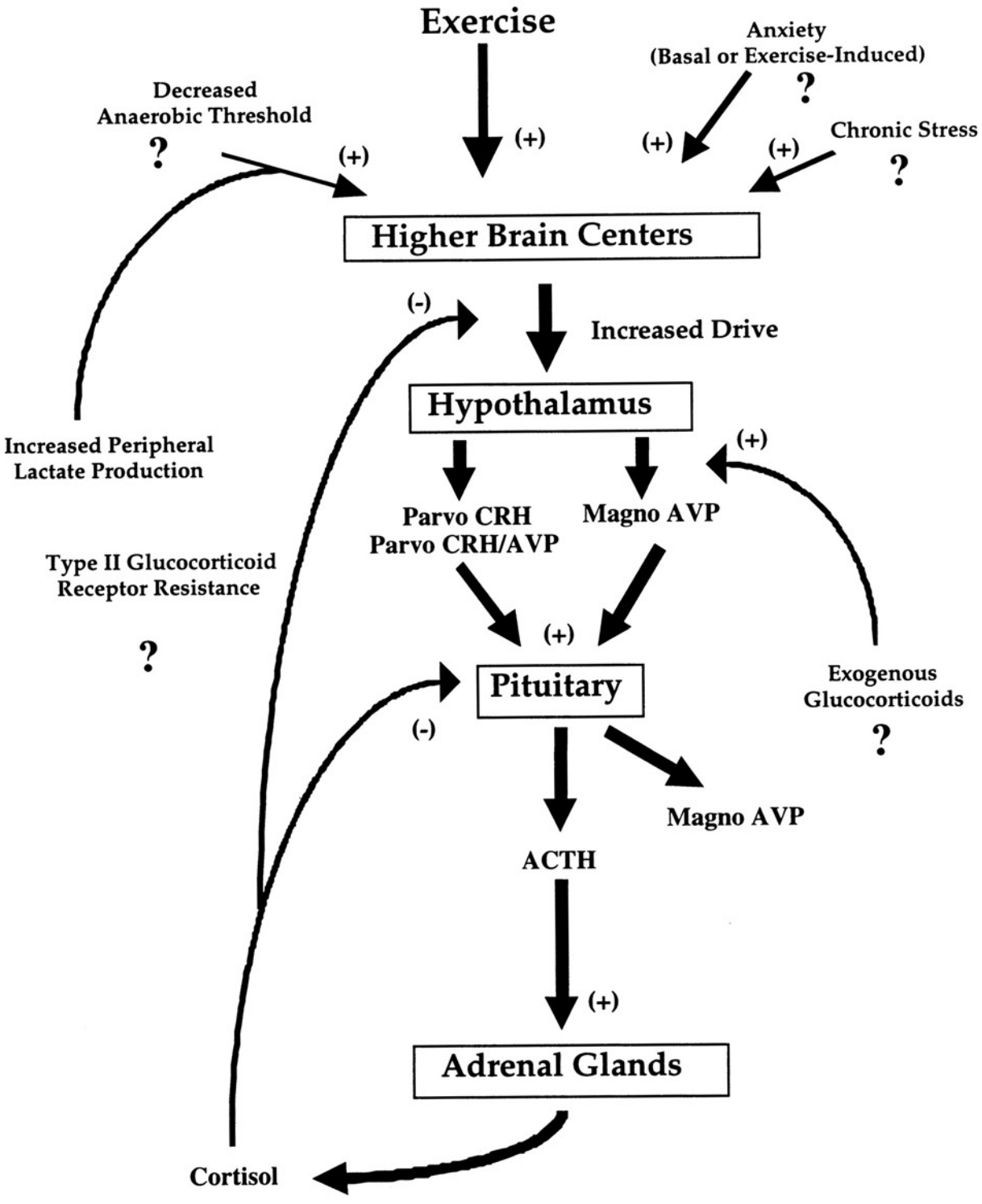
High-responders also exhibited lower anaerobic thresholds and enhanced plasma lactate concentration to a given exercise intensity. These findings may reveal two additional mechanisms that work independently or in combination to produce an exaggerated HPA response to exercise. Our *in vitro* studies showed that physiologic concentrations of lactate did not effect pituitary function or stimulate hypothalamic CRH release. However, lactate may activate higher brain centers to increase the hypothalamic drive for CRH and AVP release. In addition, having a larger difference between the relative percentage of maximal oxygen uptake for anaerobic threshold and exercise intensity may increase individual perceptions of stress, both physical and psychological, and thus produce a greater stress response (Figure 32).

It is felt that the findings of this dissertation project have increased our understanding of the basic mechanisms involved in HPA activation during high-intensity exercise. It appears that AVP release during exercise plays an important role in activating the HPA axis and could possibly be a primary adaptive mechanism for sustaining HPA function in the face of profound

Figure 32. HPA Activation of High-Responders During Exercise

High-responders show resistance to glucocorticoid suppression of exercise-induced HPA activation. In addition, they show enhanced HPA function during strenuous exercise as evidenced by higher plasma levels of AVP, ACTH and cortisol as compared to low-responders. Several mechanisms may contribute to this increased exercise-induced HPA activation. One mechanism could involve decreased negative-feedback regulation of the HPA axis through alterations in Type II glucocorticoid receptor function. Another mechanism could involve an increased drive for CRH and AVP release from parvocellular (parvo) and magnocellular (magno) neurons mediated by increased activation of higher brain centers. Factors that contribute to increased activation may include lactic acid and a lower anaerobic threshold, anxiety, chronic stress and possibly exogenous glucocorticoids stimulating magno AVP release.

High-Responders During Exercise



glucocorticoid suppression. In addition, the alteration in HPA responsiveness seen with exercise may have implications in understanding the mechanisms of HPA dysregulation, such as occurs in individuals with psychological disorders including depression, obsessive-compulsion and anxiety. Accordingly, exercise may prove to be a useful model for studying these conditions as well.

The findings of this dissertation project have raised several questions for future prospective investigations. A longitudinal project should be performed with high- and low-responders to determine the implications of differential HPA function in long term physical and mental health. This evaluation should include assessments of heart disease, hypertension, diabetes, and measures of depression, anxiety, and obsessive-compulsion. The two groups should also be further evaluated for differences in behavioral profiles including a more extensive evaluation of trait arousability and conscious perceptions. The ultimate goal of this project would be to determine the relations that exist between conscious thought and neuroendocrine reactivity to exercise and its overall contribution to health.

A second project could determine whether the differences observed here with exercise are generalized to other physiologic stimuli. For example, mental stress could be utilized to show concordance in reactivity between psychologic and physical stimuli. In addition to better defining neuroendocrine mechanisms involved in the "stress" response, this investigation may ultimately determine whether the CNS mechanisms controlling HPA reactivity are fundamentally

different in these two groups. Importantly, if they are different, can they be altered by behavioral or pharmacological treatments. Ultimately, this investigation may provide a model to explore the effectiveness of various treatments in mediating neuroendocrine reactivity in normal controls as well as in patients with psychological disorders.

Thirdly, a mechanistic evaluation for the differential responses to exercise should be performed. This investigation would, in part, determine the role of glucocorticoids in differential activation of the HPA axis. Specifically, cellular and molecular investigations should be performed to evaluate differences in the function and expression of glucocorticoid receptors in high- and low-responders. These studies could be extended *in vivo* by assessing the differential effects of glucocorticoid antagonism on HPA activation in low- and high-responders. In addition, the administration of AVP during exercise may better define the role of endogenous AVP in differential activation of the HPA axis. Overall, this investigation would clarify the mechanisms involved in differential neuroendocrine response to exercise.

Finally, it is important to determine the role of exercise intensity and different doses of DEX on HPA activation. This last project would evaluate the effects of different exercise intensities with varying doses of DEX on exercise-induced HPA activation. This project is important because it would establish a more specific criterion for defining high- and low-responders and further substantiate the basis of utilizing exercise and DEX suppression in defining

subgroups in the normal population. Importantly, this project would identify individuals who may be at risk for developing long term health problems.

Ultimately, these proposed projects would extend our understanding of exercise-induced HPA activation and establish a model for determining one's individual reactivity. Doing so could provide a mechanism by which physical and psychologic manifestations can be identified before they become detrimental to overall health.

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