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The effects of beta-endorphin on glucose homeostasis and hormonal responses during exercise

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THE EFFECTS OF BETA-ENDORPHIN ON GLUCOSE HOMEOSTASIS AND HORMONAL RESPONSES DURING EXERCISE

by

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Despite the fact that Beta-Endorphin (B-EP) has been shown to cause hyperglycemia at rest, it is not known if it influences glucose homeostasis and the glucoregulatory hormones during exercise. In an attempt to clarify the role of B-EP in glucose regulation and hormonal responses to prolonged dynamic exercise, 72 untrained, male Sprague-Dawley rats were assigned to one of three treatments: a) a control group infused with normal saline, b) a group infused with rat synthetic B-EP (0.05 $mg*kg^{-1}*h^{-1}$ after a 0.05 mg/ kg bolus), and c) a group infused with naloxone (0.4 $mg*kg^{-1}*h^{-1}$ after a 0.8 $mg*kg^{-1}$ bolus). All animals were familiarized with treadmill running for 4 days. On the fifth day they were catheterized in the jugular vein, and allowed 4 days recovery period. All recovered catheterized animals went through a second familiarization running period for 4 days. Finally, animals in each group were infused and killed either at 0 time or after 60, 90 and 120 minutes of running. Mixed venous samples were collected and subsequently analyzed for plasma glucose, lactate, insulin, C-peptide (C-P), glucagon, and B-EP.

A 3*4 ANOVA (treatment by time) revealed that plasma glucose was maintained at significantly higher levels in the B-EP group compared to the other two at 60 (142.2 $\pm$ 7.7 $mg*dl^{-1}$ vs 110.8 $\pm$ 6.4 $mg*dl^{-1}$ in the control group and vs 119.2 $\pm$ 9.2 $mg*dl^{-1}$ in the naloxone group) and 90 minutes (99.8 + 6.8 $mg*dl^{-1}$ vs 81.7 + 4.9 $mg*dl^{-1}$ in the control group and vs 84.1 + 5.9 $mg*dl^{-1}$ in the

naloxone group) of exercise. These differences were accompanied with changes in the responses of the pancreatic hormones between groups. Plasma insulin was significantly lower in the B-EP group at 60 min., while plasma C-P was significantly reduced in the B-EP group compared to the control and naloxone groups at 60 and 90 minutes of exercise. The C-P to insulin molar ratio declined significantly in the B-EP group compared to the other groups after 90 minutes of exercise, suggesting reduced insulin clearance in that group. Plasma glucagon increased significantly in the B-EP group compared to the other two groups at 60 and 90 minutes of exercise. Plasma lactate was significantly elevated in the B-EP group compared to the other two treatments at 60, 90 and 120 minutes of exercise. Plasma B-EP was significantly increased in the B-EP group as compared to the other groups at all times.

These data suggest that B-EP could act to prevent the decline in plasma glucose levels during exercise. It appears that this effect may be secondary to B-EP influence on the pancreatic hormones, insulin and glucagon, since a significant decline of insulin and increase of glucagon was observed with B-EP infusion, especially at 60 and 90 minutes of exercise.

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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CHAPTER I

INTRODUCTION

Statement of the Problem

Maintenance of plasma glucose concentration is critical to survival because it is the predominant metabolic fuel utilized by the central nervous system and red blood cells under most conditions. The central nervous system and red blood cells can not synthesize or store adequate amounts of glucose. Erythrocytes require steady supply of glucose since they lack mitochondria and therefore can not utilize fatty acid fuels. Skeletal muscle and cardiac muscle use fatty acids but they require glucose especially during strenuous activity. Cells can take up glucose by facilitated diffusion and active transport which means that blood glucose concentrations need to be maintained at a certain level. Therefore, due to the crucial role of glucose for life its blood concentration is normally kept within a relatively narrow range, roughly 60 to 150 $mg*dl^{-1}$, despite wide variations in glucose influx and efflux (Cryer, 1989).

Situations where blood glucose fluctuates from that narrow range for prolonged periods of time could be proved to be detrimental. Hypoglycemia can cause profound brain dysfunction, and prolonged, severe hypoglycemia causes brain death. Other

deleterious effects of sustained low blood glucose concentrations include loss of motivation, headache, disturbances in sensory perception, loss of coordination, sweating, involuntary tremors that progress to muscle spasms, convulsions, loss of consciousness, and finally death. However, it is also important that the blood glucose levels not rise too high for several reasons. Glucose exerts a large amount of osmotic pressure in the extracellular fluid. If the glucose concentration rises to excessive values, this can cause considerable cellular dehydration. Excessively high blood glucose concentration causes loss of glucose in the urine. This results in osmotic diuresis by the kidneys, which can deplete the body of its fluids and electrolytes.

Blood glucose concentration is kept within a narrow and normal range due to fine control by insulin and the counterregulatory hormones. Insulin lowers plasma glucose and the counterregulatory hormones increase glucose levels when it is low.

When glucose is elevated (higher than 150 $mg*dl^{-1}$), insulin release is increased resulting in glucose storage (glycogenesis) and utilization by different tissues such as liver, muscle, and adipose (Cherrington et al., 1981). At the same time the counterregulatory hormones, namely, glucagon, catecholamines (epinephrine and norepinephrine), cortisol, and growth hormone are inhibited. When plasma glucose falls to subnormal levels, insulin release is inhibited and the counteregulatory hormones are stimulated. This results in glucose production either through glycogenolysis or gluconeogenesis and inhibition of glucose uptake by the peripheral tissues (Deibert et al., 1980).

There are some interelationships between these hormones such as inhibition of insulin release and stimulation of glucagon secretion by catecholamines (Gray et al., 1980). These hormonal changes and metabolic shifts are brought about by a glucostatic mechanism that detects glucose changes both centrally and peripherally which tries to maintain glucose homeostasis.

Fat metabolism is also under hormonal control maintaining a balance between lipogenesis and lipolysis with insulin favoring the former and catecholamines, growth hormone, glucagon and cortisol supporting the latter (Issekutz, 1975; Jarhult & Holst, 1979). Increased utilization of lipids (Owen et al., 1967) and occasionally protein (Cryer, 1989) for energy production helps to spare glucose.

Exercise is a stressor where a significant metabolic acceleration is achieved due to the special metabolic demands imposed on the working muscular system. Carbohydrates and fat are the two major energy fuels used during exercise, depending on the intensity and duration of the work performed (Vranic et al., 1979). Carbohydrate utilization predominates but fats can contribute a significant supply of energy at lighter intensities and longer durations of exercise. Carbohydrate utilization is achieved at higher intensities and at the initiation of exercise (Vranic et al., 1979). During long-term work as in distance running, glucose utilization exceeds its production resulting in hypoglycemia after 90 to 120 minutes of exercise. Under these conditions glycogen stores and carbohydrate availability in general can limit performance (Fox, 1989). With aerobic exercise insulin is inhibited and glucagon and catecholamines (epinephrine

and norepinephrine) are stimulated trying to maintain plasma glucose levels from falling (Gollnick et al., 1974). Fat utilization through lipolysis and some times ketogenesis can aid to spare glucose (Gollnick et al., 1974). Exercise stimulates mechanisms that help to regulate glucose homeostasis and fat metabolism.

Beta-Endorphin (B-EP) has been suggested to play a role in the regulation of glucose homeostasis in stress situations (Giugliano, 1988). B-EP is a neurohormone peptide with potent opioid action. It is secreted by the pituitary and hypothalamus. The demonstration that B-EP is widely distributed in sites involved in glucose homeostasis, including the central and peripheral divisions of the autonomic nervous system, pancreas, gut, and hypothalamo-pituitary axis, has led to the suggestion that it may participate in the regulation of glucose homeostasis and fat metabolism (Krieger, 1980, 1983).

It has been indicated that B-EP release, either centrally or peripherally, was potentiated by induced hypoglycemia (Kjaer et al., 1993; Nakao et al., 1979) and negative energy balance situations such as starvation (Walberg- Rankin et al., 1992; Kuldeep et al., 1985). A negative correlation (- 0.91) was obtained between plasma glucose levels and plasma B-EP during insulin-induced hypoglycemia (Kjaer et al., 1993). These findings were accompanied by observations that in most cases, B-EP infusion in vivo or in vitro results in hyperglycemia (Reid et al., 1981; Feldman et al., 1983; Matsumura et al., 1984). Eventhough it has not been confirmed by all studies, data suggests that this hyperglycemic effect of B-EP could

enhance glycogenolysis (Matsumura et al., 1984) or gluconeogenesis (Matsumura et al., 1984; Dave et al., 1985) and inhibit glucose clearance (Radosevich et al., 1989). However, these observations were not consistent.

B-EP and other endogenous opioids appear to have a potent lipolytic activity in vitro (Richter et al., 1987, Vettor et al., 1993). Results from animal studies have also suggested a lipolytic effect of B-EP (Grag et al., 1977; Schwandt et al., 1979), but it is still uncertain if this occurs in humans.

Paolisso et al (1987), suggested that the induced metabolic changes induced by B-EP are secondary to its effect on hormones such as insulin, glucagon and possibly catecholamines. The action of B-EP on glucose homeostasis and on the regulation of lipid metabolism might be indirect rather than direct. Despite the fact that in vitro and in vivo studies agree that B-EP stimulates glucagon release from the pancreas (Reid et al., 1981; Feldman et al., 1983; Ipp et al., 1980), a great deal of controversy exists on the effects of B-EP on insulin and catecholamine release. Previous experimental findings point towards both stimulation (Reid et al., 1981; Feldman et al., 1983; Van Loon et al., 1981) and inhibition (Rudman et al., 1983; Matsumura et al., 1984; Mannelli et al., 1984; Farrell et al., 1991) of insulin and catecholamines.

It was reported that B-EP is increased by exercise (Blake et al., 1984; Goldfarb et al., 1987, 1991). Eventhough some investigators attributed this elevation to pain and mood regulation induced by exercise (Arentz et al., 1986; Olausson et al., 1986), few efforts were

made to explore the metabolic actions of B-EP during exercise and its role in the regulation of plasma glucose and lipolysis. There is a need to investigate if B-EP acts as a part of the metabolic mechanism during exercise since regulation of glucose homeostasis is critical not only for sports performance, but also for optimal health. In addition, if B-EP plays a role in substrate utilization during exercise, it should be determined if this action is direct or indirect. The aim of this study was to determine if B-EP affects glucose homeostasis and evaluate its effects on the major glucoregulatory hormones, namely insulin and glucagon .

Purpose

The purpose of this study was to evaluate the metabolic effects of B-EP during an acute bout of endurance aerobic exercise. This study attempted to determine how B-EP and naloxone, a drug that inhibits B-EP binding to its opioid receptors, affect plasma glucose during an acute bout of aerobic exercise. In addition, B-EP effects on the counterregulatory hormones involved in glucose homeostasis during exercise, namely insulin and glucagon were determined.

Hypotheses

The null form of hypothesis statement was chosen since previous research in the related literature is controversial and the metabolic effects of B-EP during exercise have not been investigated in depth. The researcher hypothesized that upon comparison of the three treatment groups:

1. There will be no significant differences among the three groups in plasma glucose levels during the two-hour running period.

2. There will be no significant differences among the three groups in plasma levels of C-peptide, insulin and glucagon during the two-hour running period.

3. There will be no significant differences among the three groups in plasma lactate levels during the two-hour running period.

4. There will be no significant differences among the three groups in plasma B-EP levels during the two-hour running period.

Basic Assumptions

All rats underewent surgery under anesthesia for intravenous catheter placement. The animal post-surgery recovery was completed three to five days prior to treatment intervention and it did not affect the treatment effectiveness. All rats went through an eight day familiarization period on the treadmill to insure that the rats were able to run for 120 minutes without problems. It is assumed that intravenous administration of the drugs (B-EP and naloxone) and placebo did not cause any undesired side effects to the animals so that their physiological responses and running performance were not impaired. Finally, it was assumed that the drug dosages were adequate in order to elicit the expected responses.

Limitations of the Study

Subjects of this study were untrained male Spraque-Dawley rats.

The exercise protocol used involved a two-hour endurance running activity of moderate intensity. Generalizing the findings to other exercise modes, intensities and durations may not be appropriate.

Possible changes obtained due to drug manipulation does not indicate that under normal physiological conditions they would occur.

Insulin and glucagon release were not measured. Their plasma concentration was only determined.

Delimitations of the Study

1. The subjects were untrained male Spraque-Dawley rats weighing 250-300 grams.

2. The animals ran on the treadmill for up to two hours at moderate intensity (at 22 $m*min^{-1}$).

3. The B-EP dose was 0.05 $mg*hour^{-1}*Kgr^{-1}$ of body weight.

4.The dose of the B-EP antagonist, naloxone, was 0.8 $mg*hour^{-1}*Kgr^{-1}$ of body weight.

Significance of the study

Despite the fact that research has shown repeatedly that exercise provides a stimulus for B-EP release, the physiological significance of this elevation remains unknown at present. Recent research implicates B-EP in the regulation of carbohydrate and lipid metabolism but the actual mechanisms are not understood. This study was a first attempt to assess the effects of B-EP on plasma glucose homeostasis during prolonged exercise. In addition, this study attempted to determine if B-EP control on substrate mobilization during exercise is achieved through actions on the major counterregulatory hormones insulin and glucagon.

Definitions of Terms

1. Beta-Endorphin (B-EP): A neurohormone peptide with opioid functions. B-EP is synthesized by the hypothalamus and pituitary

glands as a part of a larger precursor molecule of proopiomelanocortin (POMC). After its cleavage as a smaller fragment from POMC it acts as a neurotransmitter at nerve synapses in the brain or as an endocrine through its release in the peripheral circulation. Its action on target tissues is mediated by binding to u-opioid receptors.

2. Plasma glucose: A six-carbon molecule existing in the plasma and utilized by various tisues mainly for energy purposes. Its concentration in the plasma is normally maintained between 60 and 140 $mg*dl^{-1}$.

3. Insulin: A peptide hormone, synthesized in the beta cells of the pancreas. It is released in the bloodstream and acts on tissues to promote glucose, fat and protein storage.

4. Glucagon: A peptide hormone released by the alpha cells of the pancreas. It is released in the circulation and acts primarily on the liver in order to enhance glycogenolysis and glucose release in the plasma.

5. Naloxone: An exogenous drug which binds to the u-opioid receptors. Because of this action it can compete and prevent B-EP binding on these same receptors.

CHAPTER II

REVIEW OF RELATED LITERATURE

Mechanisms of Glucoregulation

Maintenance of plasma glucose concentration is critical to survival because plasma glucose is the predominant metabolic fuel utilized by the central nervous system and red blood cells. Brief hypoglycemia can cause brain dysfunction. Prolonged, severe hypoglycemia can cause brain death. Hyperglycemia has deleterious effects such as cellular dehydration, osmotic diuresis by the kidneys, loss of fluids and electrolytes, and glycosylation of plasma proteins. The plasma concentration of glucose is normally kept within an optimal range, roughly 60 to 150 $mg*dl^{-1}$, despite wide variations in glucose influx and efflux such as those occuring following meals and during exercise (Cryer, 1989). This narrow range of blood glucose concentration is preserved due to counterregulatory hormones that raise plasma glucose when it falls and decrease glucose when its levels are above normal. An outline of how plasma glucose is regulated is presented below.

The liver functions as an important blood glucose buffer system. When blood glucose rises to high concentration such as after a meal, as much as two thirds of the glucose absorbed by the gut is stored in the liver in the form of glycogen. During the subsequent period,

when the blood glucose concentration falls, the liver releases glucose into the (Sherwin, 1980).

It is clear that both insulin and glucagon function as the primary feedback control endocrines for maintaining normal blood glucose. When plasma glucose concentration rises, insulin release increases and in turn causes the blood glucose concentration to decrease toward normal. Conversely, a decrease in blood glucose stimulates glucagon secretion and insulin inhibition. Glucagon functions to increase blood glucose. Under normal conditions, the insulin feedback mechanism is predominant but diminished glucose intake or excessive utilization of glucose, such as during exercise stimulates the glucagon mechanism. However, the action of insulin is unique because while other counterregulatory hormones can exert action similar to that of glucagon only insulin helps to lower blood glucose (Cherrington et al., 1981).

Low blood glucose sensed by the hypothalamus stimulates the sympathetic nervous system. Epinephrine secreted by the adrenal glands can augment the release of glucose from the liver (Deibert et al., 1980). Inhibition of insulin secretion through a-adrenergic receptor stimulation by norepinephrine also occurs (Rizza et al., 1980). Beta-adrenergic stimulation of glucagon also takes place with hypoglycemia (Gray et al., 1980).

Other hormones such as growth hormone and cortisol, can be secreted in response to prolonged hypoglycemia. These hormones decrease the rate of glucose utilization by most cells and stimulate usage of proteins and fats. Together with glucagon and epinephrine

these other hormones help to stimulate the gluconeogenic process (Deibert et al., 1980). During fasting, adipocytes can also decrease their glucose utilization and satisfy their energy needs from b-oxidation of fatty acids, helping to spare glucose (Owen et al., 1967).

In summary, protection of glucose homeostasis is achieved by the coordinated action of insulin and the counterregulatory endocrines, glucagon, epinephrine growth hormone and cortisol.

Backround of Beta-Endorphin.

A. Biology of B-EP.

Beta-Endorphin (B-EP), a neurohormone peptide, is classified as an endogenous opioid. It is distributed throughout the central nervous system, pituitary gland and other peripheral tissues. The term opioid is used here to designate a group of endogenous peptides that are, to varying degrees, opium- or morphine- like in their properties. Hughes et al. (1975), succeeded in isolating the first endogenous opioids called methionine- and leucine- enkephalin. In the following years opioid peptides have been characterized in various areas of the brain (McDowell & Kitchen, 1987). B-EP was purified by Li in 1977 and has potent opioid activity.

Three distinct families of opioid peptides have been identified thus far: the enkephalins, the endorphins, and the dynorphins. Each family is derived from a distinct precursor polypeptide and has a characteristic anatomical distribution. These precursors are now designated as proenkephalin (also proenkephalin A), pro-opiomelanocortin (POMC), and prodynorphin (also proenkephalin B).

Figure 1 presents the biologically active peptides that are derived from these precursors.

POMC is an interesting precursor molecule in that it can give rise to several biologically active peptides. POMC contains the amino acid sequences for melanocyte-stimulating hormone (gamma-MSH), adrenocorticotrophin (ACTH), beta-lipotrophin (beta-LPH), B-EP, and beta-MSH. Although B-EP contains the sequence for met-enkephalin at its amino terminus, it is not converted to this peptide; instead, met-enkephalin is derived from the processing of proenkephalin. Of these, only B-EP has demonstrated opioid activity, while adrenocorticotrophic hormone (ACTH), melanotrophin (MSH), and beta-LPH posses non-opioid mediated functions.

Not all cells that make a given precursor polypeptide store and release the same mixture of active opioid (and nonopioid) peptides. These differences are thought to arise from variations in the posttranslational processing of the precursor polypeptides by peptidase enzymes depending on the needs of each individual tissue.

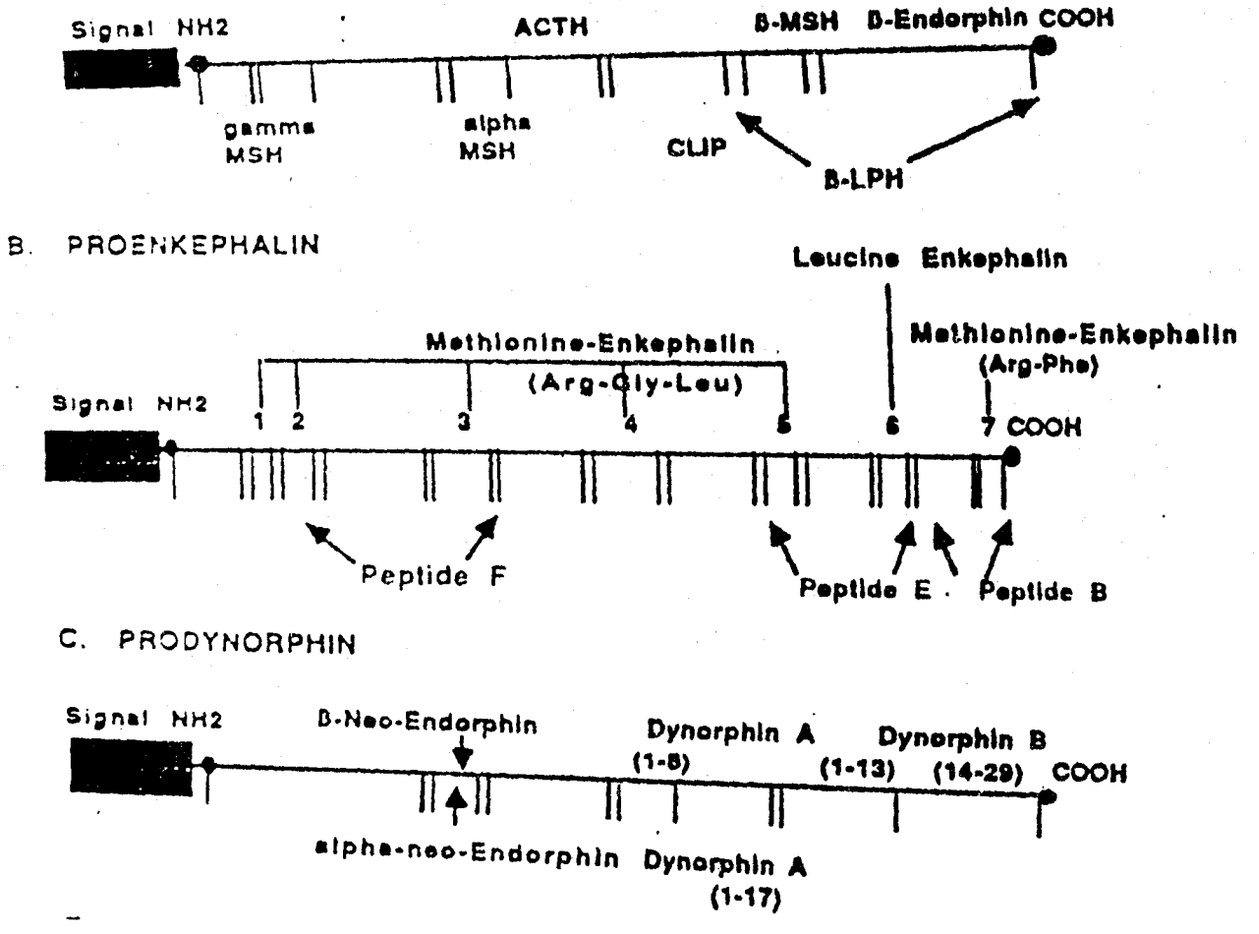


Figure 1. Schematic representation of the protein precursor structures of the three opioid peptide families.

Data are taken from Akil et al., Ann. Rev. Neurosci., 7: 223-255, 1983, and Simons, Endocr. Rev., 9: 200-212, 1988.

B. Anatomical localization.

Beta-Endorphin (B-EP), the most potent known naturally occurring analgesic agent, was first isolated and identified as one of a series of prohormone fragments present in porcine pituitary (Bradbury et al., 1975). It is now known that this opioid peptide occurs in a variety of species, ranging from the camel (Li & Chung, 1976), ostrich (Naude et al., 1980) and salmon (Kawauchi et al., 1979) to man (Dragon et al., 1977). B-EP is a 31-amino acid peptide (its amino acid sequence is shown in figure 2).

Try-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln

Figure 2. Beta-Endorphin: A 31 amino-acid sequence. Data are taken from Akil et al., Ann. Rev. Neurosc., 7: 223-255, 1984.

Beta-Endorphin is accompanied by a group of structurally related peptides that are virtually devoid of opioid activity in the brain and the pituitary gland (Smyth, 1983). B-EP appears to be transformed to its biologically active form, but can be further processed to form inactive derivatives.

In an attempt to understand the physiological role of B-EP, the distribution of its active and inactive forms has been studied in

various tissues where "immunoreactive B-EP" was known to be present. In general B-EP and its derivatives occur in low concentration, which makes their isolation and identification difficult. However, the peptides can be detected and measured readily with the aid of radioimmunoassays (RIA). Specific antibodies for B-EP have been used to localize B-EP-immunoreactive peptides by immunohistochemical methods (Pelletier et al., 1977; Watson et al., 1977). The antibodies have been used also for plasma and tissue extracts (Guillemin et al., 1977; Holt et al., 1978). When RIAs are applied in conjuction with chemical methods for purification of the peptides, the different B-EP derivatives can be identified and determined.

Recent research has revealed that the distribution of peptides from POMC is relatively large. Their location within the central nervous system includes the arcuate nucleus, which projects its fibers to the limbic area and brainstem areas. Some POMC-containig fibers descend to the spinal cord (Smyth, 1983). In the human brain the distribution of POMC is believed to correspond to areas where electrical stimulation can produce pain relief (Pilcher, 1988). Peptides from POMC occur in both the pars intermedia and the pars distalis of the pituitary (Smyth, 1983).

The precursor molecule POMC and its derived peptides are not confined to the central nervous system. The use of biochemical techniques have shown that B-EP is found in a number of tissues. B-EP has been found in the gastrointestinal tract of rats and humans (Orwoll et al., 1980; Matsumura et al., 1982). Extracts from porcine

pancreas have shown to contain B-EP-like polypeptides with a high molecular weight (Houck et al., 1981). B-EP has also been detected in human pancreas (Bruni et al., 1979) and shown to be localized in pancreatic D-islets of rat, guinea pig, and man (Watkins et al., 1980). Glucagon-containing secretory granules of rat pancreatic alpha cells were shown to contain B-EP immunoreactivity as well (Grube et al., 1978). To rule out the possibility that pancreatic B-EP is of pituitary origin and simply accumulated in alpha cells by an uptake of circulating B-EP, Grube et al (1978) examined pancreatic alpha cells of hypophysectomized rats and noted that they contained B-EP. The absence of positive immunocytochemical staining when using an antibody against ACTH provides evidence that pancreatic B-EP may not have the same biosynthetic pathway as pituitary B-EP (Bruni et al., 1979; Grube et al., 1978). However, in some studies the existence of B-EP and beta-LPH in the pancreatic tissue could not be demonstrated (Stern et al., 1982; Polak et al., 1977).

Several studies demonstrated the presence of enkephalin and B-EP-like material in the adrenal glands of several species, including humans (Imura et al., 1983). Additionally, enkephalins were found in splachnic nerves innervating the adrenal medulla (Schultzberg et al., 1978). Opiate receptors have been identified on adrenal tissue (Chevkin et al., 1979; Dave et al., 1985). B-EP has also been be detected in stomach (Tanaki et al., 1982) and placenta (Liotta et al., 1982). The extent of the distribution of B-EP suggests that B-EP is likely to be involved in both central and peripheral functions.

C. Opioid receptors.

The primary functional sites of action for the opioid peptides seem to be mu, delta, and kappa receptors. The mu and kappa receptors are activated when appropriate ligands bind to them, whereas they are inactivated when bound by competitive antagonists (Gero, 1986). Less is known about the binding characteristics of delta receptors. Table 1 shows the reported affinity characteristics of the opioid receptors for various ligands. The delta receptors have a high affinity for both enkephalin and endorphin, kappa receptors are more inclined to bind dynorphin, whereas mu receptors have a high affinity for B-EP (as well as morphine and naloxone). Herz (1984), proposed that receptor affinity may be related to peptide chain length.

While it has been proposed that m-receptors could be subdivided into mu_1 and mu_2 receptors (Pasternak and Wood, 1986), it should also be pointed out that some believe mu and delta receptors are in fact a single fluid macroreceptor, capable of different binding properties when influenced by changing cellular conditions (e.g. altered Na^+, nucleotides, or pH levels) (Blume, 1980; Bowen et al., 1981). However, kappa receptors appear to be distinctive and may act to oppose some mu supported actions (Shippenberg & Herz, 1986). Variations of these basic opioid receptors do exist (Holaday & Tortella, 1984).

Ligand	delta	mu	kappa
Agonists	High	Low	Low
Leu-Enkephalin	High	Low	Low
Met-Enkephalin	High	High	Low
B-EP	High	High	Low
Dynorphin	Low	Low	High
Morphine	Low	High	Low
Antagonists			
Naloxone	Low	High	Low

Table 1. Relative ligand interactions with opioid receptor subtypes (Adapted from Paterson et al., Br. Med. J., 39:31-36, 1983).

Naloxone.

Opioid antagonists are substances that interact with opioid receptors because of their chemical similarity to opioids while exerting little or no agonist effect. Opioid competitive antagonists are capable of displacing the opioid molecule from the receptor site, which reduces the physiologic effects of the opioid agonists. However, some of these substances may exert opioid effects.

Opioid antagonists can result in various degrees of agonistic or antagonistic effects. Naloxone, a pure antagonist, is considered the

drug of choice in most situations that require an opioid antagonist. The physiological response to opioid antagonists depends upon the following factors:

1. The degree of agonistic action of the chosen drug.

2. Whether or not opioid drugs are present at the receptor site.

3. The presence of endogenous endorphins or enkephalins at the receptor site.

4. The degree of physical dependence on opioids previously developed by the recipient.

5. Concentration at the receptor site as determined by dosage.

Naloxone is a derivative of noroxymorphone, which was originally synthesized as a structural analog of morphine. Naloxone's chemical structure has an allyl group which makes it an antagonist (figure 3 shows the chemical structure of naloxone). Naloxone has a biological active life of 20 to 60 minutes, depending administration (Kaufman et al., 1989; Ngai et al., 1976). Plasma concentration peaks within 2 to 3 minutes following an intravenous bolus, but peak pharmacological activity lags the administration by approximately 30 minutes. Simply measuring plasma naloxone concentration is inadequate for assessing the pharmacodynamic actions of naloxone. Naloxone is usually injected as a naloxone hydrochloride solution. Naloxone readily crosses the blood brain barrier (BBB) (peripherally administered naloxone has a 10 fold higher brain:blood ratio than morphine), and CNS levels turn over slower than peripheral levels. At high doses (in excess of 1 $mg*Kgr^{-1}$), naloxone may elicit some agonistic activity, but it is an essentially pure antagonist at doses

below this level. Naloxone acts primarily on the mu opioid receptors but has some affinity for the kappa opioid receptors. Naloxone is excreted by the kidney. Naloxone sometimes can cause nausea and vomiting and can aggravate hypertension.

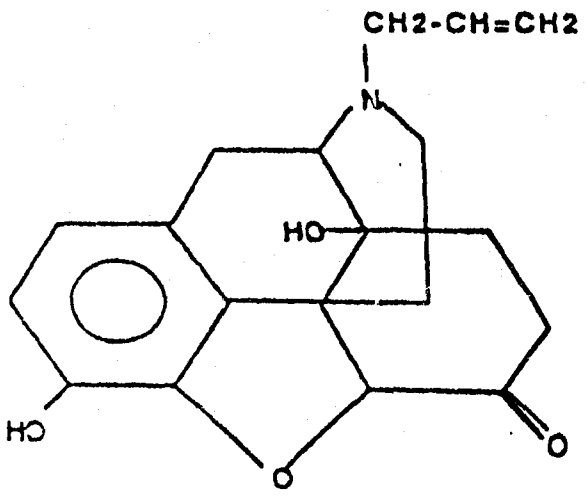


Figure 3. Chemical structure of Naloxone.

Metabolic functions of Beta-Endorphin

Claude Bernard's classic observation in 1855 that central morphine administration yields hyperglycemia provided the stimulus for work characterizing the consistent increase in blood sugar in response to morphine administration in a number of mammalian models, including humans. Following the discovery of the E.O.P. system, evidence was presented to suggest that Beta-Endorphin plays an integral role in normal fuel metabolism. Research implicates

B-EP in the metabolic regulation of both glucose and fat stores. The following discussion presents a thorough description of evidence implicating B-EP as a regulator of glucose metabolism.

Beta-Endorphin and Glucose Regulation.

The demonstration that Endogenous Opioid Peptides (EOP) are widely distributed in sites involved in glucose homeostasis, including the central and peripheral divisions of the autonomic nervous system, the pancreas, the gut, and the hypothalamopituitary axis has led to the suggestion that B-EP may participate in the regulation of glucose homeostasis (Krieger, 1980, 1983).

Responses of B-EP to hypoglycemia and negative energy balance.

Research has indicated that disturbances in glucose homeostasis and food deprivation brings about changes in the B-EP synthesis and release (Nakao et al., 1979). It was shown that hypoglycemia induced by insulin administration resulted in increased B-EP release (Nakao et al., 1979; Radosevich et al., 1988). In a more recent study, Kjaer et al (1993), noted that insulin-induced hypoglycemia stimulated ACTH and B-EP secretion (ACTH and B-EP are parts of the same precursor molecule of POMC and are co-released). In this study, a negative correlation (-0.91) was found between glucose and plasma B-EP levels. It was suggested that hypoglycemia was a potent stimulator of ACTH and B-EP secretion independent of insulin. It was indicated that this action maybe explained by the observed time lag between

insulin administration and ACTH response. The ACTH response did not occur until glucose in the cerebrospinal fluid (C.B.F.) reached low levels (Keller-Wood, 1981). Glucose clamping within the brain (infusion of glucose in the carotid arteries) during insulin infusion eliminated the ACTH response (Keller-Wood, 1981). These findings confirm other experimental results that insulin-induced hypoglycemia increased the pituitary POMC mRNA levels and this increased response can be inhibited by B-EP infusion (Suda et al., 1992). However, a few studies failed to observe any elevations in plasma B-EP in response to insulin-induced hypoglycemia (Liotta et al., 1978; Suda et al., 1978).

Insulin was the major tool to cause hypoglycemia in most studies conducted in this area. In the study of Zelissen et al. (1991) where diet was manipulated in order to alter plasma glucose levels, both a predominantly carbohydrate diet or a predominantly fat diet did not succeed to elicit a significant change of plasma B-EP concentration. In contrast, one week of carbohydrate deprivation resulted in a significant elevation of central and peripheral B-EP levels in rats (Fatouros et al., unpublished observations, 1994). In this study a significant negative correlation between B-EP and plasma glucose levels was obtained.

However, not only induced hypoglycemia was found to elicit a significant response in B-EP release, but starvation or food deprivation conditions were found to drastically change B-EP release both centrally and peripherally. It has been demonstrated that a negative energy balance during exercise resulted in a greater

increase in B-EP concentration than when the subjects were in energy balance (Walberg-Rankin et al., 1992). An even more drastic alteration of energy balance, such as that induced by starvation, resulted in an increase of pituitary B-EP (Margules, 1979). A significant increase (16-43%) in the pituitary B-EP was observed 2-4 days following food deprivation and similar increases were seen in B-EP located in hypothalamus and striatum but not in plasma (Kuldeep et al.,1985). It was suggested that the stimulation of food intake in rats exposed to 24 hours of food deprivation is associated with increased plasma B-EP (Davis et al.,1983). Finally, when food deprived rats were fed normal food for 24 hours, B-EP returned to basal levels, indicating that the food deprivation B-EP response was reversible (Kuldeep et al., 1985).

Proposed mechanisms of the B-EP elevation in hypoglycemia.

Histamine, a central nervous system neurotransmitter, has been proposed by a number of investigators as a possible mediator of B-EP elevation in response to insulin-induced hypoglycemia. Insulin-induced hypoglycemia increased the turnover rate of neuronal histamine in the hypothalamus (Kjaer et al., 1993). It has also been shown that hypoglycemia elevates the release of histamine from hypothalamic tissue in vitro (Kjaer et al., 1986). However, histamine has no direct effect on the release of ACTH and B-EP from pituitary tissue in vitro (Hashimoto et al., 1979; Vermes et al., 1980). This indicates that the effect of histamine in hypoglycemia on B-EP is

exerted by an action at the level of the hypothalamus. This observation is in agreement with the finding that postsynaptic blocking of histamine and blocking of histamine synthesis attenuated the B-EP and ACTH responses to insulin-induced hypoglycemia (Kjaer et al., 1993).

An alternative hypothesis is that hypothalamic releasing factors are the mediators of B-EP and ACTH elevation in hypoglycemia. Since lesioning of the mediobasal hypothalamus inhibits the ACTH response to hypoglycemia (Mezey et al., 1984; Jesova et al., 1987; Aizawa et al., 1981; Karteszi et al., 1982). Support for this hypothesis is the observation that hypoglycemia stimulates the release of the Corticotrophic Releasing Factor (CRH) in the pituitary portal blood (Caraty et al., 1990; Plotsky et al., 1985; Guillaume et al., 1989; Engler et al., 1989) and in the peripheral plasma (Sumitomo et al., 1987; Baylis et al., 1980). In addition, Suda et al. (1992), suggested that B-EP can inhibit the insulin-induced hypoglycemia elevation of POMC mRNA levels. This effect was due to inhibition of CRF release (through negative feedback inhibition) by hypothalamus since CRF induces POMC expression.

Recent research links together the above two hypotheses. Central administration of histamine or histamine agonists led to increased levels of CRF in peripheral plasma and pituitary portal blood (Kjaer et al., 1991, 1993), and immunoneutralization of endogenous CRF inhibited the ACTH and B-EP responses to histamine (Kjaer et al., 1992). This was further supported by the finding that histaminergic neurons originating in the posterior hypothalamus

project to the paraventricular and supraoptic nuclei (Panula et al., 1989; Inagaki et al., 1988) where CRF neurons are localized (Swanson et al., 1983; Piekut et al., 1986; Whithall et al., 1988). Naloxone, a B-EP antagonist, reduced the insulin-induced hypoglycemia increase of B-EP indicating that the hypoglycemic release of B-EP might be mediated through mu opioid receptors (Nush et al., 1989).

B-EP responses to hyperglycemia.

Sustained hyperglycemia did not affect plasma levels of B-EP and this result was not affected by prior exercise (Farrell et al., 1986). B-EP concentration was unaffected by a hyperglycemic clamp (Ritter et al., 1990). In contrast, oral or intravenous glucose administration resulted in elevated plasma B-EP (Getto et al., 1984; Scavo et al., 1987).

In summary, from the research findings presented no definitive conclusions can be drawn about the effects of hyperglycemia on the release of B-EP. It appears that hypoglycemia (usually caused by insulin administration) and starvation (food deprivation) is a powerful stimulus for the increase of B-EP both centrally or peripherally. The physiological significance of this B-EP response is not clear although a role as a counterregulatory agent has been speculated. The effects of B-EP on the glucoregulatory mechanism needs to be examined.

Beta-Endorphin Effects on Glucose Regulation.

A. In humans.

In two of the classical studies in this area, a single intravenous bolus of B-EP in normal human subjects resulted in hyperglycemia (Reid et al., 1981; Feldman et al., 1983). It was unclear if this was a direct B-EP effect or mediated by the pancreatic hormones or sympathetic hormones. B-EP-induced hyperglycemia is probably not mediated through growth hormone since in acute experiments growth hormone does not cause hyperglycemia (Fineberg et al., 1984; Sirek et al., 1979).

B. Central effects of B-EP in animals.

B-EP-induced hyperglycemia was observed not only in humans but also in animal and in vitro experiments with both central and peripheral administration of B-EP. A rise in blood sugar was obtained with a small dose of B-EP injected into a lateral cerebral ventricle (Borison et al., 1962; Feldberg, 1972). Intraventricular injection of B-EP or morphine leads to a rapid elevation of blood glucose in cats and rats (Borison et al., 1962; Feldberg, 1972). These observations agree with the finding that direct injection of B-EP into the rat brain produces prolonged, naloxone-blocked elevations of blood glucose (Appel et al., 1987; Brown et al., 1979). Central infusion of B-EP after 2-deoxy-glucose administration increased glucose utilization in the brain (Ableitner et al., 1992). Naloxone, a B-EP antagonist, was able to diminish the hyperglycemic response to central nervous system glucose deprivation (Ipp et al., 1984). Feldberg (1985), suggested

that the site of hyperglycemic action of B-EP and other opioids after intraventricular and intracisternal injection is the ventral surface of the brainstem. The central action of B-EP seems to involve cholinergic mediation, since prior intracisternal administration of hemicholinium-3, a substance which dramatically decreases acetylcholine concentration in the brain, inhibits increases in blood glucose by centrally infused B-EP (Van Loon et al., 1981).

Another possible mediator of central action of B-EP could be somatostatin since intracisternal administration of this neurohormone also blocks the hyperglycemic effect of central B-EP (Van Loon et al., 1981). A third mechanism of centrally-induced hyperglycemia by B-EP involves sympathetic mediation. The evidence for this is based on the observation that intracisternal administration of synthetic human B-EP in conscious adult male rats increased plasma epinephrine concentration, while adrenal denervation totally inhibited the catecholamine response to the opioid (Van Loon et al., 1981). In addition, morphine or B-EP induced hyperglycemia was reduced by sectioning the sympathetic ganglia or adrenal ablation (Borison et al., 1962; Feldberg, 1972). From these observations it seems that the central effects of B-EP on plasma glucose levels are not directly mediated.

Peripheral administration of naloxone was found to block the hyperglycemic effect of central B-EP, suggesting the involvement of specific opiate receptors (Van Loon et al., 1981). In contrast, in cat, the effects of centrally infused morphine (also an opiate agonist) were not antagonized by naloxone (Ramahadran et al., 1990). In

dogs, a high dose of central naloxone administration attenuated hyperglycemia produced by intracerebroventricular injection of 2-deoxy-D-glucose (Ipp et al., 1984). More work needs to be done in this area in order to confirm the central actions of B-EP.

C. Peripheral effects of B-EP in animals.

Peripheral administration of B-EP has not produced as consistent results compared to centrally administered B-EP. B-EP intravenous infusion in rats increased basal glucose levels from 170 to 250 $mg*dl^{-1}$ ten minutes after the infusion and returned to about the initial level after 20 minutes (Matsumura et al., 1984). In rabbit, B-EP caused a rise in blood glucose when it was given intravenously (Rudman et el., 1983). However, the intravenous infusion of B-EP in dogs failed to observe a rise in plasma glucose concentration (Radosevich et al, 1984, 1989).

D. In vitro experiments.

In vitro studies have complicated the picture even more. Increased glucose release in the medium was noted when liver cells from fed rats were incubated in the presence of B-EP (Matsumura et al.,1984). Similar results with isolated hepatocytes were obtained when B-EP, but not morphine, was added in the medium (Allan et al., 1983; Leach et al., 1985). In contrast, Brubaker et al. (1987) could not replicate these observations with physiological and supraphysiological doses of B-EP to isolated rat hepatocytes. In this study, B-EP did not alter hepatic glucose production and glycogen

phosphorylase-a activity (a key glycogenolytic enzyme). Discrepancies were explained by methodological differences between the aforementioned studies such as mediums used, species selected, doses, etc.

Table 2 presents a summary of the effects of different modes of B-EP infusion on plasma glucose concentration.

Study	Infusion	Species	Effect on glucose
Reid, 1981	i.v.	Humans	Increase
Feldman, 1983	i.v.	Humans	Increase
Borison, 1962	i.c.v.	Cats	Increase
Feldberg, 1972	i.c.v.	Rats	Increase
Appel, 1987	i.c.v.	Rats	Increase
Brown, 1979	i.c.v.	Rats	Increase
Ableitner, 1992	i.c.v.	Rats	Increase
Van Loon, 1981	i.c.	Dogs	Increase
Radosevich, 1989	i.c.v.	Dogs	Increase
Matsumura, 1984	i.v.	Rats	Increase
Rudman, 1983	i.v.	Rabbits	Increase
Radosevich, 1984	i.v.	Dogs	Decrease
Radosevich, 1989	i.v.	Dogs	No change
Allan, 1983	in vitro	Rats	Increase
Leach, 1985	in vitro	Rats	Increase
Brubaker, 1987	in vitro	Rats	No change
Matsumura, 1984	in vitro	Rats	Increase

Table 2. Effects of B-EP infusions on glucose concentration. i.v. denotes intravenous, i.c.v. denotes intracerebroventricular, and i.c. denotes intracisternal.

Proposed mechanisms for the B-EP induced hyperglycemia.

Plasma glucose concentration reflects only the balance of the rate of glucose production or appearance and the rate of glucose clearance or uptake of glucose by peripheral tissues. If B-EP has a hyperglycemic affect, one would expext to either increase glucose production, decrease glucose clearance or uptake or both. Radosevich et al. (1989) reported that intracerebroventricular infusion of B-EP caused an increase of glucose production and a decline of glucose clearance leading to hyperglycemia. A similar infusion protocol, by Nush et al. (1989), showed that B-EP led to an increase in both glucose production and utilization. This resulted in no change of plasma glucose concentration. However, in the latter study B-EP was administered after insulin-induced hypoglycemia. Another study, which employed peripheral B-EP administration, observed an inhibition of both glucose production and glucose uptake (El-Tayeb et al., 1985). Increased glucose production was also reported by a number of studies (Matsumura et al., 1984; Allan et al., 1983; Leach et al., 1985). However, two studies, one in vivo (Radosevich et al., 1989) and one in vitro (Brubaker et al., 1987) could not confirm these findings. These two studies showed that B-EP did not affect plasma glucose or glucose turnover.

The findings presented in the last paragraph suggest that B-EP increases glucose synthesis or production. In order for B-EP to increase glucose synthesis or production one of two mechanisms must operate: either glycogenolysis or gluconeogenesis. Enkephalin,

another endogenous opioid peptide, induced an increase in glycogenolytic rate in rats in vitro. This effect was independent of cAMP stimulation since cAMP levels in the liver were unchanged (Allan et al., 1983). Matsumura et al. (1984) demonstrated that B-EP was capable of inducing glycogenolysis. This effect was dose-dependent and cAMP-related. The results by Matsumura et al. (1984) were indirect evidence since it was shown that B-EP can bind to nonopiate receptors in rat liver which are coupled to adenylate cyclase. Activation of these receptors resulted in increased level of cAMP (Dave et al., 1985). Brubaker et al. (1987) observed no alteration in glycogen phosphorylase-a activity after B-EP addition in rat hepatocyte medium.

Gluconeogenesis is the other metabolic pathway of increasing glucose production. Morphine infusion in rats, an opiate which binds to the same opioid receptors as B-EP, resulted in an increase of glucose synthesis by liver slices. This was a result of both an induction of a number of gluconeogenic enzymes and a suppression of pyruvate kinase (Wong et al., 1981). Enkephalins induced an elevation in glucose synthesis from pyruvate[2-^{14}C] in vitro but morphine did not (Allan et al., 1983). In this study, naloxone had no effect on either the basal or stimulated rate of glucose output, although it significantly depressed the basal rate of incorporation of label into glucose and decreased the degree of stimulation by enkephalin by 30%. These results suggested that B-EP could have similar action. Indeed, the first evidence came from the observation that naloxone infusion was able to decrease glucose production in the

conscius dog despite a rise in plasma glucagon and no change in plasma insulin (Werther et al., 1981). This may indicate that the gluconeogenic pathway is under some degree of tonic control by opioid receptors. It was demonstrated that B-EP induced gluconeogenesis in vitro, after the addition of L-lactate in the medium from isolated rat cells (Matsumura et al., 1984).

In summary, research suggests that central administration of B-EP induces hyperglycemia. A concesus has not been reached concerning peripheral effects of B-EP on glucose homeostasis. In order to explain the hyperglycemic effect of B-EP, an increase in glucose production or an inhibition of glucose clearance or both have been proposed. Nevertheless, there is no agreement in this area. In the following paragraphs the research backround obtained on hormonal control will be presented.

Beta-Endorphin Effects on Pancreatic Hormones.

The effects of B-EP on plasma glucose concentration supports the notion that B-EP-induced hypoglycemia is secondary to its effect on pancreatic hormones. Thus, researchers attempted to assess the effects of B-EP on insulin and glucagon. A great deal of controversy exists, at least, for the B-EP effect on insulin synthesis and release.

A. In humans.

B-EP infusion at low pharmacological doses (0.05 $mg*h^{-1}$) under pancreatic clamp with somatostatin infusion, did not affect plasma glucose turnover rate (production, utilization and clearance) (Paolisso

et al. 1987). However, in the same study, in the absence of the clamp, B-EP at the same dose caused hyperglycemia, indicating that the metabolic effects of B-EP are a consequence of the changed hormonal millieu and not the expression of intrinsic metabolic properties of the opioid. Thus, B-EP-induced hyperglycemia in normal subjects is probably a secondary effect to its regulation on pancreatic hormones. A single intravenous bolus (2.5 $mg*kg^{-1}$) of B-EP increased plasma insulin and glucagon levels in women (Reid et al., 1981). Feldman et al. (1983) found a bell-shaped dose-response curve for the effects of B-EP on insulin. The threshold dose for B-EP was 5 ug, with a maximal insulin response obtained after a 50 ug bolus. The highest dose tested (2.5 mg) produced only small changes in plasma insulin.

It is still uncertain whether B-EP effect on glucose homeostasis is direct or indirect. Research presented in the last paragraph suggested that, in humans, the opiates stimulate the release of both insulin and glucagon but they increase glucagon more than that of insulin. This results in an increased glucagon to insulin molar ratio which may play a major role in the opioid-induced hyperglycemia (Reid et al., 1981; Feldman et al., 1983).

B. In vivo experiments.

In contrast with the Paolisso et al. (1987) study in humans, Radosevich et al. (1984) showed that a low dose of B-EP (0.2 $mg*h^{-1}$) in conscious dogs resulted in a fall in plasma glucose concentration. This effect was observed in the absence of changes in circulating levels of insulin, glucagon, catecholamines, and cortisol.

This was controlled, in part, by clamping pancreatic hormones at basal levels with somatostatin plus intraportal replacement of insulin and glucagon. These authors suggested that the hypoglycemic effect of B-EP was not mediated by other hormonal changes.

There are several studies that used a central B-EP infusion to examine its role in regulating glucose homeostasis. Intracisternal infusion of B-EP in rat increased plasma insulin and its half-life. This decreased blood glucose but was reversed by naloxone (Bartolome et al., 1989). Intracerebroventricular administration of B-EP in dogs, increased peripheral insulin two fold while glucagon remained the same (Radosevich et al., 1989). However, in these two studies, central B-EP produced hypoglycemia since insulin release was not accompanied by glucagon stimulation. Beta-LPH injected intravenously at 10 $mg*kg^{-1}$ in fed rabbits increased plasma insulin within ten minutes (Schwandt et al., 1981). Green et al. (1980) suggested that the stimulatory effect of opioids on insulin secretion has been found to be rapid and transitory during continuous perfusion of the pancreatic islets with enkephalin and morphine (but not B-EP in this study). Insulin returned to baseline during the non-infusion period (Green et al., 1980).

Numerous other studies produced opposite results, describing an insulinopenic (fall of plasma insulin) effect of B-EP both in vivo and in vitro: in the rat, B-EP infusion decreased plasma insulin and increased plasma glucagon and somatostatin (Matsumura et al., 1984). In dogs, B-EP administration attenuated the epinephrine-induced release of glucagon and insulin but did not modify basal

pancreatic alpha and beta cell secretion (Tannenlbaum et al., 1979). In rabbit, B-EP inhibited insulin release, while it potentiated glucagon release (Schleicher et al., 1987). B-EP caused inhibition of insulin secretion which was not blocked by naloxone (Rudman et al., 1983). This suggested that the effect of B-EP on insulin was not mediated by μ-receptors. In addition, the lack of response to D-ala^2-D-leu^5-β_h-endorphin or etorphine appears to exclude a delta or epsilon receptor. Furthermore, the same study, noted that almost the entire free B-EP structure (1-31) was required for the in vivo insulinopenic response.

Finally, several studies have noted that B-EP does not cause any changes in the release of insulin. Intravenous B-EP infusion in dogs did not affect insulin and glucagon release (Radosevich et al., 1989). Infusion of B-EP at 0.5 mg*h^{-1} did not affect plasma insulin. This occured despite plasma glucose increasing (Paolisso et al., 1987). Passive immunization of rats with B-EP antibodies did not change basal plasma insulin release. These authors have questioned the physiological role of B-EP in the regulation of insulin secretion (El-Tayeb et al., 1985).

C. Studies with naloxone.

Naloxone has been used to evaluate if B-EP influences pancreatic endocrine function through a mu opioid receptor activation. Unfortunately, this methodology added to the discrepancy that exists in this field. Studies with naloxone indicate that endogenous opioid peptides stimulate insulin secretion which could result in decreased

insulin sensitivity (Locatelli et al., 1985; Schusdziarra et al., 1984). Naloxone was able to reverse the effects of centrally administered B-EP, lowering insulin release (Bartolome et al., 1989). However, some hormonal effects of B-EP are resistant to naloxone (Feldman et al., 1983).

In contrast, injection of naloxone in dogs did not block glucose, glucagon, and insulin responses to B-EP (Tannenbaum et al., 1979). B-EP-induced inhibition of insulin secretion was not blocked by naloxone in rabbits showing that the insulinopenic effect of B-EP was not mediated by receptor subtypes associated with opioid activity (Rudman et al., 1983). Naloxone failed to produce any effect on basal somatostatin and glucagon release. The authors indicated that there was not a persisting "tonic" in vitro action of endogenous opioid peptides that can be blocked by the antagonist, but does not exclude such an action in vivo in an intact pancreas (Ipp et al., 1984). In contrast, naloxone was found to stimulate the release of insulin in dogs without affecting glucagon (Ipp et al., 1978).

In general, naloxone partially reverses the action of opioids on pancreatic hormone secretion in vitro (Green et al., 1980, 1983; Hermansen et al., 1983; Ipp et al., 1978; Kanter et al., 1980; Pierluissi et al., 1981; Ryder et al., 1980). The effects of B-EP on insulin secretion from rat and rabbit pancreas are not antagonized by naloxone (Green et al., 1983; Rudman et al., 1983). Naloxone by itself did not affect either glucagon or somatostatin release from pancreatic tissues (Hermansen et al., 1983; Ipp et al., 1978; Pierluissi et al., 1981).

At present, the effect of naloxone on insulin secretion remains unresolved with positive (Green et al., 1983; Hermansen et al., 1983; Pierluissi et al., 1981) and negative findings (Kanter et al., 1980; Rudman et al., 1983). Feldman et al. (1983), offered three reasons why naloxone may not block B-EP effects: a) the dose of naloxone was too small relative to the dose of B-EP. It has been shown that a 10 to 25 fold molar excess of naloxone is needed to block B-EP effects in animals (Rivier et al., 1977; Chicara et al., 1978), b) B-EP effect on human pancreas can be mediated through a non-mu-opioid receptor, c) B-EP might act through a non-opiate pathway. Non-opiate receptors for B-EP have been described in lymphocytes and mouse neuroblastoma cells (Gilman et al., 1982; Hammonds et al., 1981). The presence of specific non-opiate receptors for B-EP in cultured human lymphocytes (Hazum et al., 1979), and the ability of small amounts of B-EP to inhibit specific [^{3}H]-etorphine binding to bovine adrenomedullary membrane preparations (Dumont et al., 1983) strengthen the hypothesis that the peripheral action of B-EP may be mediated through non-opiate receptors. These B-EP receptors are not antagonized by other opiate agonists or by opiate antagonists (Dave et al., 1985). However, the fact that morphine acts through an opiate receptor to produce hyperglycemia argues in favor of an opiate receptor (Ipp et al., 1980).

Research indicates the possibility of non-opiate binding of B-EP to specific non-opiate receptors. B-EP was reported to bind to nonopiate receptors which were coupled to adenylate cyclase in rat liver. Activation of these receptors resulted in increased intracellular

cAMP levels (Dave at al., 1985; Giugliano et al., 1989). Dave at al. (1985) showed that the B-EP-induced formation of cAMP was linear for 45 minutes. Maximal activation of adenylate cyclase activity (148%) was at $0.5*10^{-10}$M of B-EP. Neither the N-terminal acetyl group nor the C-terminal amino acids of B-EP were essential for binding to the peripheral B-EP non-opiate receptors, since [125I]-AchB-EP(1-27) competed with rB-EP(1-31) for binding on these sites. The midportion area of B-EP was critical for optimal receptor binding. In vitro, B-EP increased cAMP in liver cells in a dose-dependent manner (Matsumura et al., 1984). B-EP in low concentrations increased basal cAMP levels in rat islets of Langerhans but it reduced cAMP at high concentrations (Ehrenreich et al., 1986). Furthermore, Jean-Baptiste et al. (1980), reported that B-EP can induce lipolysis through cAMP activation. Beta-adrenergic blockade during exercise impaired exercise time and induced an earlier onset of B-EP release (Schwartz et al., 1989).

A few studies have suggested that there is an interaction between the opiate receptor and the beta-adrenergic receptor systems since naloxone reduced water uptake induced by isoproterenol (a beta-receptor agonist) (Brown et al., 1981), and reduced the hypotensive effect of atenolol, a $beta_1$-adrenergic agonist (Laskey et al., 1989). However, other investigators suggested that opiates inhibit adenylate cyclase activity through functionally coupled receptors, guanine nucleotide-binding protein (G protein) (Sharma et al., 1975; Kuroseet al., 1983; Abood et al., 1985). The Gi inhibitory subunit of G-protein appears to be involved in the opioid

receptor-mediated inhibition of adenylate cyclase (Ui et al., 1984). This was supported when B-EP was reported to decrease cAMP in a dose-dependent manner in rat luteal cells (Kato et al., 1992). The possibility that B-EP interacts with non-opiate receptors, probably beta-adrenergic receptors, might partially explain B-EP actions, such as B-EP-induced liver glycogenolysis or gluconeogenesis.

Table 3 presents a summary of the B-EP or naloxone effects on insulin and glucagon plasma concentrations.

B-EP Studies	Dose	Species	Effect
Feldman, 1983	5-50 μg iv bol.	Humans	Inc. Insulin, Glucagon
Reid, 1983	2.5 mg iv bolus	Humans	Inc. Insulin, Glucagon
Paolisso, 1987	0.5 mg/h iv	Humans	No change
Bartolome, 1989	0.15-1.5 mg/gr	Rats	Inc. Insulin
Radosevich, 1989	0.2 ml/h i.c.v.	Dogs	Inc. Insulin
Ipp, 1978	5.5 mM in vitro	Dogs	Inc. Insulin, Glucagon
Matsumura, 1984	5 μg/100gr iv	Rats	Dec. Insulin, Inc. Gluc.
Rudman, 1983	9 nM iv	Rabbits	Dec. Insulin
Radosevich, 1989	.06 mg/kg/h iv	Dogs	No change
Khawaja, 1990	0.1 mg/kg ip	Mice	Dec. Insulin
Naloxone studies	**Dose**	**Species**	**Effect**
Locatelli, 1985	0.2 mg/kg iv	Humans	Dec. Insulin
Bartolome, 1989	15 μg/gr brain	Rats	Dec. Insulin
Feldman, 1983	120nmol/kg/h	Humans	No effect
Rudman, 1983	$2.9*10^{-6}$M iv	Rabbits	No effect
Ipp, 1984	5 mg/min icv	Dogs	Dec. Glucagon
Ipp, 1978	11 mmol/l iv	Dogs	Inc. Insulin

Table 3. Effects of B-EP and naloxone on insulin and glucagon in plasma. iv denotes intrvenous, icv denotes intracerebroventricular, ip denotes intraperitoneal infusions..

Proposed mechanisms of B-EP action on pancreatic hormones.

There is general agreement thar B-EP stimulates glucagon release but its effects on insulin are equivocal. This discrepancy with insulin is due in part, to the dose-dependent effect of B-EP on insulin. It is also affected by the mode of B-EP administration. It was demonstrated that a dual dose-dependent effect of B-EP on insulin secretion in man occurs (Giugliano et al., 1987). An intravenous bolus (50 ug) of B-EP raised B-EP plasma levels more than 200 fold above basal values. It also raised plasma insulin. In contrast, constant infusion (50 ug$*h^{-1}$) of B-EP i.v. raised plasma B-EP concentration (7 to 8 fold) and inhibited insulin secretion. The latter response was associated with a rise in B-EP that was physiological. Insulin release was also inhibited. This is similar to the B-EP response in exercise (Goldfarb et al., 1987).

Different kinetics obtained with the two routes of intravenous administration appear to affect insulin release. Intravenous injection of B-EP bolus produces high plasma B-EP concentration within ten minutes, while an intravenous infusion progressively increases plasma B-EP concentration (Paolisso et al., 1987). Different experimental species used in the studies presented above could have contributed to some of the discrepancy.

Investigators have attributed B-EP effect on insulin and glucagon release to an inhibition of pancreatic somatostatin (somatostatin usually inhibits insulin and glucagon secretion by the beta and alpha cells respectively). Morphine and B-EP stimulated

insulin and glucagon release while inhibited somatostatin from perfused dog pancreas (Ipp et al., 1978). In this study, somatostatin inhibition by B-EP preceded insulin and glucagon elevations, suggesting the possibility that the primary event in opioid action upon the islets is the inhibition of the secretory activity of the somatostatin-secreting D-cells. However, two in vivo studies do not support a major role for somatostatin as the mediator of the B-EP effects on pancreatic hormone release (Schusdziarra et al., 1983).

Another theory why insulin could be stimulated by B-EP is the hyperglycemia effect. A secondary rise in insulin can result in hypoglycemia (Reid et al., 1981). Chronic opioid administration has been associated with a paradoxical hypoglycemia and hyperinsulinism (Schmid et al., 1953; Reed et al., 1973). This mechanism could also explain the fall of plasma glucose that some investigators observed (Radosevich et al., 1984).

Furthermore, it was suggested that B-EP synthesized in the pancreas can regulate insulin secretion via a paracrine mechanism. This action involved acute membrane effects as opposed to effects on the biosynthesis of insulin (Curry et al., 1987).

An alternative mechanism is that peripherally administered B-EP diffuses into the central nervous system where it produces its effects on pancreas. The circumventricular organs into the brain which lack the blood brain barrier can allow B-EP to be taken up (Pardridge et al., 1981; Radosevich et al., 1989). This hypothesis was also supported by the finding that there was a significant brain uptake of labeled B-EP (Rapoport et al., 1980). However, Houghten et

al. (1980), noted that intravenously injected ^{3}H-B-EP was degraded rapidly at the blood brain barrier prior to entry into the brain hemispheres. This is in contrast to plasma B-EP which remained in plasma 45 minutes after injection. Therefore, based on these findings it does not seem likely that B-EP can diffuse into the brain area from the peripheral circulation. In addition, the uptake of the labeled B-EP was minimal (3-5 %). Therefore, it appears to be improbable that B-EP crosses the blood brain barrier in a significant amount when it is given intravenously, making it difficult for peripheral B-EP to exert actions on the brain (Houghten et al., 1980; Radziuk et al., 1978; Rossier et al., 1977; Marynic et al., 1980).

A more plausible explanation, by Giugliano et al. (1987), is that the prevailing glycemic state determines if B-EP will have a stimulatory or an inhibitory effect on insulin secretion. This hypothesis seems to be supported by a number of experimental findings. In vitro, B-EP increased insulin secretion in isolated islets from both lean and obese mice. However, during a glucose load insulin was inhibited and this effect was blocked by naloxone (Khawaja et al., 1990). In humans, naloxone, decreased insulin response to a transient glucose challenge (Locatelli et al., 1985). Furthermore, naloxone pretreatment decreased the insulin response in obese patients compared to lean controls, after an oral glucose challenge (Giugliano et al., 1987; Vettor et al., 1985). In contrast, in rabbits, when B-EP was given simultaneously with an intravenous glucose load, the glucose-stimulated rise of insulin was prevented and the clearance of the glucose load was delayed (Rudman et al.,

1983). These observations suggest that the discrepancy that exists on how B-EP affects insulin secretion could be due to the differences in the subject's glycemic state at the time of the experiment. There is a trend in the research findings cited in this paragraph towards a stimulation of insulin secretion by B-EP when a hyperglycemic state prevails. One would assume that the opposite would be true (insulin inhibition) in a hypoglycemic condition. However, this remains to be established.

In summary, B-EP effects on insulin secretion is equivocal. Research results appear to agree that B-EP stimulates glucagon release. However, B-EP action on the pancreas has not been determined whether is mediated by an opiate or a non-opiate pathway.

Effects of Beta-Endorphin on Catecholamines.

Catecholamines play an important role in the counterregulation of plasma glucose. Catecholamines stimulate liver and muscle glycogenolysis and lipolysis, or enhance gluconeogenesis. Catecholamines also stimulate the release of glucagon and inhibit the secretion of insulin. Researchers have examined the interelationship between B-EP and catecholamines (norepinephrine and epinephrine) both at the central and peripheral level.

Several studies demonstrate the presence of enkephalin and B-EP like material in the adrenal glands of several species, including humans (Govoni et al., 1981; Linnoila et al., 1980; Lundberg et al., 1979). Opioid peptides were also found in splachnic nerves supplying

the adrenal medulla (Schultzberg et al., 1978). Opiate receptors have been localized on adrenal tissue (Chevkin et al., 1979; Dave et al., 1985). These authors have suggested an interaction between B-EP and catecholamines.

A. In vivo and in vitro experiments.

Sectioning of the sympathetic ganglia reduced the hyperglycemic effect of morphine (Dey et al., 1975; Feldberg and Shaligram, 1972; Feldberg et al., 1983). Peripheral hyperglycemia induced by opioid agonist peptides such as morphine and B-EP, has been speculated to be mediated by an increased central sympathetic outflow from the adrenal medulla and peripheral sympathetic endings (Giugliano, 1988). This was supported by findings that intracisternal administration of B-EP, in conscious adult male rats, increased plasma epinephrine concentration, while adrenal denervation or intracisternal injection of naloxone totally inhibited the catecholamine response to the opioid (Van Loon et al., 1985). Central B-EP activation was reported to act on the paraventricular nucleus of hypothalamus to release catecholamines (Kiritsy-Roy et al., 1986; Gunnion et al., 1988). In contrast, elevated nor-adrenergic activity was found to be attenuated in the dienkephalon by endogenous opioid peptides (Gold et al., 1984). This indicated that opioid effects on catecholamines might vary from one brain area to the other. Finally, the hyperglycemic effect of morphine was found to be depressed when catecholamines were infused centrally (Feldberg, 1972). This suggested some kind of negative feedback inhibition on

the release of B-EP by catecholamines.

Feldberg et al. (1972) noted that morphine stimulated the epinephrine-induced increase in plasma glucose. This raised the possibility that B-EP may contribute to the stimulation of epinephrine release. In fact, morphine was reported to induce hyperglycemia by stimulating the release of epinephrine. This action appeared to be mediated through the central nervous system although the specific site of action was unclear (Feldberg et al., 1974; Vassale et al., 1961). Radosevich et al. (1984) confirmed these findings that morphine administration increased plasma catecholamines. An intracerebroventricular infusion of B-EP caused an increase in peripheral catecholamines (Radosevich et al., 1989). However, another study failed to demonstrate any morphine effect on plasma catecholamines in vivo (Taborsky et al., 1982).

A considerable body of literature which used infusions of both B-EP and its antagonist, naloxone, dispute the findings presented in the previous paragraph. These articles support an inhibitory role of B-EP on the release of peripheral catecholamines. Morphine was reported to inhibit catecholamines (Taborsky et al., 1979). B-EP infusion in dogs attenuated the epinephrine release of glucagon (Tannenbaum et al., 1979), indicating a possible inhibition of epinephrine release by B-EP. Infusion of B-EP in dogs modulated the effects of epinephrine in the liver and inhibited catecholamine-stimulated glucose production (El Tayeb et al.,1985). In vitro results suggested an inhibitory role for B-EP on catecholamine release (Mannelli et al., 1984). Sympathetic reflex response to both pressor

and depressor stimuli were attenuated by morphine or opioid analogs and potentiated by naloxone (Montrastrug et al., 1981; Petty et al., 1981). The inhibitory effect of opioids was further supported by a number of other studies (Illes et al., 1987; Karras et al., 1981; Konishi et al., 1979; Williams et al., 1979).

B. Changes in humans.

Increases in circulating epinephrine and norepinephrine during various stress conditions tend to be greater after B-EP antagonism in humans. Naltrexone, a B-EP antagonist, significantly elevated epinephrine compared to controls, but not norepinephrine during exercise (Farrell et al., 1986). Activation of peripheral sympathetic outflow during isometric exercise was enhanced by naloxone (Farrell et al., 1991). In addition, blocking B-EP with naloxone increased epinephrine at high but not low B-EP doses (Estilo et al., 1982; Manelli et al., 1984). Intravenous naloxone infusion during insulin-induced hypoglycemia produced a decreased hypoglycemic response which was associated with increased epinephrine, glucagon and cortisol (El Tayeb et al., 1986). Finally, naloxone had no effect on resting plasma catecholamines but decreased the insulin-induced hypoglycemia response (Nush et al., 1989).

C. Effects of catecholamines on B-EP secretion.

A small number of studies examined how catecholamines or adrenergic agonists, such as isoproterenol, affect B-EP secretion. In animal experiments, isoproterenol, a beta adrenergic agonist,

resulted in a dose-dependent increase in B-EP (Berkenbosch et al., 1981; Sweep et al., 1989). According to this finding, beta adrenergic blockade should decrease B-EP release. However, it was shown that beta adrenergic blockade during exercise increased plasma B-EP (Schwartz et al., 1989). El-Tayeb et al. (1985) noted that repeated epinephrine infusions did not change plasma B-EP in dogs. Therefore, catecholamines effect on plasma B-EP is not clearly established.

It is certain that more work is needed in this area in order to understand the interrelationship of B-EP and catecholamines.

Lipolytic Function of Beta-Endorphin.

Fat metabolism is a major deteminant of energy utilization both during resting conditions and during stress such as exercise. It is regulated by central and peripheral factors. Many hormones and neurotransmitter peptides are involved in the mediation of both lipogenic and lipolytic metabolic pathways. Insulin is a major lipogenic hormone and catecholamines, growth hormone, ACTH, glucagon are major lipolytic regulators. There is a significant interelationship between carbohydrate and fat metabolism. Excess carbohydrates are converted and stored as fat, and when plasma glucose and liver or muscle glycogen become limited, fat metabolism is enhanced to spare glucose. During the last decade, a substantial amount of experimental evidence has accumulated that implicates B-EP in the lipolytic regulation. Therefore, lipolysis is another area of metabolism that appears to be influenced by B-EP.

Margules (1979), suggested that increased responsiveness of the endogenous opioid system in starvation might conserve energy by inhibiting sympathetics. However, an alternative view (Feldberg et al., 1972) supported the notion that norepinephrine was responsible for short-term lipolysis during starvation. After some time norepinephrine does not solely participate in this function and other substances are involved (Brodie et al., 1963; Goodman et al., 1959; Maickel et al., 1977; Stern et al., 1963). B-EP appears to be one of the substances that might be involved in long term regulation of lipolysis. A number of other candidates for this role exist, such as epinephrine, growth hormone, and ACTH. However, the involvement of B-EP in lipolysis is beyond the scope of this study.

Beta-Endorphin Responses During Exercise

Exercise intensity increases the plasma levels of most hormones. The explanation for an increase in many of the endocrines can be linked either directly or indirectly with exercise metabolism. However, there is not enough evidence to implicate the endogenous opioid system with exercise metabolism. A major question related to the exercise-B-EP literature is the role of B-EP in metabolic regulation. It is difficult to assign any direct significance of plasma B-EP to tissue responses during exercise. Careful studies need to be conducted in order to determine the role of B-EP on metabolism during exercise.

A. Effects of Exercise on Central Beta-Endorphin.

Although knowledge of peripheral opioid functions is rapidly accumulating, the best characterized opioid systems are still in the central nervous system. Despite this fact, most research into exercise and opioids has determined peripheral effects. Only a limited number of studies have investigated central opioid activity with exercise. Exercise appears to influence central endorphin function, but no systematic conclusions can be drawn at present. In addition, most of this work has employed animal models, making implications for human responses difficult.

Prolonged exercise was shown to alter opioid peptide content in discrete brain areas, but not throughout the brain (Blake et al., 1984). B-EP and leu-enkephalin in the nucleus acumbens were increased by exercise. However, this could indicate either an increase synthesis or a decreased release. Twenty to thirty minutes of swimming resulted in a significant decrease in hypothalamic B-EP in rats (Wardlaw et al., 1980). However, this finding was not confirmed by a study which used sprint running (Metzger, 1984). Furthermore, Sforzo et al. (1986), found increased binding of an opioid antagonist (^{3}H-diprenophine) in five brain regions following two hours of warm water swimming. This could indicate a decreased opioid release following exercise providing decreased competition to the injected ligand for available receptors. It also could suggest B-EP release to other areas or increased receptors. Decreased C-carfentanil (a μ-receptor agonist) binding following treadmill exercise was observed using positron emmission tomography (Wagner et al., 1986).

Hoffmann et al. (1990), observed a significant increase in B-EP concentration in cerebrospinal fluid of rats trained to run spontaneously compared to sedentary controls.

In summary, reports on exercise-induced changes in central B-EP are inconsistent. More information is needed to confirm that exercise influences central B-EP response.

B. Effects of exercise intensity on Peripheral Beta-Endorphin.

Peripheral B-EP levels increased from resting values after a graded exercise test in all studies. The magnitude of this exercise-induced increase in plasma B-EP ranges from 1.5 fold (Goldfarb et al., 1987) to 7 fold (Olehansky et al., 1990). The variation in findings might be partly due to methodology since both the intensities and durations at each stage varied. In addition, different types of exercise and different protocols to determine intensities were used. Another explanation for the varying increases in B-EP levels might be a variable degree of exhaustion and method to measure B-EP.

Investigators have suggested that exercise-induced increase in B-EP is intensity dependent (Colt et al., 1981; Donevan et al., 1987; McMurray et al., 1987; Rahkila et al., 1987; Goldfarb et al.,1990), whereas others had contradictory findings (Farrell et al., 1982; Goldfarb et al., 1987). DeMerleir et al. (1986), included lactate as an intensity-related metabolic parameter. It was assumed that B-EP levels in blood were dependent upon anaerobic metabolic demand. During a graded exercise test, at a RER of 0.85 and a lactate

concentration of 3 mmol*l^{-1} no significant changes in B-EP levels were noted. In contrast, at exhaustion, B-EP levels increased in parallel to lactate values. It was also shown that B-EP increased significantly during exercise above the lactate threshold (75% of VO2max) and after the cessation of exercise almost 3.6 fold (Schwartz et al., 1990). Thus, according to the authors, the point of overproportional increase in lactate concentration and B-EP level is coincident. In addition, maximal increases in B-EP correlated with the maximal lactate levels suggesting a connection between the extent of the B-EP increase and anaerobic metabolism. Mougin et al. (1987) reported a significant correlation (0.6) between plasma lactate and B-EP concentrations after a long distance nordic ski event. However, it must be underlined here that a significant correlation between the B-EP and lactate responses does not establish a cause and effect relationship. In addition, these high correlations between B-EP and lactate concentrations were observed during non-steady state conditions and were not obtained during steady state exercise. Goldfarb et al. (1991), observed a dissociation between B-EP and lactate concentrations during the later stages of prolonged exercise. In that study, increases of lactate levels at 60% of VO2max were not accompanied by plasma B-EP elevation. Furthermore, endurance exercise at a lactate steady state was not able to stimulate a peripheral B-EP increase (Sforzo et al., 1988).

An acute anaerobic exercise increased plasma B-EP 2-4 fold which was lower than that obtained with graded exercise test (Brooks et al., 1988; Rahkila et al., 1987; Schwartz et al., 1990).

As it was pointed out by Goldfarb et al. (1987), B-EP and exercise intensity do not have a linear relationship. It might be best described by a curvilinear model or more probably by a threshold driven release. Figure 4 demonstrates how B-EP response changes with exercise intensity and Tables 4 and 5 list the changes for B-EP in studies which employed a graded exercise test or acute anaerobic bout of exercise (less than 2-3 minutes), respectively.

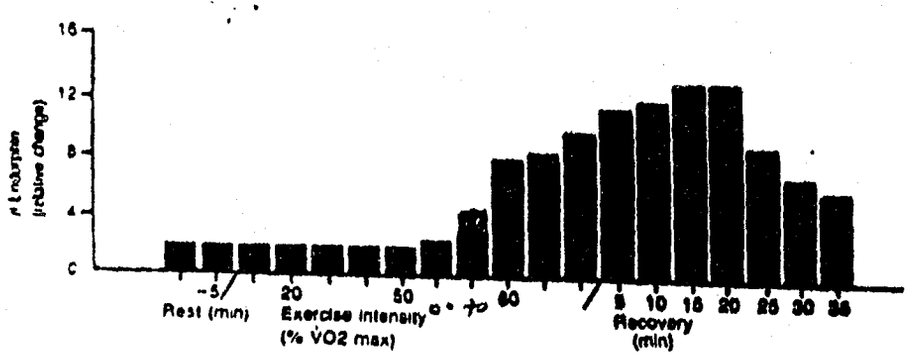


Figure 4. Responses of B-EP to varying intensities of exercise. (From G.A. Sforzo, Sports Medicine, 7: 109-124, 1988).

Reference	Exercise	Intensity	Change in B-EP
Goldfarb, 1987	Cycling	Exhaustion	Increase
Goldfarb, 1990	Cycling, 30 min.	70-80%	Increase
Rahkila, 1988	Running, 10 min.	< 80%	No change
		> 80%	Increase
Elias, 1989	Running	At exhaustion	Increase
McMurray, 1987	Cycling, 20 min.	<70%	No change
		>80%	Increase
Farrell, 1987	Running, 7 min.	60%	No change
Brooks, 1988	Sprint, 30 sec.	100%	Increase
Sonka, 1975	Running	<70%	No change
		>90%	Increase

Table 4. Plasma B-EP responses to varying exercise intensities. (LA denotes lactate concentration).

Reference	Exercise	Lactate Conc.	Change in B-EP
Brooks, 1988	Running, 30 sec	LA=15.6 mmol/L	Increase
Farrell, 1987	Running, 300 sec.	HRmax =196 bpm	Increase
Rahkila, 1987b	Running, 65 sec	LA =11.5 mmol/l	Increase
Kraemer, 1989	Cycling, 195 sec	LA =10 mmol/l	Increase
	Cycling, 6-45 sec	LA = 4-8 mmol/l	No change
Schwarz, 1990	Cycling, 60 sec	LA = 12 mmol/l	Increase

Table 5. Changes in Plasma B-EP associated with an acute bout of anaerobic exercise (less than 3 minutes). LA denotes lactate concentration.

Kraemer et al. (1993), showed that heavy resistance exercise increased plasma B-EP. It appears that the duration of the force production and the length of the rest periods between sets were important variables that influenced plasma B-EP with lifting exercises.

Most investigations indicated that B-EP levels continue to rise for 5-15 minutes into recovery from heavy exercise and gradually return to baseline over the next 45 minutes (Sforzo et al., 1988).

There appears to be a great intraindividual (Sheps et al., 1988) and interindividual variability (Donovan et al., 1987; Goldfarb et al., 1987; Howlett et al., 1984) in B-EP response to exercise. Gambert et al. (1981), indicated that men had greater B-EP responses than women to a 20 minute run on the treadmil at 80% of the predicted

maximal heart rate. However, their sample size was very small.

Finally, emotional stress of the task, i.e. the stress of competition or danger might contribute to the rise of circulating endorphins in some exercise settings (Adams et al., 1987; Oltras et al., 1987). Stress specific increase in plasma B-EP and ACTH levels were often not associated with increase in brain B-EP concentration (Rossier et al., 1977).

In summary, it appears that incremental graded exercise using both aerobic and anaerobic energy sources or short term maximal exercise, increases B-EP levels. There is probably a threshold exercise intensity that is required to elicit an increase in B-EP response which is above 60% $V0_2max$ depending on duration.

C. Duration of Exercise and peripheral B-EP.

Whereas in the relationship between exercise intensity and B-EP response little or no conflicting results exist, there is some controversy in the findings on the relationship between B-EP response and exercise duration. It has been reported both unchanged (DeMeirleir et al., 1986; Elias et al., 1989; Kelso et al., 1984; Kraemer et al., 1989; Langenfeld et al., 1987) and markedly increased B-EP responses (Bortz et al., 1981; Carr et al., 1981; Dearman et al., 1983; Farrell et al., 1982; Gambert et al., 1981; Schwartz et al., 1989) during prolonged exercise. Methodological differences of the above studies could have resulted in this inconsistency: The antibody used by some studies (Carr et al., 1981; Farrell et al., 1982; Gambert et al., 1981) had a high cross-reactivity to beta-LPH. Farrell et al. (1982),

used only a small number of subjects. Little information was presented for intensity of exercise, just maximal heart rate (Carr et al., 1981; Gambert et al., 1981). This workload varies from person to person. Some authors with the same intensity of exercise (80-85% of maximal heart rate) did not show any changes in B-EP levels (Bullen et al., 1984; Elias et al., 1989).

One factor that might have to be considered in this discussion, is the intensity of the work performed during prolonged exercise. There might be a threshold intensity below which no changes in circulating B-EP are observed. DeMeirleir et al. (1986), reported no increase in B-EP plasma levels after one hour of cycle ergometer exercise at submaximal workload below the lactate threshold of 4 $mmol*l^{-1}$. Comparable results were obtained by McMurray et al. (1987), who showed that B-EP increased only at an intensity of 80% of maxVO2. Goldfarb et al. (1990), used 12 college-aged men who completed three submaximal sessions of cycle ergometer exercise, on separate days at intensities of 60, 70, and 80% of maxVO2. Cycling at 60% of maxVO2 did not alter plasma B-EP levels whereas at 70% and 80% max V02 increased B-EP significantly. Two other studies found that long-term exercise at 60% of maxVO2 did not alter plasma B-EP levels after one hour (Langerfeld et al., 1987; Rahkila et al., 1987). Furthermore, endurance exercise at a lactate steady state was not able to stimulate a peripheral B-EP increase (Sforzo et al., 1988).

In summary, plasma B-EP increase with duration of exercise depends on exercise intensity. (Table 6 presents experimental results on how B-EP changes in response to prolonged exercise).

Reference	Exercise	Intensity	Change in B-EP
Carr, 1981	Cycling, 60 min.	85% of HRmax	Increase
Gambert, 1981	Running, 20 min.	80% of HRmax	Increase
Farrell, 1982	Running, 30 min.	60% of VO2max	Increase
		80% of V02max	No change
Bullen, 1984	Cycling, 60 min.	50-80% of HRmax	Increase
Kelso, 1984	Cycling, 120 min.	50% of VO2max	No change
De Merleir, 1986	Cycling, 60 min.	LA = 2.5 mmol/l	No change
Elias, 1989	Running, 20 min.	80% of HRmax	No change
Langenfeld, 1987	Cycling, 60 min.	60% of VO2max	No change
McMurray, 1987	Cycling, 20 min.	40% of VO2max	No change
		60% of VO2max	No change
		80% of VO2max	Increase
Kraemer, 1989	Running, 30 min.	HR = 55 bpm.	No change
Schwarz, 1989	Cycling, 89 min.	63% of V02max	Increase
Goldfarb, 1990	Cycling, 30 min.	60% of V02 max	No change
		70% of V02max	Increase
		80% of V02max	Increase

Table 3. Changes in plasma B-EP in response to endurance exercise. (La denotes lactate concentration, HRmax denotes Maximal Heart Rate, and VO2max denotes maximal oxygen consumption)

D. Adaptations of Beta-Endorphin to Training.

At this point in time, there is not a substantial amount of evidence to support a certain direction of B-EP or opioid peptides in general adaptation to chronic exercise. Met-enkephalin release was mitigated following eight weeks of training (Howlett et al., 1984). No rise in circulating enkephalin was shown following exercise in trained individuals but no pretraining comparisons were made (Farrell et al., 1987; Mougin et al., 1987). Exercise training was reported to augment B-EP response to heavy exercise (Carr et al., 1981; Farrell et al., 1987; Mougin et al., 1987). However, it must be questioned whether greater absolute exercise alone, attainable by trained athletes, was responsible for the differences between the groups in cross sectional studies. Howlett et al. (1984), in a longitudinal study failed to support an adaptive enhancement of B-EP release with training. In contrast, another study showed that maximally attained B-EP levels were inversely related to maxVO2 (Donovan & Andrew, 1987). Goldfarb et al. (1991), reported no difference in B-EP reponse between trained and untrained subjects at similar relative intensity of exercise.

Blake et al. (1984), indicated that training decreased opioid activity in the amygdala of trained rats. Also, a 22-week period of treadmill running resulted in downregulation of the number of opioid receptors in rats (Houghten et al., 1986). Recent studies indicate that different types of training may bring about different effects on the release of B-EP during exercise with anaerobic or sprint-type training producing the largest hormonal increase

(Kraemer et al., 1989). It was suggested that opiates produced in the body during exercise are degraded more slowly in the blood of trained individuals compared to the untrained subjects (Jaskowski et al., 1989). Finally, Hoffmann et al. (1990), observed a significant increase in B-EP concentration in cerebrospinal fluid of rats which were trained to run spontaneously compared to sedentary controls

In summary, the experimental evidence is equivocal concerning training effects on B-EP levels both centrally and peripherally. Some studies demonstrated an increase, others no change and still others a decrease. This may be in part, due to the type of training, the tissue examined and methodology.

Metabolic Effects of Beta-Endorphin During Exercise.

Exercice-associated increases of peripheral blood B-EP levels were related with changes in mood state and pain perception (Arentz et al., 1986; Jungkunz et al., 1983; Olausson et al., 1986; Paulev et al., 1989; Pertovaara et al., 1982; Shyu et al., 1981). This made it possible to assign well known sports related phenomena, such as exercise-induced analgesia and "runners-high" phenomenon to a biochemical substance. Unfortunately, studies examining this topic have reported contradictory results using naloxone as the primary investigative tool (Cumming &Wheeler, 1987). A review published by Cumming and Wheeler (1987), concluded that "claims of exercise-related improvements in mood as mediated via opioidergic pathways are largely hypothetical".

In addition to the changes of mood state found with exercise, there are indications that exercise-induced changes of pain perception are connected with increased levels of B-EP in the periphery. Modulation of pain was described after exercise with a predominantly anaerobic component (Arenz et al., 1986; Kempppainen et al., 1985). The authors suggested that the analgesic effect of endogenous opioids occurs in relation to the metabolic condition of increased lactate acidosis. Considering the correlation between exercise-induced increases in B-EP and lactate levels, the acidosis tolerance might therefore be influenced by endogenous opioids. However, these conclusions are speculative, because so far there is no evidence that this occurs.

While no conclusive results have been presented for the above hypothesized functions of B-EP during exercise, evidence is mounting to suggest that opioid peptides, particularly B-EP, play an important role in resting metabolism. B-EP seems to have a part in both glucose and fatty acid metabolism. Considering the metabolic acceleration that accompanies exercise, the adaptations that follow physical training, and the opioids' potential input for these changes, it is surprising that opioid-regulated mechanisms have not been researched in respect to exercise metabolism.

Negative energy balance during exercise resulted in a greater B-EP increase compared to when energy balance was normal (Walberg-Rankin et al., 1992). This finding suggests that B-EP responded to alterations of human metabolism during exercise. However, hyperglycemia, did not stimulate a rise in circulating B-EP and prior

exercise did not affect this lack of response (Farrell et al., 1986). This could mean that either a positive glucose balance does not stimulate a B-EP response or a hypoglycemic condition such as that obtained in prolonged exercise beyond two hours might be required in order to elicit B-EP release.

Studies describing correlations between ACTH, B-EP and lactate (Buono et al., 1987; Farrell et al., 1983), assumed that substances resulting from anaerobic metabolism could stimulate the hypothalamus-pituitary axis via chemoreceptors in the muscle. Sympatho-adrenal activity is needed for a high glycolytic flow rate since glucose must be present in adequate amounts to supply this pathway. Animal experiments indicated that B-EP increased glycogenolysis and gluconeogenesis from L-lactate (Allan et al., 1983; Matsumura et al., 1984). Thus, it appears that the physiological significance of B-EP rise during exercise maybe to mediate glucoregulation.

However, few studies have attempted to examine this possibility in human subjects. They have used naloxone infusion as their major research tool. Naloxone, however, can not always inhibit the actions of B-EP. Also, since B-EP could act indirectly and at multiple locations, more invasive, basic, and extensive procedures are needed. Both central and peripheral responses of B-EP must be examined since actions of centrally located B-EP probably are anatomically and functionally different.

It was reported that cerebrospinal fluid glucose declined and B-EP and ACTH increased towards the end of exercise (Radosevich et

al., 1989). These authors suggested that there was an inverse relationship between cerebrospinal fluid B-EP and ACTH with exercise intensity. The elevations in cerebrospinal B-EP and ACTH were not proportional to elevations in the plasma. Farrell et al. (1988), determined that naloxone did not affect glucose levels during exercise. However, plasma glucose levels were not a good indicator of B-EP involvement in glucose regulation since exercise causes significant alterations in plasma glucose concentration only after prolonged work to exhaustion lasting over 120 minutes. These conditions were not met in this study. Despite the fact that there is some evidence to hypothesize that B-EP might act as a glucoregulatory agent, there is limited information to support this model.

Lipid metabolism seems to have association with B-EP. Exercise induces both adipocyte and muscular triglyceride breakdown. There have been reports of in vivo and in vitro B-EP-stimulated lipolysis (Richter et al., 1983). Naloxone was examined to determine if the opioid system was involved with exercise-induced lipolysis (Vettor et al., 1987). Rats swam for 60 minutes in warm water. Free fatty acid release was attenuated following naloxone injection when compared with saline-injected exercised animals. The authors suggested that increased lipolytic activity during exercise may, at least, partially be under opioid control. They suggested that naloxone apparently had a more general antilipolytic effect. Some evidence indicates a postreceptor antilipolytic effect of naloxone (Jean-Baptiste et al., 1980). Although this data establishes a non-causal relationship

between B-EP and exercise-induced lipolysis, opioid peptides might be involved in triglyceride metabolism during exercise. At this time no investigation of a potential opioid mediation of lipolysis with exercise appears in the literature.

Beta-Endorphin mediated control of glucose and lipid metabolism during exercise might be either direct or indirect. By affecting other agents B-EP may act indirectly. Limited information has been published dealing with the issue concerning the B-EP relationship with the glucoregulatory hormones.

Antagonism with naloxone was used to examine if B-EP might affect insulin during exercise. During sustained hyperglycemia after exercise, the exercise-naloxone group had reduced insulin levels compared to the exercise-saline group and resting-naloxone groups but not with the resting-saline group (Farrell et al., 1988). They suggested a stimulatory role of endogenous opioid peptides on insulin secretion. However, this study showed naloxone effects after and not during exercise and also used a hyperglycemic manipulation. As it has been previously stated, glycemic state of the subjects can influence B-EP response. Insulin secretion was also shown to be inhibited by naloxone after a prior stress (Merrill et al., 1987). No data exist on how B-EP directly affects insulin during exercise. Naloxone could not alter glucose metabolism and glucoregulatory hormone responses (insulin and glucagon) in a number of exercise studies (Bramnert, 1988; Farrell et al., 1986; Grossman et al., 1984; Staessen et al., 1988). These studies however, used exercise protocolas which lasted less than 60 minutes. Glucose metabolism

during prolonged exercise is disturbed only after 60-90 minutes. In the only study that used a 90 minute protocol, naloxone caused relative hyperglycemia while it did not alter insulin or glucagon concentrations in the plasma (Hickey et al., 1994). Nevertheless, it is not safe to assume that B-EP elicits the opposite results or has no effect on the glucoregulatory hormones due to problems with naloxone effectiveness a blocking agent previously mentioned.

Prior work in both humans (Bouloux et al., 1985; Farrell et al., 1986; Lam et al., 1986) and animals (Imai et al., 1988), demonstrated that increases in circulating norepinephrine during various stresses tend to be greater after B-EP antagonism with naloxone; yet these increases were not always statistically significant. At all times during exercise, naltrexone (another B-EP antagonist) significantly elevated epinephrine compared to controls, but not norepinephrine (Farrell et al., 1986). Lipolysis or plasma free fatty acids were unchanged in this study. Activation of peripheral sympathetic outflow during isometric exercise was enhanced by opioid antagonism with naloxone (Farrell et al., 1991). Eventhough this body of literature seems to agree on a inhibitory role of B-EP on peripheral catecholamine release, it must be remembered that B-EP action might be different at the brain level. Also, none of the above studies employed a B-EP infusion, either centrally or peripherally, to explore the direct effects of B-EP on catecholamine metabolism. Futhermore, in non-exercise settings, central infusion of B-EP caused catecholamine activation both centrally and peripherally (Van Loon et al., 1981, 1984; Radosevich et al., 1989), and pripheral infusion of B-EP gave contradictory

results. It does not seem reasonable that B-EP should inhibit catecholamine release and at the same time cause hyperglycemia or lipolysis, unless it substitutes or competes with catecholamines for these actions.

Finally, there is no data to support or refute that B-EP affects exercise performance or running time. One study used naloxone which did not affect running performance (Farrell et al., 1988).

In summary, evidence has accumulated to suggest a role for B-EP in the control of metabolism. The application of this information to exercise models is sparse.

Final considerations.

Kjaer et al. (1989) elegantly demonstrated that afferent sensory input from exercising muscle is active in stimulating circulating levels of B-EP in humans. However, the question as to the extent to which central B-EP is elevated during exercise is still unanswered. Work with animal models suggests that central and peripheral opioid systems are indepedently regulated, and that elevations in peripheral levels can not be taken as an indication of concomitant central responses (Sforzo et al., 1985). Pharmacological evidence clearly suggests centrally mediated effects on pancreatic, adrenal, and hepatic responses (Giugliano, 1984), However, reliance on measurement of peripheral opioid peptides as an index of the activity of the system as a whole is problematic. The use of naloxone is "suspicious". It has the distinct benefit of rapid blood brain barrier (BBB) accessibility and can antagonize both central and peripheral

effects. Its non-selective nature suggests that it would antagonize most B-EP mediated responses. Different subclasses of opiate receptors may act in a modulatory or interactive fashion and non-specific blockade may obviate the significant of these interaction. In addition, some B-EP effects may be mediated through non-opiate receptors.

In summary, exercise of adequate intensity (> 60 % of VO_2max) and duration (> 20 minutes) resulted in significant elevations of B-EP concentration at least in the periphery. However, it is not certain how training state, diet, and acute effects of preceding stressors affect this relationship. Despite the fact that a number of exercise manipulations appear to be stimulatory toward B-EP secretion, little is known about the physiological role that this peptide has under these circumstances. Data from naloxone studies suggest that B-EP may be involved in the subjective response in exercise, and may have a role in exercise capacity as judged by decreases in endurance time (11-13%) (Hickey et al., 1992; Surbey et al., 1984). B-EP also appears to modify adrenal and pituitary hormone responses to exercise (Grossman et al., 1984). More recent data has implicated B-EP in post-exercise insulin secretion in an animal model, and suggested that B-EP could inhibit exercise-induced sympathetic activity (Farrell et al., 1988, 1991).

Metabolic aspects are less clear; few investigations have employed exercise which is a significant fuel stressor, to determine the B-EP effect on metabolic regulation. Thus, the role of the B-EP in fuel mobilization under conditions of elevated fuel demand during

exercise has yet to be elucidated. In conclusion, the B-EP effect on glucose, insulin and glucagon during exercise needs to be determined.

CHAPTER III

METHODS AND PROCEDURES

This chapter will include the following headings: experimental design, subjects, sampling, treatment and analysis of samples, and statistical analysis.

Experimental Design

The purpose of this study is to investigate the effects of B-EP in the regulation of glucose metabolism during prolonged exercise. After catheterization surgery, rats were randomly assigned to one of three treatments (twenty four rats per group). The groups were as follows: a) B-EP infusion, b) naloxone infusion, and c) placebo infusion (normal saline).

Subjects

Animal subjects will be used for this study since administration of B-EP to human subjects is prohibited by F.D.A regulations. Ninenty six male Spraque-Dawley rats, weighing 250-300 grams, were purchased from Harlan Company, IN (It was determined by power statistical analysis that the animal number necessary to obtain statistical significance at the 0.05 level is 72. The rest of the animals

were used for pilot experiments and practice of the catheterization technique). Animals that did not recover from the surgical operation for catheter placement or did not survive the running protocol were replaced so that at least 76 animals completed the experiment. Animals were individually caged in the animal care facility of the University of North Carolina at Greensboro. The room was kept on a 12-12 light-dark cycle at a temperature of 22 $\pm$ 1^{o}C. Animals were fed a standard laboratory rat chow diet and tap water was provided ad libitum. Body weight and amount of food consumed was monitored daily in order to assure proper growth of the experimental animals. Approval for this study by the Institutional Animal Care and Use (IACUC) committee of the University of North Carolina at Greensboro was granted to the investigator.

The animals arrived at the animal facility and randomly assigned to groups of 24. A pilot study with at least five rats was conducted by the investigator before the commencement of the actual experiment, in order to assure the proper performance of the protocol. This enabled him to familiarize himself with the animals, procedures, and instrumentation.

Three days after arrival, rats were familiarized with treadmill running on a motorized rodent treadmill by running daily for 10 minutes at 22 meters per minute at 0% grade for four days (Farrell et al., 1988). After this familiarization period, animals were catheterized according to the procedures described by Gao et al. (1992). The cannulas and surgical instruments were sterilized overnight using sterilizing and disinfecting solutions. Animals were

anesthetized with ketamine (60 $mg*kg^{-1}$) and xylazine (2 $mg*kg^{-1}$) by intramuscular injection. These anesthetic agents provide appropriate anesthesia and analgesia and a quick recovery. The skin at the ventral and dorsal portions of the neck and the abdomneen was shaved and blotted with 70% isopropyl alcohol. A 25 mm longitudinal skin incision was made over the right external jugular vein. The vein was exposed (10 mm) and the salivary glands and subcutaneous tissue were moved slightly to allow placement of the cannula. The cannula was composed of 20 gauge polyethylene (PE 20) tubing. A loop was made at the end of the cannula in order to minimize the movement of the tube in the vessel. The cannulas were channeled subcutaneously and through the skin on the back of the neck with a trocar instrument. The jugular vein was punctured with a 27 gauge hypodermic needle. The cannula was inserted into the hole and advanced 5-10 mm into the vessel. The cannula's placement in the vein was checked by withdrawing blood from the cannula. The loop of the tube was placed under the salivary glands. The cannula was anchored to the midline neck muscles just anterior to their attachement at the sternum using a 4-0 silk thread previously tied to the PE 20 tube. A small drop of "super glue" was applied to the tubing and the underlying connective tissue. The connective tissue beneath the skin was adhered along the midline with "super glue" and the skin incision was closed using a 4-0 silk thread. The outside portion of the cannula was connected to the skin behind the neck with a 2-0 silk thread. The cannulas were capped with segments (15 mm) of a stainless steel stylet (wire). The animals received penicillin

(60 mg*kg^{-1} i.m.) after the surgery and returned to their cages and observed until they recovered from anesthesia. The patency of the cannulas was maintained by daily flushing. The cannulas were washed with 0.2 ml of normal saline and filled with heparinized saline (100 U*ml^{-1}). All surgical and cannula maintenance procedures were performed under aseptic conditions to reduce the possibility of infection.

After the surgery animals were allowed to recover for 5 days and then went through a second identical familiarization running period. Only rats who surpassed their preoperative weight and demonstrated similar to preoperative weight gains were used in the experiments. During the first day after catheterization, most rats tended to lose some weight but after 2 days they started regaining body weight.

Familiarization running continued after surgery for 3-6 days. The infusions took place 24 hours after the last bout of familiarization running.

Food was withdrawn the night before, which was 7 hours before the commencement of an infusion. During the experiment, catheters were exposed for infusion. On the day of the experiment inserted cannulas were exposed and connected to syringes (27 gauge plastic) with tubing of PE 20. These syringes were connected to a Harvard Aparratus infusion pump. Synthetic B-EP (Sigma Chemicals, MO) was given by constant intravenous (right jugular vein) infusion at 0.05 mg*kg^{-1}*h^{-1} after an initial intravenous bolus at 0.05 mg*kg^{-1}. B-EP was dissolved in 500 ul distilled water that previously had its

oxygen extracted by bubbling nitrogen into the water. Then, 50 ul aliquots were prepared from the above solution to make 280 ul of 0.9% saline solution. Giugliano et al. (1987), found that constant intravenous infusion at this dose of B-EP raised plasma concentrations of B-EP 7-8 fold, similar to the B-EP elevation observed during stress conditions such as exercise (Goldfarb et al., 1987, 1990, 1991). Naloxone (Sigma Chemicals, MO) was infused intravenously (right jugular vein) at 0.4 $mg*kg^{-1}*hour^{-1}$ after an initial bolus infusion (0.8 $mg*kg^{-1}$). Naloxone was also dissolved in normal saline as B-EP. Jaffe et al. (1990), suggested that doses of naloxone, 0.6-0.8 mg, were able to block μ-receptors while higher doses can produce agonist effects. Normal saline (0.9%) was given intravenously (right jugular vein) at a volume equivalent to the B-EP volume.

During the experimental period, animals were grouped in blocks of six. Each block went through the experimental stages described previously.

Rats run on a motor driven treadmill (Bringham Young, Utah). The speed was initially set at 22 $m*min^{-1}$ at an elevation of 0% for 90 minutes and then it was raised to 28 $m*min^{-1}$ for the last 30 minutes in order to exhaust the animals after a total of 120 minutes. This type of workload represents an intensity of 60-70% of VO_2max of untrained rats (Goldfarb et al., 1981). Endurance running for two hours has been shown to produce a significant decline in plasma glucose concentration (Bergstrom et al., 1973). The rats were enticed to run by mild shock at the back of the treadmill.

Sampling

Prior to and at 60, 90, and 120 minutes of running, six rats in each group were killed by decapitation and mixed venous blood samples were obtained. Mixed venous blood was collected into chilled tubes containing EDTA. Blood samples were immediately centrifuged (3000 RPM for 20 minutes), and plasma was collected and stored at -90^{o}C until analyzed.

Analysis of Samples

Plasma was assayed for glucose, lactate, insulin, C-peptide, glucagon and B-EP. Insulin, glucagon, C-peptide and B-EP were analyzed by commercial radioimmunoassay kits. Glucose and lactate were analyzed by standard spectrophotometric procedures.

1. Plasma Glucose. It was analyzed by an enzymatic colorimetric procedure which utilizes the enzymes glucose oxidase and peroxidase. A Sigma Diagnostics glucose kit (# 510) was used. This method is a modification of the procedure described by Raabo and Terkildsen (1960). The sample was added to a mixture containing glucose oxidase, peroxidase and o-dianisidine. The reaction was allowed to proceed to completion in approximately 30 minutes at 37^{o}C. The final color intensity is proportional to the glucose concentration.

$$\text{Glucose} + 2H_2O + O_2 \text{ ---> Gluconic Acid} + 2H_2O_2$$

$$H_2O_2 + \text{0-dianisidine ---> oxidized o-dianisidine}$$

The following steps were followed:

a) Add 10 ul of standard or unknown duplicate to tubes.

b) Add 1 ml of of combined enzyme - color reagent solution (500 I.U. of glucose oxidase, 100 purpurogallin units of horseradish peroxidase, a buffer, 100 ul distilled water, 1.6 ml of 0-dianisidine solution) and mix all tubes thorougly.

c) Incubate all tubes at 37^{o}C for 30 minutes.

d) Read absorbance at 450 nm. Use water and the combined enzyme - color reagent solution as the blank.

e) A standard curve was drawn and the concentrations of the unknown samples were determined from this curve.

2. Lactate. For the determination of lactate a standard spectrophotometric assay was used (Sigma kit, #826). This procedure is based on the convertion of L-lactate to pyruvate by lactate dehydrogenase (LDH) with reduction of an equivalent amount of NAD. The assay included the following steps:

a) Add standards (0, 10, 20, and 30 ul) and unkowns (10 ul) to the corresponding labeled tubes.

b) Add distilled water to standards (500, 490, 480, and 470 ul) and unknowns (490 ul).

c) Add 500 ul of Glycine buffer (pH, 9.2) to all tubes.

d) Add 25 ul of NAD to all tubes and mix.

e) Add 5 ul of LDH to all tubes and mix.

f) Incubate samples at room temperature for 20-30 minutes.

g) Read absorbance ar 340 nm. The increase in absorbance at 340 nm is proportional to lactate concentration. Unknown concentration were calculated from the standard curve.

3. Plasma Insulin. It was measured by a standard double antibody ^{125}I-radioimmunoassay designed specifically for the quantification of plasma or serum insulin. The kit was purchased from ICN (CA). The procedure included the following steps:

a) Assay was set up in duplicate in 10*75 disposable glass tubes. 200 ul of standards and samples were added into the tubes.

b) Diluent bufer was added into the Non Specific Binding (NSB) tubes (0.4 ml), into the standards (0.2 ml) and samples (0.2 ml).

c) Anti-insulin (antibody) (0.2 ml) was added next into all tubes except the NSB tubes.

d) Next, samples and standards were mixed and incubated at 37^{o} C for one hour.

e) After incubation, 0.1 ml of second antibody was added into all tubes. Then, samples were mixed and incubated at room temperature for at least one hour.

f) After incubation, tubes were centrifuged at 2300-2500 rpm for 15 minutes.

g) Then, the supernatant was decanted and the precipitates were counted in a gamma counter.

4. C-peptide. It is a double-antibody ^{125}I-radioimmunoassy. The kit was purchased from Linco Research Inc, (St. Louis, MO). It utilizes an antibody made specifically aginst rat C-Peptide. Sensitivity of 30 pM can be easily achieved when using a 100 µl palsma sample in a 2-day disequilibrium assay (400 µl total volume). The procedures include the following steps:

a) Assay buffer (300 ul) was added to the NSB tubes while 200 ul of the same buffer were added to the 0 standard and 100 ul to the rest of the standards and samples.

b) 100 ul of standards and samples were added into the tubes.

c) 100 ul of rat C-peptide antiserum was added to all tubes except the NSB and total count tubes and solutions were mixed.

d) Samples were mixed and incubated for 24 hours at 4°C.

e) 100 ul of ^{125}I-Rat-C-Peptide was then added to all tubes.

f) Tubes were then mixed and incubated for 24 hours at 4°C.

g) After incubation, 1.0 ml of precipitating reagent was added into all tubes. Then, samples were mixed and incubated for 20 minutes at 4°C.

h) Samples were then centrifuged for 15 minutes at 3000*g.

i) The supernatant was then decanted and the precipitate was counted in a gamma counter.

5. Plasma Glucagon. Total plasma Glucagon was analyzed using ICN plasma Glucagon RIA kit (# 07-152101). Blood was collected into chilled (EDTA) tubes. The assay was performed in duplicate in glass test tubes. Blanks, eight standards (0, 25, 50, 100, 250, 500, 1000, and 2000 pg*ml^{-1}), and two controls were processed in duplicate. The following steps were followed:

a) Add 0.2 ml of ultra pure water to the blanks.

b) Add 0.2 ml of standard or unknown plasma sample to the tubes.

c) Add 0.2 ml of anti-glucagon to all tubes (except blanks).

d) Samples will be mixed and incubated for 6 hours at $4^{o}C \pm 2$.

e) Add 0.1 ml of ^{125}I-Glucagon to all tubes. Tubes are mixed and incubated at least 16 hours at $4^{o}C \pm 2$.

f) Add 0.2 ml of second antibody-PEG mixture to all tubes and mix.

g) Add 1 ml of cold distilled water to all tubes.

h) Centrifuge at 2300-2500 rpm for 15 minutes and then decant the supernatant.

i) ^{125}I in the precipitates will be counteed in a Packard gamma scintilation counting system for at least 2 minutes per tube.

The average counts of all duplicate tubes were determined. The average blank (NSB) counts were then subtracted from the average counts obtained. This yields the corrected counts which are then divided by the corrected 0 standard counts to obtain the % bound (B/B_0). The % bound is then plotted vs the concentration of Glucagon for all standards. This yielded a standard curve. Sample values were then compared to this curve. The standard curve was drawn on a semi-log paper.

6. Plasma B-EP. Total plasma B-EP was analyzed using INCSTAR plasma B-EP RIA kit (# 46065). Blood was collected into chilled (EDTA) tubes. The assay was conducted in duplicate in glass test tubes. Blanks, five standards (0, 7.1, 14.4, 29.1, 60.9, and 120.0 $pmol*l^{-1}$), and two controls were analyzed in duplicate. The whole procedure consisted of two steps. The first involved extraction of B-EP from plasma using specific absorption particles. This absorbed B-

EP was then easily and quickly eluted from the particles for immediate measurement in a sensitive RIA (minimum detectable amount is < 3 pmol*l^{-1}). The following steps were followed:

a) Add 0.5 ml of well-mixed suspended Sepharose particles to each of the chromatographic coloumns. Allow the supernatant of the Sepharose to drain through the coloumns. The remaining supernatant was forced through the columns using a rubber bulb.

b) Tightly capped columns received 1 ml of each standard, control plasma, or sample1.0 ml of 0 standard for the NSB tubes were used.

c) Each column will be capped and then rotated for 4 hours at 2-8^{o}C in a centrifuger.

d) Columns was placed upright in a rack . Allow the plasma to drain through the coloumns. A rubber bulb helped to force the remaining plasma through the columns.

e) Each coloumn was washed 3 times with 0.85% saline, being careful to rinse both sides of the column. The columns were rinsed until the Sepharose is forced at the bottom of the coloumn. Force all remaining saline from the coloumns using the rubber bulb.

f) Eluting of the B-EP occurred by rinsing the entire surface of the lower column with 250 ul of 0.025 N HCL. This exposed all the Sepharose parrticles to the HCL. Let stand 1 minute, then force the O.025 N HCL through the column into the tubes using the rubber bulb. This step was repeated and a total volume of 0.5 ml was obtained. Immediately assay the eluate according to the following RIA procedures.

g) Place a rack with 12 * 75 mm tubes on crushed ice.

h) Add 0.2 ml of extracted standard or unknown sample in the labeled tubes.

i) Add 01 ml of neutralizing buffer to all tubes except the total count tubes.

j) Add 0.1 of B-EP antiserum to all tubes except the total count and NSB tubes.

k) Mix the tubes without foaming and incubate for 16-24 hours at 2-8^{o}C.

l) Add 0.1 ml of ^{125}I-B-EP to all the tubes.

m) Mix gently without foaming and incubate for 16-24 hours at 2-8^{o}C.

n) Vigorously mix the precipitating complex and add 500 ml to all the tubes except the total count tubes.

o) Mix the tubes without foaming and incubate for 15-25 minutes at 2-8^{o}C.

p) Centrifuge the tubes for 20 minutes at a minimum of 760*g at 20-25^{o}C.

q) Decant the supernatant from all the tubes except the total count tubes and allow them to drain for a minimum time of 2 minutes. Blot the tubes on absorbent paper to remove any drops of supernatant before turning the tubes upright.

r) A Packard gamma scintilation counting system was used to measure the ^{125}I in the pellet of each tube.

The average counts of all duplicate tubes were determined. The average blank (NSB) counts was subtracted from the average counts

obtained. This yielded the corrected counts which were then divided by the corrected 0 standard counts to obtain the % bound (B/B_0). The % bound was then plotted vs the concentration of B-EP for all the standards. This yielded the standard curve. Sample values were then determined from this curve. The standard curve was drawn on a semi-log paper. This procedure has < 5% cross reactivity with b-LPH and <0.01% with α-endorphin, leucine and methionine enkephalins.

Statistical Analysis

A factorial, two-way Analysis of Variance (3*4 ANOVA, treatment by time) was performed in order to analyze the data. Statistical analysis did not include any repeated measures design. When significant interactions were noted in the 3*4 ANOVA, the Neuman-Keuls post-hoc procedure was used in order to clarify the interaction. Comparisons between groups and over time were made by utilizing multiple contrasts between cells. The level of significance was set at $p < 0.05$ level. All data are presented as mean ± S.E.

CHAPTER IV

RESULTS

Subject Characteristics

The experimental design of the present study involved three different treatments (placebo, B-EP, and naloxone). Ninety six rats were initially purchased for the study. Thirteen rats were used for pilot work and for the practice of the surgical catheterization technique. Six animals in each group were sacrificed at four different times (0, 60, 90, and 120 min.). The desired number of at least six animals per treatment for each specific time examined was reached or even exceeded in some cases. The final sample size for the saline group was 26 animals, for the B-EP group was 29 animals, and for the naloxone groups was 28 animals.

The means of initial and final body weights, operative weights, and weight gain of the rats during the experimental period are presented in Appendix B. The summary statistics for main effects obtained from the 3*4 ANOVA for these variables are also given in Appendix B. There were no significant differences between groups or times or a significant interaction effect on the animal weight variables (Appendix B).

The results of the anesthesia and the surgical techniques were satisfactory. The cannulation surgery lasted about 30 minutes per animal. There were no skin infections. The original position of the cannulas was maintained during the entire study. All the animals that underwent successful catheterization operation recovered completely and they were successfully infused (100% recovery rate). The animals' health status was monitored daily. The animals were considered fully recovered by their health status and by their body weight surpassing its preoperative value. All animals' final body weights were larger than the preoperative ones (Appendix B).

In summary, animals in all groups had the same initial and final body weights. The rats gained similar amounts of weight as well. All subjects surpassed their preoperative body weight at the time of the experiment. Therefore, the above factors are unlikely to have affected the outcome of the experiment.

Experimental Characteristics

Animals in all three groups went through the same experimental stages for exactly the same amount of time as it was described in the methodology section (in blocks of six). All rats completed the second familiarization running period with no difficulty despite the fact they were catheterized. All animals were fasted overnight for 7 hours before the initiation of the infusion experiment. This manipulation was intended to minimize variability of carbohydrate content in the animals at the time of the experiment. All experimental procedures took place between 7.00 A.M. and 12.00 P.M. in order to avoid

diurnal variations of the major glucoregulatory hormones (such as cortisol) and B-EP.

Infusion pumps were calibrated before each experiment in order to insure an adequate delivery of the infused substances. Patency of the venous cannulas was maintained for all the animals throughout the entire study byflushing with normal saline (0.9%) daily in order to avoid clotting. No problems occured while the animals were running when they were being infused. In both the pilot and actual experiments, animals which were assigned to run were able to run for the entire 120 minutes. However, signs of exhaustion were obvious for animals that ran 120 minutes. Very mild electrical shocks were used in some instances to motivate the animals to run (primarily in the 120 minute run groups). In summary, no unusual technical problems were presented to the investigators during the performance of the experiments.

Plasma Glucose Concentrations

The means ± S.E. for plasma glucose concentrations in the three groups across time are in Appendix A. Graphic representation of the plasma glucose data in the three groups is in Figure 5.

A 3*4 ANOVA indicated that there were treatment, time but not an interaction of treatment*time main effect ($p < 0.05$) on plasma glucose concentrations (Table 7).

Table 7.

Summary Statistics on Plasma Glucose concentrations

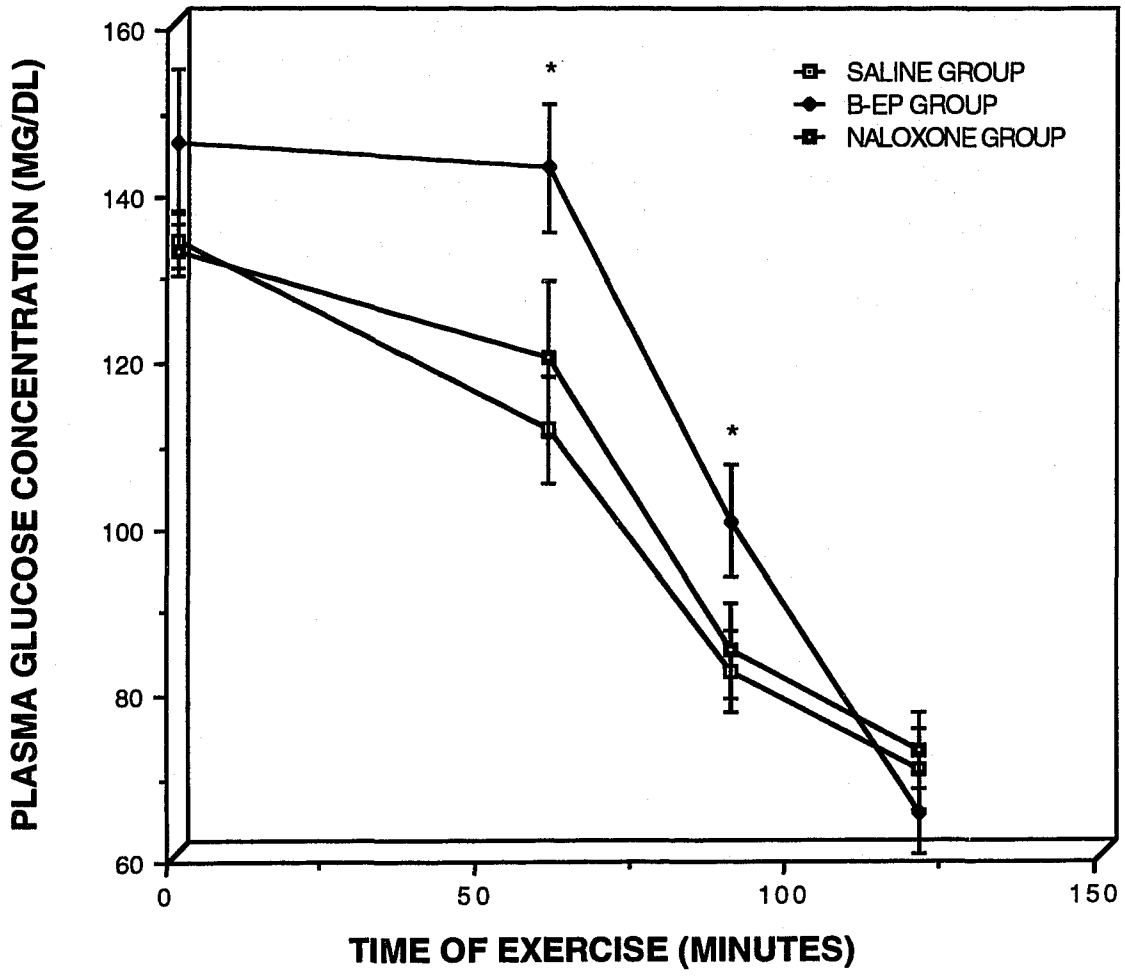
Source	DF	SS	MS	F	Pr > F
Treatment	2	2871.5	1435.8	7.3	0.0015
Time	3	54711.9	18237.3	92.2	0.0001
Treat*Time	6	2566.4	427.7	2.2	0.0592
Error	60	11869.9	197.8		

(Significance at $p < 0.05$)

Plasma glucose concentration declined significantly in all groups across time as compared to resting values (Figure 5). More specifically, in the saline group, plasma glucose levels dropped significantly after 60 minutes of exercise, while in the other two groups, plasma glucose levels started declining significantly after 90 minutes (Figure 5). In general, plasma glucose levels declined by 48% in the saline group, by 56% in the B-EP group, and by 45% in the naloxone group after 120 minutes of running compared to rest.

B-EP infusion resulted in significantly higher plasma glucose levels than the other two groups at 60 minutes of running. Plasma glucose values were still significantly higher in the B-EP group at 90 minutes compared to the other two groups. No other significant differences were noted between treatments at the other times during exercise (Figure 5).

Figure 5. Plasma glucose concentration changes in the three groups over time. * denotes significance differences between the B-EP group and the naloxone and saline groups ($p < 0.05$).



Plasma Lactate Concentrations

The means ± S.E. for plasma lactate concentrations in the three groups across time are in Appendix A. Graphic illustration of the plasma lactate levels in the three groups is in Figure 6.

There were treatment and time main effects ($p < 0.05$) but not an interaction of treatment*time effect on plasma lactate concentrations as indicated by the 3*4 ANOVA (Table 8).

Table 8.

Summary Statistics of Plasma Lactate Concentrations.

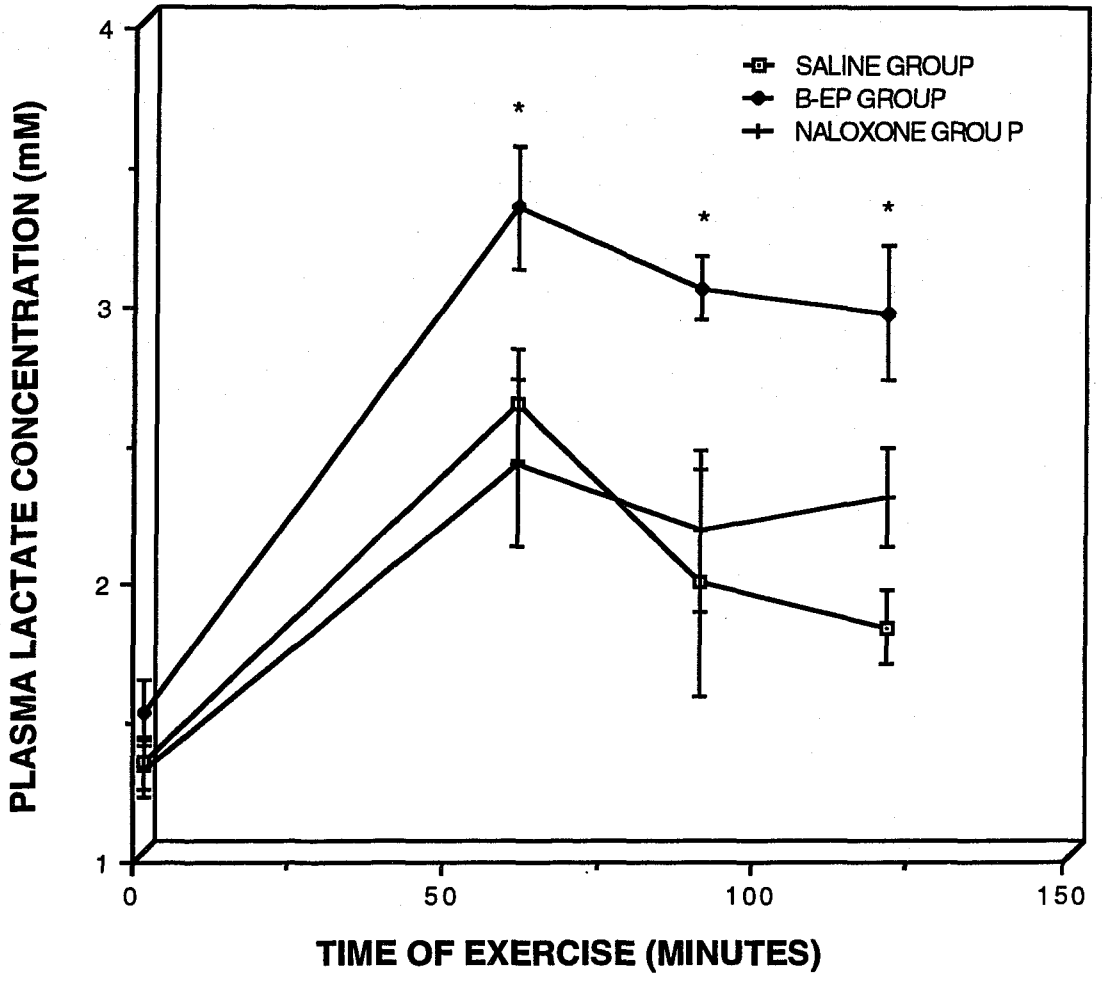
Source	DF	SS	MS	F	Pr > F
Treatment	2	8.9	4.4	14.5	0.0001
Time	3	18.6	6.2	20.3	0.0001
Treat*Time	6	1.9	0.3	1.1	0.3801
Error	60	18.3	0.3		

(Significance at $p < 0.05$)

Plasma lactate concentration increased significantly in all groups over time compared to resting values (Figure 6). Peak plasma lactate concentrations were reached at 60 minutes of exercise in all groups. Thereafter, plasma lactate levels declined slightly but did not return to resting values. In general, plasma lactate concentration increased by 97.8% in the saline group, by 121.3% in the B-EP group, and by 84.6% in the naloxone group (maximal lactate change during exercise).

The B-EP group had significantly higher plasma lactate levels than the other two groups at 60 minutes. This response was maintained at 90 minutes with the B-EP group plasma lactate values higher than the other two groups. At 120 minutes of running, B-EP group had higher plasma lactate levels than the saline group and naloxone group. No other differences between groups were detected (Figure 6).

Figure 6. Plasma lactate concentration changes in the three groups over time. * denotes significant differences between the B-EP group and the saline and naloxone groups ($p < 0.05$).



Plasma Insulin Concentration

The means ± S.E. for plasma insulin concentration in the three groups across time are in Appendix A. Graphic representation of the plasma insulin levels is in Figure 7.

There was a treatment and time main effects ($p < 0.05$) but not a significant interaction effect of treatment*time on plasma insulin concentrations as indicated by the 3*4 ANOVA (Table 9).

Table 9.

Summary Statistics of Plasma Insulin Concentrations.

Source	DF	SS	MS	F	Pr > F
Treatment	2	45.0	22.5	3.7	0.0309
Time	3	2825.0	941.6	154.3	0.0001
Treat*Time	6	48.7	8.1	1.3	0.2577
Error	60	366.3	6.1		

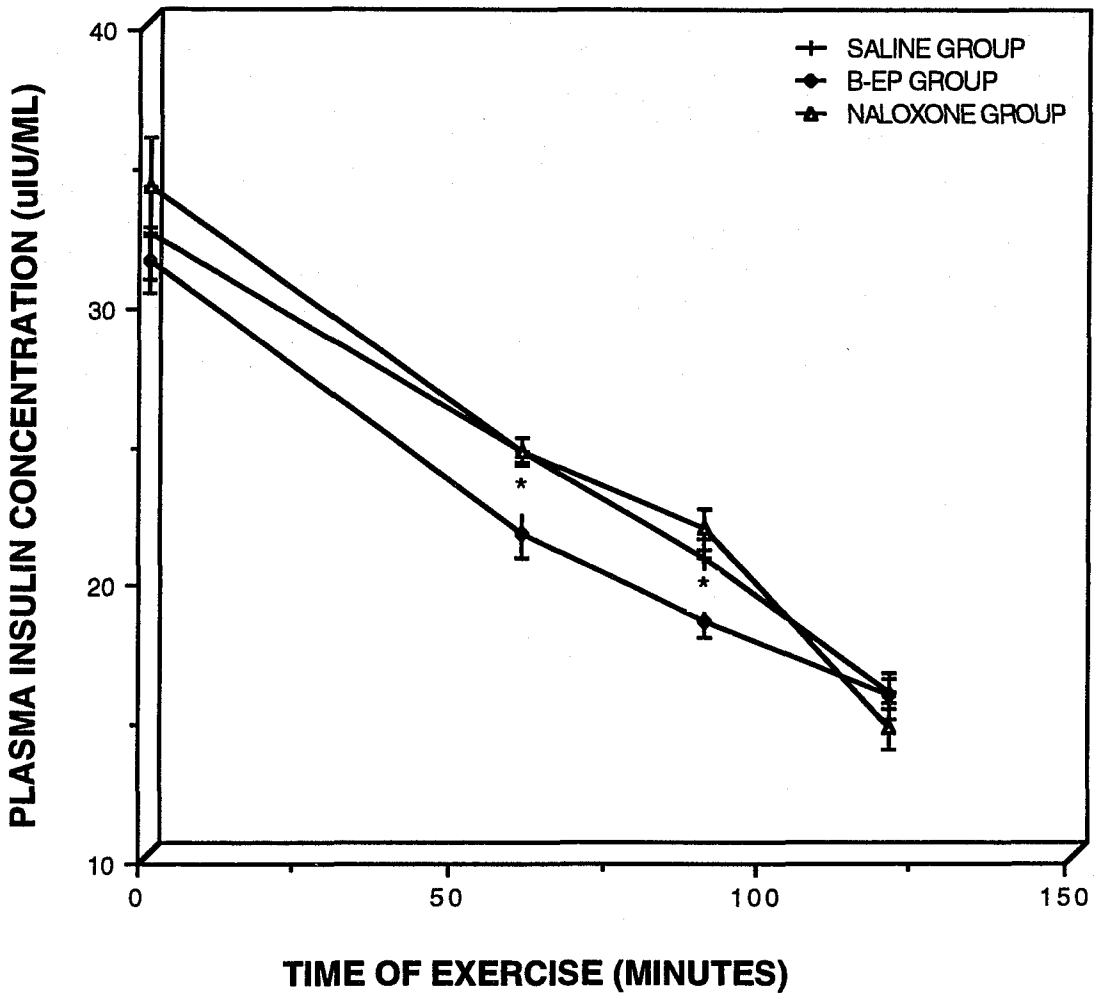
(Significance at $p < 0.05$)

Plasma insulin concentration declined significantly across time compared to resting values in all groups (Figure 7). In general, plasma insulin concentration declined by 51% in the control group, by 50% in the B-EP group and by 57% in the naloxone group by 120 minutes of running.

The B-EP group had significantly lower levels of plasma insulin at 60 minutes of exercise compared to the other groups (Figure 7). In addition, the B-EP group had significantly lower plasma insulin levels

than the naloxone group at 90 minutes (Figure 7). No other significant differences in plasma insulin levels were noted between groups.

Figure 7. Plasma insulin concentration changes in the three groups over time. * denotes significant diferences between the B-EP and the saline and naloxone groups ($p < 0.05$).



Plasma C-Peptide concentration

Plasma C-Peptide (C-P) concentration in the three groups across time are in Appendix A. Graphic representation of the plasma C-P levels is in Figure 8.

A 3*4 ANOVA indicated that there were treatment and time main effects ($p < 0.05$) but not a significant interaction effect of treatment*time on plasma C-P concentrations (Table 10).

Table 10.

Summary Statistics of C-P Concentrations.

Source	DF	SS	MS	F	Pr > F
Treatment	2	52081.4	26040.7	20.2	0.0001
Time	3	1661561.5	553853.8	430.7	0.0001
Treat*Time	6	21217.9	3536.3	2.75	0.0199
Error	60	1812024.9	1286.1		

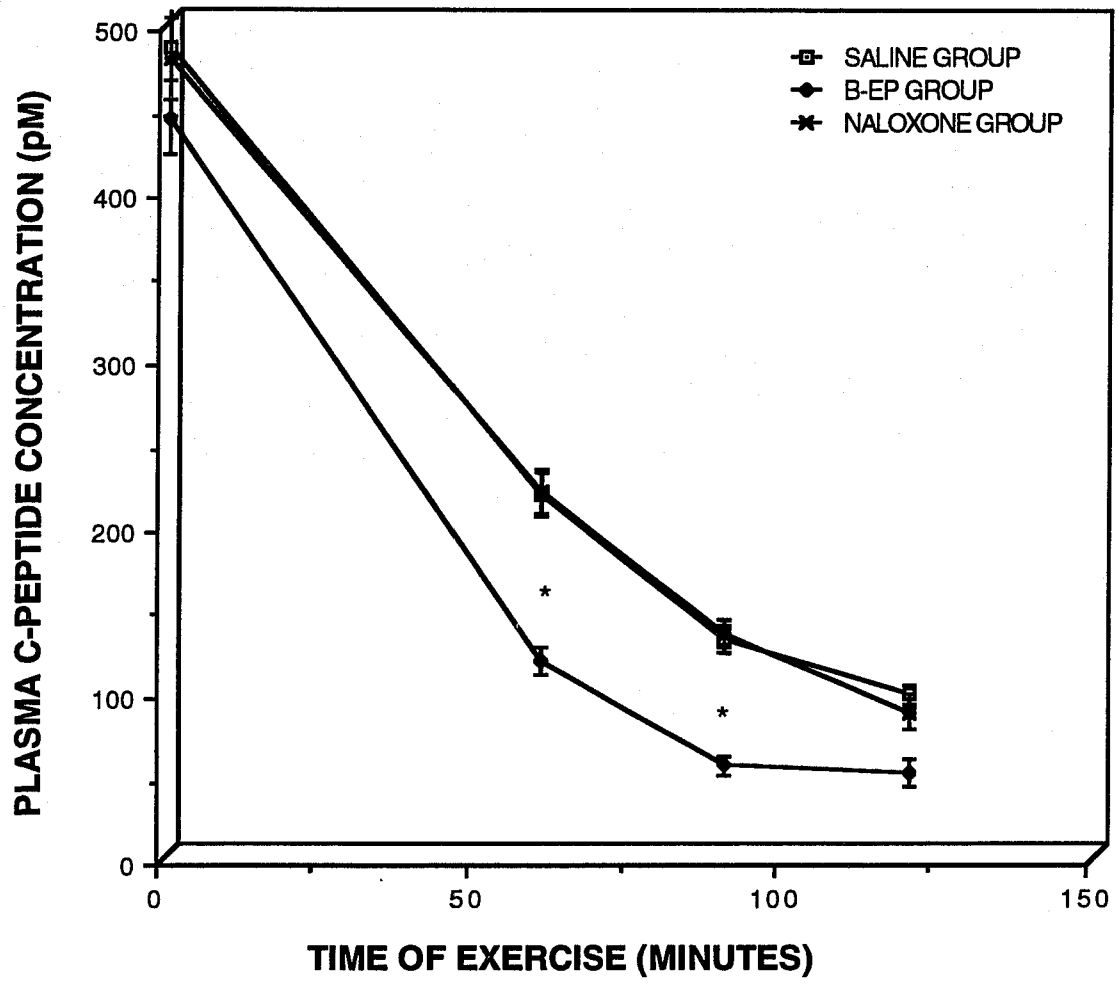
(Significance at $p < 0.05$)

Plasma C-P levels decreased significantly in all three groups with time (Figure 8). This decline was significant after 60 minutes of exercise and continued until the end of the run in all groups (Figure 8). In general, C-P declined by 80% in the saline group, by 89% in the B-EP group, and by 82% in the naloxone group by 120 minutes of running.

Multiple contrasts, following the 3*4 ANOVA for treatment main effects revealed that the B-EP group C-P levels were

significantly lower than the control and the naloxone group at 60 minutes and 90 minutes Figure 8. No other significant differences between groups were detected.

Figure 8. Plasma C-P concentration changes in the three groups over time. * denotes significant differences between the B-EP group and the saline and naloxone groups ($p < 0.05$).



C-Peptide to Insulin Molar Ratio

The means ± S.E. for C-Peptide to insulin molar ratio (C-P/INS) in the three groups across time are in Appendix A. Graphic representation of the C-P/INS is in Figure 9.

A 3*4 ANOVA indicated that there were treatment and time main effects ($p < 0.05$) but not a significant interaction effect of treatment*time on C-P/INS (Table 11).

Table 11.

Summary Statistics of C-P/INS.

Source	DF	SS	MS	F	Pr > F
Treatment	2	1.3	0.64	10.7	0.0001
Time	3	21.9	7.32	122.0	0.0001
Treat*Time	6	0.6	0.09	1.6	0.1603
Error	60	3.6	0.06		

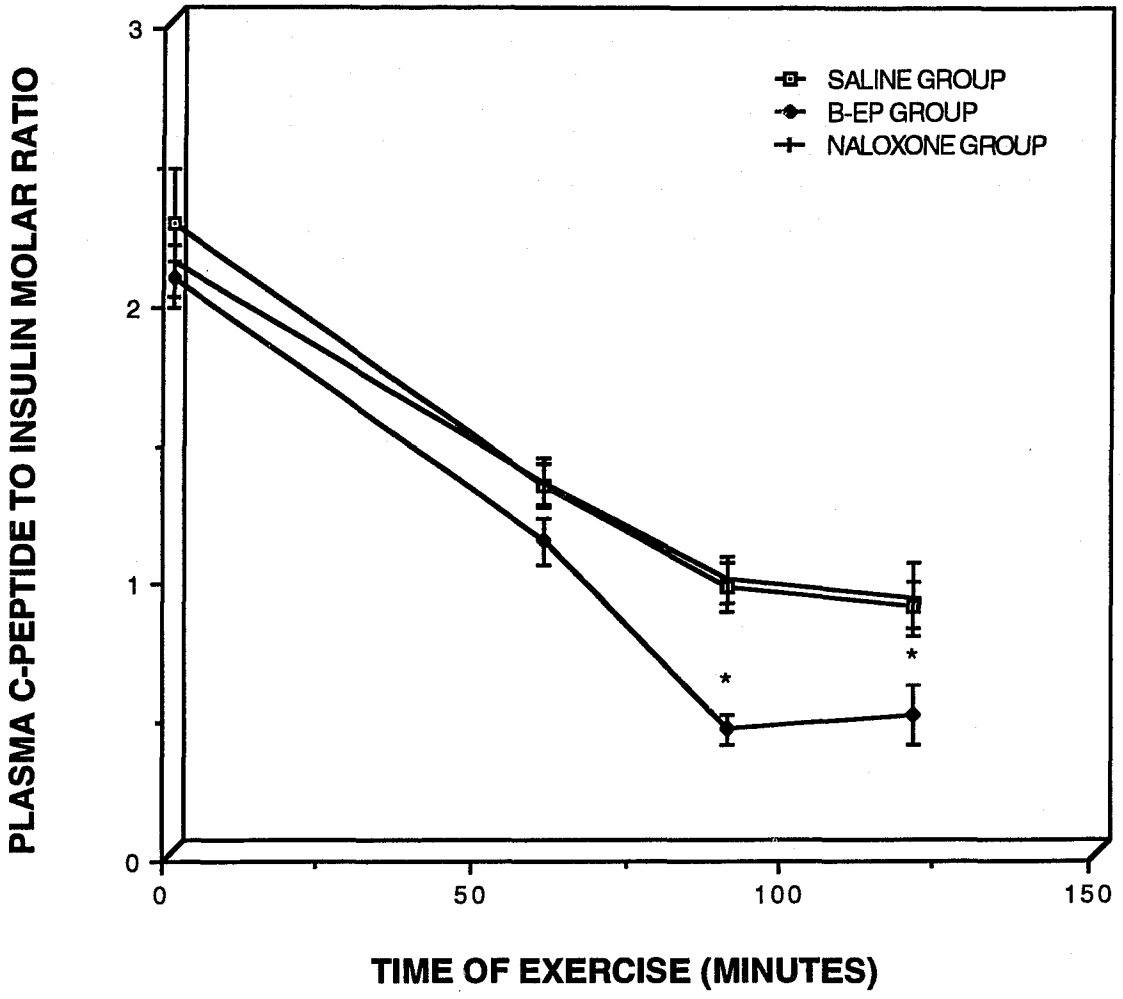
(Significance at $p < 0.05$)

C-P/INS decreased significantly in all groups after 60 minutes of exercise and continued to decline until the end of exercise (Figure 9). In general, C-P/INS declined by 60.6% in the control group, by 76.3% in the B-EP group, and by 57% in the naloxone group by 120 minutes of running.

Multiple contrasts following the 3*4 ANOVA for treatment main effects revealed that the B-EP group had significantly lower C-P/INS values than the other two groups at 90 and 120 minutes of exercise

(Figure 9). No other differences between groups were detected in C-P/INS values.

Figure 9. C-P/INS changes in the three groups over time. * denotes significant differences between theB-EP group and the saline and naloxone groups ($p < 0.05$).



Plasma Glucagon Concentration

Plasma glucagon concentrations in the three groups across time are in Appendix A. Graphic representation of the plasma glucagon levels in the three groups is in Figure 10.

A 3*4 ANOVA indicated that there were treatment and time main effects ($p < 0.05$) but not an interaction of treatment*time effect on plasma glucagon concentrations (Table 12).

Table 12.

Summary Statistics of Plasma Glucagon Concentrations.

Source	DF	SS	MS	F	Pr > F
Treatment	2	283685.5	141842.8	12.8	0.0001
Time	3	4067819.8	1355939.9	122.3	0.0001
Treat*Time	6	63848.8	10641.5	1.0	0.4603
Error	60	665384.3	11089.7		

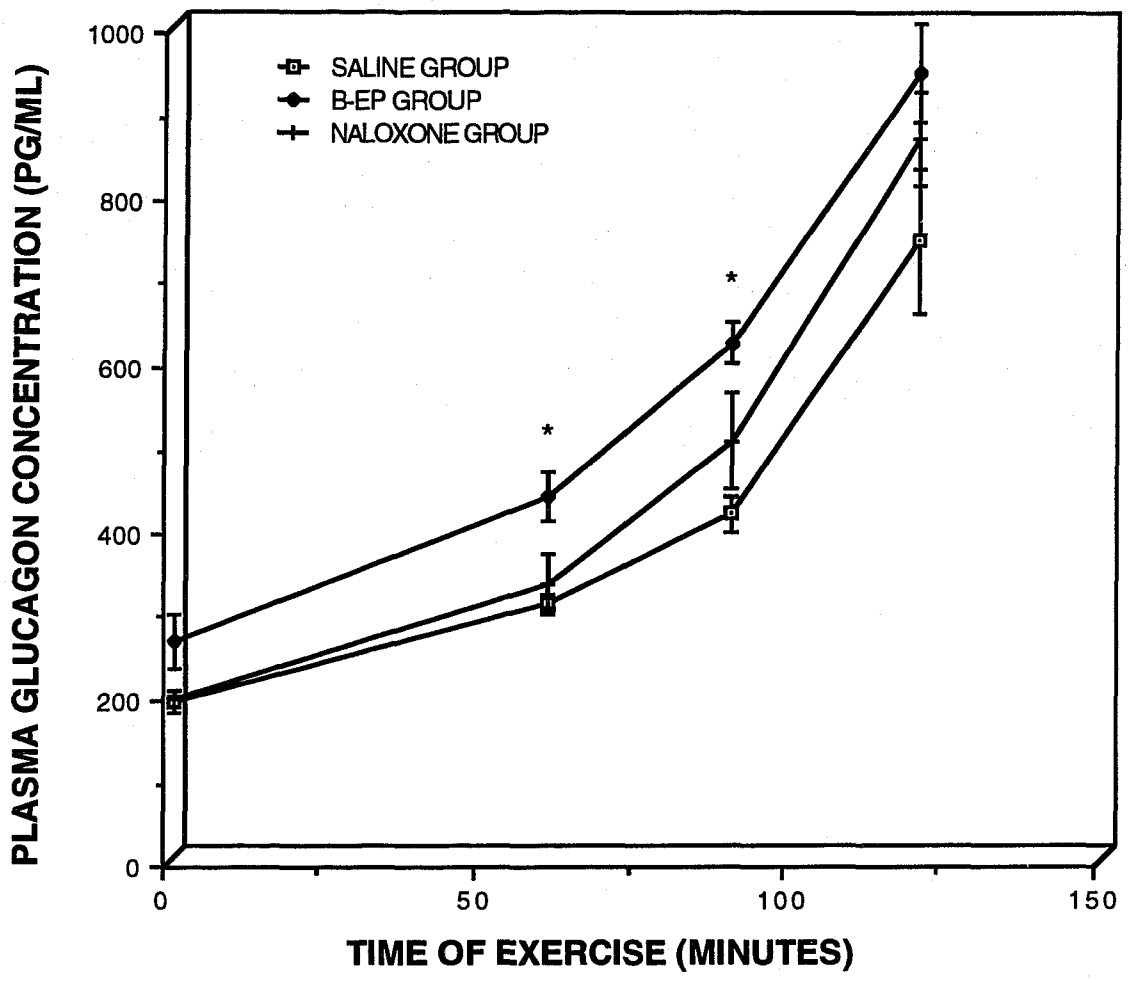
(Significance at $p < 0.05$)

Plasma glucagon concentration increased significantly after 60 minutes of exercise and continued increasing until the end of exercise in all groups (Figure 10). In general, plasma glucagon concentration increased almost four fold in all groups.

At 60 and 90 minutes, the B-EP group had significantly higher plasma glucagon levels than the saline and naloxone groups (Figure 10). At 120 minutes the B-EP group had significantly higher plasma glucagon levels than the saline group but not compared to the

naloxone group. No other significant differences between treatments were detected.

Figure 10. Plasma glucagon concentration changes in the three groups over time. * denotes significant differences between the B-EP group and the saline and naloxone groups ($p < 0.05$).



Plasma B-EP Concentration

Plasma B-EP concentrations in the three groups over time are in Appendix A. Graphic illustration of the plasma B-EP levels in the three groups is in Figure 11.

A 3*4 ANOVA indicated that there were treatment and time main effects ($p < 0.05$) but not an interaction of treatment*time effect on plasma B-EP concentrations (Table 13).

Table 13.

Summary Statistics of Plasma B-EP Concentrations.

Source	DF	SS	MS	F	Pr > F
Treatment	2	3901.2	1950.6	87.8	0.0001
Time	3	10500.7	3500.2	157.6	0.0001
Treat*Time	6	195.9	32.6	1.5	0.2038
Error	60	1332.7	22.2		

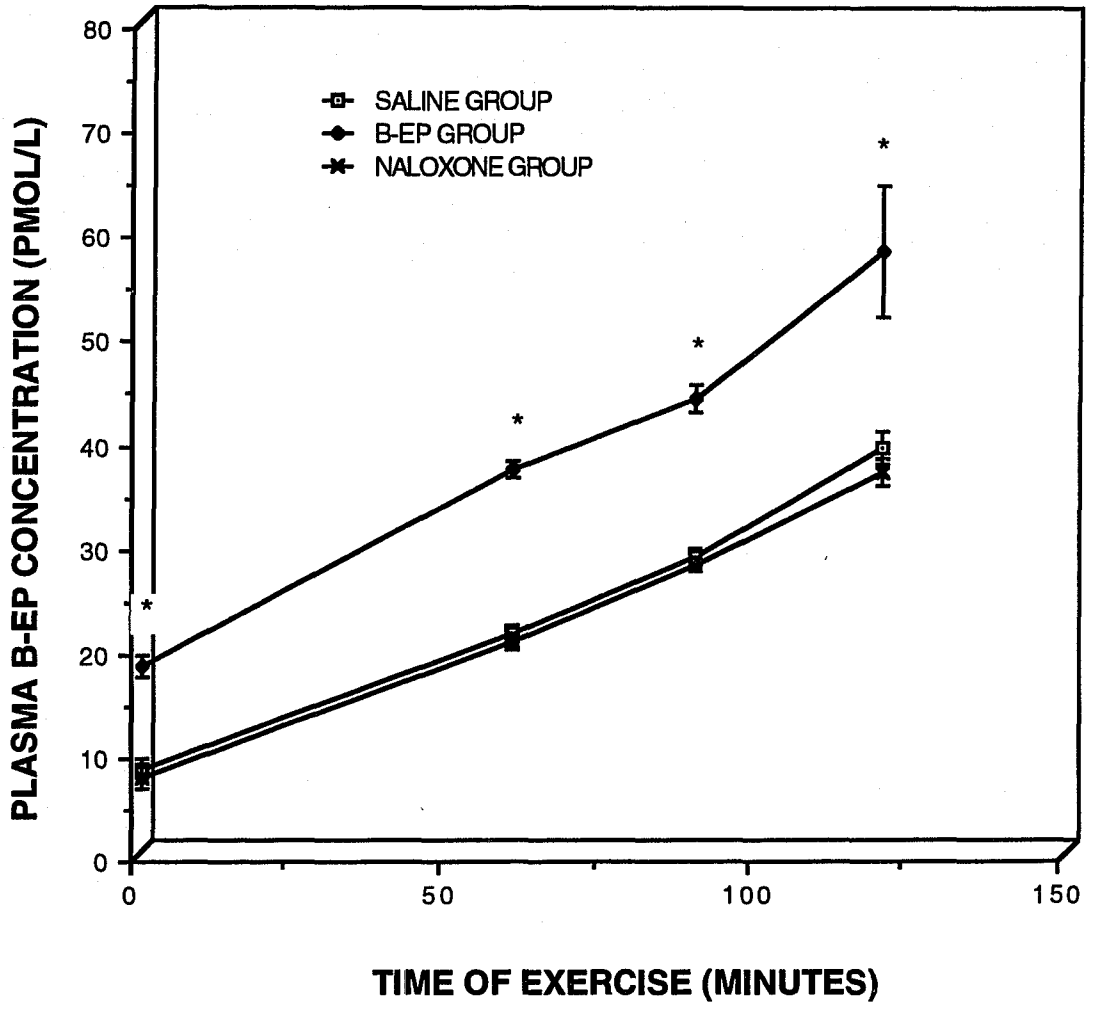
(Significance at $p < 0.05$)

Plasma B-EP concentration was raised significantly after 60 minutes compared to rest and was further elevated at 90 and 120 minutes in all groups (Figure 11). In general, B-EP increased five fold in the saline and naloxone groups and three fold in the B-EP group. However, all groups had increased B-EP by approximately a similar absolute level (25-30 $pmol*l^{-1}$).

The B-EP group maintained significantly higher levels of plasma B-EP than the other two groups at all times (Figure 11). There were

no significant differences between the saline and naloxone groups at any time during exercise.

Figure 11. Plasma B-EP concentration changes in the three groups over time. * denotes significant differences between the B-EP group and the saline and naloxone groups ($p < 0.05$).



Infusion of B-EP and naloxone at rest

In order to separate the drug effect from the exercise effect, a small number of animals was infused with B-EP (n = 3) or naloxone (n = 3) for two hours while animals were resting. The infusion rates were identical to the ones used during exercise. Analysis of the data revealed that infusion of B-EP and naloxone at rest for two hours did not affect any of the variables examined except for the B-EP increase in the B-EP group.

Summary of the Results

All animals which underwent catheterization surgery surpassed their pre-operative weight and demonstrated full recovery. No differences were detected between animals in different groups or different times on their initial and final body weights as well as on their weight gain. While plasma glucose declined with exercise in all groups, the B-EP group showed significantly higher plasma glucose concentrations at 60 and 90 minutes of exercise than the saline and the naloxone group. Plasma lactate increased significantly across all time periods in all groups. Plasma lactate levels were significantly higher in the B-EP group compared to the other two groups throughout the exercise period but not at rest. Plasma insulin was lower in all groups at each time point of exercise. Plasma insulin concentration was significantly lower in the B-EP group compared to the other two treatments at 60 minutes of exercise. At 90 minutes of exercise, the B-EP group had lower levels of plasma insulin than the naloxone group but not the saline group. Plasma C-peptide levels

were significantly lower at 60 and 90 minutes of exercise compared to time 0 in all groups, with the B-EP group demonstrating the lowest plasma C-P values ($p < 0.05$) at these two time points. C-P/INS declined with time in all groups and the B-EP group responded with the lowest values at 90 and 120 minutes ($p < 0.05$). Plasma glucagon increased with exercise in the three groups. However, the B-EP group had higher values at 60, 90, and 120 minutes of exercise ($p < 0.05$). B-EP concentration in plasma increased significantly during exercise in all groups, but as it was expected the B-EP infusion group had significantly higher B-EP levels at all times.

CHAPTER V

DISCUSSION

A major premise of this investigation was that prior research had not employed a model which involved a direct B-EP infusion during exercise to determine its role in glucose regulation during exercise. B-EP has already been documented to increase with exercise of sufficient duration and intensity (Goldfarb et al., 1987, 1990). However, its role in glucoregulation could be just permissive rather than stimulatory. A B-EP infusion should elevate plasma B-EP concentration in order to amplify its actions on glucose homeostasis during exercise. All previous work in the area of opioid involvement in the regulation of substrate mobilization and more specifically in the regulation of glucose homeostasis during exercise, used naloxone infusion either in human or animal models. However, the use of naloxone is dubious. Despite its potential to antagonize both central and peripheral effects of opioids and of B-EP in particular, it has a non-selective receptor action. Different subclasses of opioid receptors may act to modulate or interact with the opioid to produce its effect. A non-specific blockade of the receptors with naloxone could obscure the significance of the opioid effect. Therefore, a direct intervention with B-EP infusion is needed in order to determine whether this

neuropeptide is involved in the regulation of glucose metabolism during exercise.

A second major consideration of this study was that past research had not utilized a model which represented a significant metabolic demand. Thus, this study was designed to examine B-EP effects on the regulation of glucose homeostasis under what is considered a significant fuel mobilizing stressor. In a bout of prolonged duration, substrate flux and subsequent utilization becomes increasingly depedent upon inter-organ communication between muscle, liver, adipose tissue and the related hormonal milieu (Galbo, 1992; Romjin and Wolfe, 1992). Substrate flux and utilization represents a balance between availability, substrate utilization at the level of the exercising muscle, and the underlying homeostatic demand to avoid hypoglycemia. B-EP has been repeatedly demonstrated to increase in response to stress (Goldfarb et al., 1987, 1990), and as such, a regulatory implication for B-EP may take place under conditions that substantially tax body fuel supplies. A protocol of 120 minutes of prolonged running, as the one employed in the present study, meets these requirements (Vranic et al., 1976).

Plasma Glucose Responses

The hyperglycemic effect of morphine, a known opiate which acts in a similar fashion and through the same receptors as B-EP, has been known for many years (Araki, 1891; Feldberg et al., 1972). More recently, studies conducted with humans, animals or in vitro confirmed the hyperglycemic effect of B-EP (Feldman et al., 1983;

Matsumura et al., 1984). However, the major question in this study was if B-EP, which has been repeatedly shown to increase during exercise of a certain intensity and duration (Goldfarb et al., 1987, 1989, 1990), is involved in the regulation of glucose metabolism during prolonged exercise and if so what functions is B-EP influencing?

In contrast to the established hyperglycemic effect of B-EP at basal conditions, there is limited and inconclusive research on the involvement of B-EP on the regulation of glucose metabolism during exercise. Aerobic exercise of moderate intensity is a condition during which substrate mobilization and glucoregulatory hormone responses are drastically accelerated. A few studies that attempted to study the opioid effects on glucose homeostasis during exercise used only opioid antagonism with naloxone and only some of these studies support a role for the endogenous opioid peptides (EOP) in substrate mobilization during exercise (Bramnert, 1988; Farrell et al., 1986; Grossman et al., 1984; Staessen et al., 1985, 1988). The present study represents the first attempt to study the opioid (B-EP) involvement in substrate mobilization during exercise directly by utilizing a continuous B-EP infusion without relying only on indirect observations based on opioid antagonism with naloxone.

The present study demonstrated that there were no differences in the plasma glucose concentration at rest. In the present investigation, animals in the control group showed a significant decline of plasma glucose. Under most physiologic conditions, the rates of glucose utilization by the muscle are precisely matched on a

minute-to-minute basis by similar increments of glucose production (Vranic et al., 1976). During exercise of 60 minutes, in well trained animals or humans, an increased supply of glucose from the liver to the muscle can occur essentially without changes in the concentration of plasma glucose because the increased rate of glucose production by the liver and the increased rate of glucose utilization are sychronous and proportional (Vranic et al., 1969). However, our animals were untrained and were exercised after a 7 hour fast. In contrast to the control group, the B-EP and the naloxone groups did not demonstrate a significant decline of plasma glucose levels after 60 minutes. Beyond the 60 minute mark, all groups demonstrated a significant decrease in their plasma glucose levels with time.

Previous research has shown that prolonged exercise longer than 60 to 90 minutes reduces plasma glucose concentration significantly (Galbo et al., 1976; Vranic et al., 1976). After 60 to 90 minutes of exercise, the need of hepatic glucose production to maintain glucose homeostasis is primarily influenced by continuous, peripheral glucose utilization. Hepatic glucose production declines eventually with prolongation of exercise when hepatic glycogen stores are depleted since the muscle continues to oxidize glucose at a constant rate, hypoglycemia can result. Brain hypoglycemia is presumably the final cause for the inability to continue prolonged physical activity (Issekutz et al., 1970). Thus availability of circulating glucose becomes a limiting factor for any sustained work performance (Astrand et al., 1970; Issekutz et al., 1970).

A major premise of this study was that in order to examine the opioid (B-EP) involvement in fuel utilization, a protocol of adequate duration should be employed. Previous attempts in the same area with naloxone infusion failed to detect a major counteregulatory role for opioids (Bramnert, 1988; Farrell et al., 1986; Grossman et al., 1984; Staessen et al., 1988). We believe that this was in part due to protocols which used exercise duration shorter or up to 60 minutes. This kind of duration might not be an adequate stimulus for opioid involvement in glucose regulation. A running protocol of 120 minutes as the one used in the present study represents a major perturbation of glucose homeostasis. In one study, when a longer than 60 minutes exercise protocol (90 minutes) was used (Hickey, et al., 1994) opioid antagonism with naloxone affected glucose regulation.

The major finding of this study is that plasma glucose levels were maintained significantly higher after a B-EP infusion than after infusion of either saline or naloxone at 60 and 90 minutes of exercise. This study is the first to show a hyperglycemic effect of B-EP during exercise. The present results are in contrast with previous research which employed opioid antagonism with naloxone. Several investigators failed to show any significant alterations of glucoregulation when they infused their subjects with naloxone (Bramnert, 1988; Farrell et al., 1986; Grossman et al., 1984; Staessen et al., 1988). It was inferred that opioids do not play a major role in glucoregulation. However, their experimental protocols were different than the one used here. First, naloxone was infused and not B-EP. Second, exercise protocols were significantly different. Staessen

et al. (1985), showed no changes in glucoregulation during exhaustive graded exercise subsequent to naloxone administration. In another study (Staessen et al., 1988), moderate supine exercise for 20 minutes during naloxone infusion did not change the plasma glucose status. Farrell et al. (1986), reported non significant increases in blood glucose levels during 30 minutes of exercise at 70% VO_{2max} on naltrexone, an opiate antagonist.

It appears from these studies that when plasma glucose homeostasis is only mildly disturbed, no glucoregulatory role for the EOP was noted. It is also suggested from these studies that exercise of longer duration is probably needed to demonstrate a role for the EOP. In contrast to our results, Hickey et al. (1994), showed that opioid antagonism with naloxone caused significant elevation of plasma glucose during a 90 minute run (between 60 and 90 minutes). This indicated that opioids probably work towards decreasing glucose levels during prolonged exercise. In the present study, direct opioid infusion with B-EP caused significant elevations of plasma glucose at 60 and 90 minutes of exercise. In addition, naloxone administration did not alter glucose homeostasis relatively to the saline group at any time during rest or exercise. This was similar to other studies (Staessen et al., 1988, 1985; Farrell et al., 1986; Grossman et al., 1984). However, it must be noted that although the naloxone group did not have significantly higher plasma glucose levels than the control group, its mean plasma glucose value did not decrease significantly at 60 minutes as the control group. As in the Hickey et al. (1994) study, naloxone maintained higher plasma

glucose levels than the control group. However, this did not happen at 90 minutes of exercise where both the control and the naloxone groups had similar glucose levels. Furthermore, the present results confirm data from studies that reported a hyperglycemic effect of B-EP infusion either centrally or peripherally during basal conditions (Feldman et al., 1983; Reid et al., 1981; Matsumura et al., 1984; Rudman et al., 1983; Borison et al., 1962; Van Loon et al., 1981).

Naloxone failed to alter glucose homeostasis in the present investigation. Naloxone has been unable to block the hyperglycemic effect of B-EP (Feldman et al., 1983), or other B-EP effects (Rudman et al., 1983) as it occurred in this study. Naloxone was also unable to alter metabolic responses during exercise in a number of studies (Bramnert, 1988; Farrell et al., 1986; Grossman et al., 1984; Staessen et al., 1988). Despite the ineffectiveness of naloxone to inhibit B-EP actions peripherally, it did reverse B-EP effects centrally with or without concommitant central infusion of B-EP (Appel et al., 1987; Brown et al., 1979; Ipp et al., 1984; Van Loon et al., 1981). It seems that naloxone can block B-EP actions centrally but not peripherally in a number of studies. Therefore, the observation that naloxone did not affect plasma glucose levels in the present study or previous experiments with exercise does not necessary mean that B-EP is not involved in the regulation of glucose metabolism but that B-EP might have different pathways of action peripherally than centrally.

Several factors could contribute to the discrepancy on the effectiveness of naloxone to either antagonize B-EP peripherally or alter glucoregulation. First, naloxone has a non-selective nature.

However, different subclasses of opiate receptors may act in an interactive fashion and a non-specific blockade may obscure the significance of this interaction. Second, the dose of naloxone could be too small relative to the B-EP concentration. It has been shown that a 10 to 25 fold molar excess of naloxone is needed to block B-EP effects in animals (Rivier et al., 1977). Differences in naloxone doses could explain the discrepancy of research data. Farrell et al. (1986) as well in the present study used a dose of 0.8 $mg*kg^{-1}*h^{-1}$ whereas Hickey et al. used 0.1 $mg*kg^{-1}*h^{-1}$. Third, some B-EP effects could be mediated through a non-mu-opioid receptor (Feldman et al, 1983). B-EP shows a great affinity for mu-receptor, the original morphine receptor, although it is also active at delta and epsilon receptors (Giugliano et al., 1988). Unlike the mu-receptor, the delta receptor is resistant to naloxone (Giugliano et al., 1988). Fourth, B-EP might act through a non-opiate pathway. Non-opiate receptors have been found in lymphocytes and mouse neuroblastoma cells (Gilman et al., 1982; Hammonds et al., 1981) and these receptors are not blocked by opioid antagonists (Dave et al., 1985). Research also indicates the possibility of non-opiate binding of B-EP to Beta-adrenergic systems which might interact with opioid receptors (Brown et al., 1981; Sharma et al., 1975; Abood et al., 1985). Giugliano et al. (1989) showed that B-EP might interfere with cAMP in human pancreas. These observations suggest that B-EP may express its actions through receptors that are not antagonized, fully or partially, by naloxone.

Responses of Pancreatic Hormones

Previous research attempted to determine whether the hyperglycemic effect of B-EP during basal conditions was direct or mediated by parallel changes in pancreatic hormone levels. Paolisso et al. (1987) evaluated the influence of B-EP infused at a dose similar to the one used here (0.05 $mg*h^{-1}$) on glucose homeostasis in normal human subjects using the euglycaemic clamp technique. In that study, endocrine pancreatic function was fixed at its basal levels with somatostatin together with replacement of basal insulin and glucagon by the exogenous infusion of the hormones. In this metabolic condition, B-EP failed to have significant influence on the tracer-determined glucose metabolism (production, utilization, and clearance). Interestingly enough, the same B-EP dose caused plasma glucose levels to rise when infused in humans in the abscence of the clamp (Paolisso et al., 1987). This seems to indicate that the metabolic effects of B-EP are a consequence of the changed hormonal "millieu" and not the expression of intrinsic metabolic properties of the opioid. At basal conditions, B-EP-induced hyperglycemia in normal subjects appears to be secondary to its stimulation on pancreatic hormones release.

A. Insulin Response

In the present study, B-EP infusion (0.05 $mg*kg^{-1}*h^{-1}$), changed glucose homeostasis by maintaining significantly higher plasma glucose levels until 90 minutes of the 120 minute run. This study was organized to examine if B-EP infusion was accompanied by simultaneous changes of pancreatic hormones as previous research at

basal conditions suggested. First, all groups demonstrated a significant decline of plasma insulin concentrations with time as previous research has reported (Galbo, 1981; Bloom et al., 1976; Issekutz et al., 1980; Martin et al., 1981). This significant decline was first noted at 60 minutes of exercise in the present study. Vranic et al. (1975) showed plasma insulin radioimmunoreactivity (IRI) decreases in a gradual manner during moderate exercise, while during strenuous exercise a near maximal decrease occurs within minutes after beginning running. The protocol employed in the present study represents a rather strenuous stress for untrained rats. All groups demonstrated a final insulin decline of about 50% over time which is not different to the 45% decline usually noted with strenuous exercise (Vranic et al., 1975).

Insulin levels in plasma were lower in the B-EP group than the other two groups at 60 and 90 minutes of exercise. However, statistical significance was obtained only at 60 minutes of exercise. At 90 minutes, the B-EP group had statistically significantly lower levels of plasma insulin only compared to the naloxone group. These data suggest that B-EP lowers plasma insulin during exercise. There is limited information on how opioids affect insulin homeostasis during exercise. Farrell et al. (1988) found that opioid antagonism with naloxone had a stimulatory effect on plasma insulin during sustained hyperglycemia after exercise. This suggested an insulinopenic effect for E.O.P. Merrill et al. (1987), showed that insulin secretion was inhibited by naloxone after a stress. More recently, Hickey et al. (1994) showed that naloxone did not affect

plasma insulin levels during 90 minutes of exercise. Our results suggest that B-EP could act to lower plasma insulin concentration during exercise. This occured in conjuction with higher plasma glucose in the B-EP group.

A number of studies have confirmed that opiates and opioid peptides may influence the secretion of the endocrine pancreas in different animal species (Giugliano, 1984). Experimental results, however, have been conflicting. Both stimulation (Reid et al., 1981; Feldman et al., 1983) and inhibition (Matsumura et al., 1987; Giugliano et al., 1989) of plasma insulin secretion has been demonstrated after peripheral B-EP infusion. The conflicting data is apparently related to the system used, the species investigated, the dose administered and last but not least, the concentration of glucose in the medium. The prevailing plasma glucose levels might alter the reactivity of the beta-cells to B-EP (Giugliano et al., 1988). In situations where plasma glucose declines as in prolonged exercise, B-EP appears to protect glucose concentration by inhibiting insulin secretion (Giugliano et al., 1988). A dual, dose-depedent effect of B-EP on insulin secretion has also been demonstrated. An i.v. bolus (50 ug) of B-EP which produced plasma levels of more than 200-fold the basal values for B-EP raised plasma insulin levels, while the i.v. infusion (0.05 $mg*h^{-1}$) raised plasma B-EP 7-8 fold and inhibited insulin (Giugliano et al., 1987). Literature indicates that stress such as exercise is associated with a 3-10 fold increase in plasma B-EP levels (Rossier et al., 1977; Dubois et al., 1981, Goldfarb et al., 1987). Our data are in agreement with these data since after continuous i.v.

infusion of B-EP at 0.05 $mg*h^{-1}$, plasma B-EP was elevated about 8-10 fold after 120 minutes of running and insulin was inhibited. The inhibitory effect of B-EP on insulin especially at 60 minutes, is further suggested by the failure of plasma insulin to rise in response to the hyperglycemia that accompanied the opioid infusion in the first 60 minutes. Similar findings were obtained at basal conditions with peripheral B-EP infusion (Giugliano et al., 1989). However, it must be noted here that while the control and naloxone groups showed a significant decrease of plasma insulin levels from 90 to 120 minutes, the B-EP did not present the same degree of plasma insulin decline at this time. The fall of plasma insulin concentration from 90 to 120 minutes of exercise was not statistically significant at the 0.05 level. It must be mentioned, however, that the B-EP had significantly higher plasma glucose levels than the naloxone group and the control group at 90 minutes (almost 20 $mg*dl^{-1}$ more). It is possible that the higher plasma glucose levels in the B-EP group at that time caused this attenuated insulin response at this period of time in the exercise. Reactive response of insulin to the higher glucose level effect of B-EP has been suggested before (Reid et al., 1981).

In the present experiment naloxone did not alter insulin responses. This has been demonstrated by previous exercise studies with naloxone infusion (Hickey et al., 1994). A few studies showed that naloxone infusion at normal conditions can lower plasma insulin levels (Locatelli et al., 1985; Schusdziarra et al., 1984; Bartolome et al., 1989). In contrast, others showed that naloxone infusion did not block the insulinopenic effect of B-EP or change pancreatic function

in any way (Tannenbaum et al., 1979; Rudman et al., 1983; Ipp et al., 1984). In vitro studies demonstrated that the effects of B-EP on insulin secretion from rat and rabbit pancreas are not antagonized by naloxone (Green et al., 1983; Rudman et al., 1983). A number of possible reasons for the inability of naloxone to alter the effects of B-EP were given previously.

The large and variable hepatic extraction of insulin is a major obstacle to our ability to quantitate insulin secretion rates accurately. However, the evidence that C-peptide (C-P) is secreted from the b-cells in equimolar concentration with insulin (Rubenstein et al ., 1969; Horwitz et al., 1975), but not extracted by the liver to any significant degree (Stoll et al., 1970; Polonsky et al., 1981), has provided a firm scientific basis for the use of peripheral C-P concentrations as a semiquantitative marker of beta-cell secretory activity in a variety of situations.

The main cause of the reduction in the plasma insulin levels during exercise is the supression of its secretion (Wright et al., 1969). Indirect measurements of decreased insulin secretion is the reduction in C-P concentration and in the molar ratio between C-P and plasma insulin (Hilsted et al., 1980; Wirth et al., 1981; Menshikow et al., 1983). Results from the present study agree with previous observations since C-P levels were significantly reduced at all times of exercise in all groups.

Reasons for the decrease of C-P levels and insulin secretion during exercise are the fall of plasma glucose with time (Galbo et al., 1981; Felig et al., 1979; Ahlborg et al., 1977; Bonen et al., 1977) and

the increase in alpha-adrenergic activity (Porte et al., 1970; Galbo et al., 1977,1976).

In the belief that the hepatic extraction of C-P is negligible under all circumstances, peripheral C-P to insulin molar ratios have been assumed to reflect changes in insulin clearance. However, Polonsky and Rubenstein (1984) have cautioned the use of this ratio as an index of insulin removal. These authors have suggested that it is likely that alterations in the pattern and speed of equilibration of both insulin and C-P in their respective compartments can exert important influences on their relative plasma concentrations. There is little information on how exercise affects these parameters. However, recent data suggest that exercise does not alter C-P kinetics (Levitt et al., 1993). Another criticism is that differences in the plasma half lives of C-P (Sonksen et al., 1973) can further complicate the interpretation of the significance of changes in the C-P to insulin molar ratio. It is not certain how exercise influences the half-lives of these two peptides.

Since there is not evidence to suggest that exercise alters C-P kinetics during exercise as compared to rest, the C-P to insulin molar ratio could be used as an indicator of insulin clearance during exercise. This ratio has been shown to decrease during exercise (Hilsted et al., 1980; Wirth et al., 1981, Brisson et al., 1971). These observations agree with our findings that the peripheral plasma C-P to insulin molar ratio (C-P/INS) was reduced significantly with time in all three groups suggesting a reduction of insulin clearance. This observation suggests that insulin clearance is reduced with exercise.

In contrast, in depancreatized dogs, similar or even increased insulin clearance (as compared to resting values) has been observed (Vranic et al., 1975). Reasons for this observed enhanced insulin clearance rate may be due to promoted binding of insulin to its receptors or augmented hepatic catabolism of the hormone. The discrepancy between this last study and the earlier-mentioned studies may be due to differences in relative exercise intensity or species. In addition, exogenous insulin was used in the later study. This exogenous insulin probably decreased endogenous insulin secretion, making calculations of clearances difficult.

In the present study plasma C-P concentration decreased significantly. Additionally, C-P/INS decreased significantly as well. These data suggest that both insulin secretion and insulin clearance were reduced with exercise. However, plasma insulin concentration, which is the product of its secretion vs its clearance or elimination from the plasma, was significantly reduced with time. This means that the degree of reduction in insulin clearance rate was less than the decrease in its secretion rate. A closer examination of the results obtained on these two variables in the present investigation reveal that while C-P/INS or insulin clearance decreased by an average of 65% in the three groups, C-P levels or insulin's secretion rate was reduced by an average of 85% in the three groups.

While naloxone has been rarely found to alter insulin kinetics (secretion vs elimination from the plasma compartment), B-EP in this study appears to have an effect on these parameters. B-EP was found to decrease plasma insulin concentration significantly at 60 and 90

minutes. This observation coincided with the finding that B-EP suppressed insulin secretion rates as judged by plasma C-P levels. However, this significant depression of plasma insulin and C-P levels in the B-EP group as compared to the control and naloxone groups did not last until the end of exercise. Along with this attenuation in the decrease of insulin secretion after 90 minutes, a significant reduction of glucose removal from plasma was noted in the B-EP group compared to the other two groups after 90 minutes. This observation is based on the fact that the reduction in C-P/INS was similar in the three groups until 90 minutes of exercise. C-P/INS became significantly higher in the B-EP beyond the 90 minutes. Therefore, B-EP decreased plasma insulin by reducing its secretion until 90 minutes of exercise, but then it attenuated this reduction by reducing, directly or indirectly, its clearance from the plasma. This change could in part explain the largest decline of plasma glucose concentration in the B-EP after 90 minutes of exercise in the present study (35% drop in the B-EP group vs 14% in the other two). Giugliano et al. (1989) demonstrated a suppresive effect of B-EP on plasma insulin levels at rest. These authors, like in their earlier research (Giugliano et al., 1983), attributed this effect mostly on a depression of insulin secretion rates based on C-P levels rather than in changes of insulins' metabolic clearance. To a certain degree the present study confirms this observation since we observed that B-EP reduced only C-P levels before 90 minutes of exercise. However, B-EP effect after 90 minutes of exercise moved towards reducing metabolic clearance of insulin. Since prolonged exercise poses

different metabolic demands compared to a resting situation as in the Giugliano et al. (1989) study, additional factors may come into play.

It is not surprising that B-EP affected plasma insulin levels in the present study. Early research (Ipp et al., 1978) suggested that B-EP could alter somatostatin release from the D-cells in the pancreas. Somatostatin however, inhibits insulin release (Ipp et al., 1978) and in most cases as in the present study B-EP has been shown to decrease insulin release. In contrast, two in vivo experiments did not support a major role of somatostatin as a mediator of the B-EP effects on insulin release (Schusdziarra et al., 1983). A paracrine mechanism of B-EP action in the endocrine pancreas seems more plausible. In the late 70's it was shown that B-EP can be found in pancreatic cells (Bruni et al., 1979; Grube et al., 1980). Curry et al. (1987) later demonstrated that B-EP could exhibit membrane effects as opposed to effects on the biosynthesis of insulin. More recent observations (Giugliano et al., 1989) suggested that B-EP inhibits insulin release through a possible interaction with adrenergic receptors and cAMP.

Another attractive hypothesis offered by Giugliano (1987) speculates that the prevailing glycemic state determines if B-EP will have a stimulatory or an inhibitory effect on insulin secretion. It could be that B-EP stimulates insulin when hyperglycemia was observed in studies using a glucose challenge or obese subjects (Khawaja et al., 1990; Locatelli et al., 1985) and B-EP inhibits insulin release in situations where plasma glucose tends to decline such as in exercise.

B. Glucagon response

The glucagon content of plasma increases gradually during exercise (Vranic et al., 1975; Felig et al., 1972; Issekutz et al., 1980). This change is found to be depedent on both intensity and duration of the exercise (Galbo et al., 1975). A strenuous protocol of long duration, especially with untrained subjects, as the one used in this study, has been found to elicit a significant increase of plasma glucagon (Vranic et al., 1975; Winder et al., 1978). An important peculiarity of blood glucagon response is the lag period from the onset of exercise until the appearance of a rise in its concentration. At the level of 50-70% of VO_2max, the time of rise in plasma glucagon is 45-60 minutes (Luyckx et al., 1978; Winder et al., 1978). This time is thought to be a species-depedent phenomenon. In man it is well pronounced, but in rats and sheep an increase in plasma glucagon levels can be seen immediately at the onset of the exercise (Galbo, 1981; Winder et al., 1979). Our findings agree with these earlier observations since plasma glucagon concentration was significantly increased after 60 minutes of exercise and kept increasing until the end of the run in all three groups. This increase in plasma glucagon is believed to occur primarily due to a fall in blood glucose levels (Galbo et al., 1977). The effect of the fall in plasma glucose can either be direct on pancreatic alpha-cells (Muller et al., 1972) or indirect by causing sympathetic stimulation (Richter et al., 1980). In rats alpha-adrenergic stimulation is more significant (Richter et al., 1980). However, even during prolonged exercise, increments in catecholamine levels cannot fully account for the rise

in glucagon concentration (Galbo et al., 1975).

Despite the fact that studies performed at rest consistently noted a stimulation of alpha-cell secretion of glucagon (Giugliano et al., 1989; Reid et al., 1981; Feldman et al., 1983; Matsumura et al., 1984; Paolisso et al., 1987;Schleicher et al., 1987), even during a glucose challenge (Giugliano et al., 1989), studies that utilized an exercise model with naloxone infusion did not show any significant effects on glucagon levels (Grossman et al., 1984; Hickey et al., 1994). Variability in exercise protocols could in part explain these results. At all times during exercise, B-EP infusion elevated plasma glucagon levels at a significantly higher degree compared to the other two groups in the present study. This is the first study to demonstrate that B-EP stimulates glucagon secretion during exercise. On the other hand, naloxone did not alter glucagon metabolism during exercise as in previous studies (Grossman et al., 1984; Hickey et al., 1994). Possible explanations as to why naloxone was unable to alter glucagon response and other variables involved in the regulation of glucose homeostasis have been given previously. Whether the effects of B-EP (on insulin and glucagon) reflect a direct action on the pancreas or a centrally mediated process indirectly requires further studies. However, B-EP is known to influence the pancreatic hormonal secretion in vitro (Giugliano et al., 1984), and therefore, it seems conceivable that its effects on peripheral insulin and glucagon concentrations represent the result of the impact on a- and b-cell secretion. Furthermore, B-EP has been shown to be localized in pancreatic cells (Bruni et al., 1979; Grube et al., 1980).

Plasma Lactate Response

Blood lactate concentration increased after 45-60 minutes of prolonged exercise of submaximal intensity (Pruett et al., 1970; Galbo et al., 1977) and then (90-120 minutes) this rise subsides while it is still higher than resting levels (Galbo et al., 1977; Winder et al., 1982). This later decrease in blood lactate accumulation has been found to be due to its increased removal and use for gluconeogenic pathways at later stages of prolonged exercise (Brooks, 1985). Our results on plasma lactate agree with previous observations since plasma lactate increased significantly after 60 minutes and remained higher than resting levels throughout the exercise period in all three groups.

Lactate is a product of glycogenolysis and glycolysis. The intracellular enzymes which process carbohydrates produce lactic acid as a function of their metabolism. This is because the terminal enzyme of the glycolytic pathway lactate dehydrogenase (LDH) has the greatest catalytic activity (V_{max}) of any glycolytic enzyme (Brooks, 1985). Additionally, the catalytic activity of LDH exceeds by many times the combined catalytic activities of enzymes involved in alternative pathways for pyruvate metabolism (Everse et al., 1973; Karlsson et al., 1974). Therefore, substrate levels of pyruvate are enough to support maximal catalytic activity of LDH for the production of lactate, during exercise.

In the present experiment, B-EP was found to elicit significantly higher concentration of lactate in the plasma as compared to the other two groups while naloxone had similar values to the saline

group. De Meirleir et al. (1986), noticed that at exhaustion, B-EP levels increased parallel to lactate values. Studies describing correlations between B-EP and lactate (Buono et al., 1987; Farrell et al., 1983) assumed that substances from anaerobic metabolism could stimulate the hypothalamus-pituitary axis via chemoreceptors in the muscle. This speculation seems to agree with observations which suggest that B-EP increased glycogenolysis and gluconeogenesis from L-lactate (Allan et al., 1983; Matsumura et al., 1984).

In the present investigation B-EP caused a significant rise in plasma glucose concentration until 90 minutes of exercise. Increased plasma glucose levels could have increased the glucose uptake by the muscle. This in turn could enhance glucose flux into the working muscle. Since lactate is formed as a result of the glycogenolytic and glycolytic pathways, accelerated flux might have occurred in the present study. Therefore, LDH would see higher substrate levels resulting in increased muscle lactate production and inevitably more plasma lactate would appear. Finally, circulating concentrations of lactate reflect a balance between two opposing processes; production and removal. Any aspect of either production or removal, including redox buffering, alterations in regional blood flow and/or alterations in the hormonal millieu, could contribute to transient changes in lactate concentration. It is unclear as to the exact mechanism for the elevated lactate.

Plasma B-EP Response

It is well substantiated that plasma B-EP increases with exercise of adequate intensity and duration (Goldfarb et al., 1987,1990,

1991). The results in all three groups of this study confirm this notion. B-EP in plasma increased gradually in all groups with the highest levels obtained at 120 minutes. As it was expected, plasma B-EP was significantly elevated in the B-EP infused group compared to the other two groups at all times. The naloxone group had plasma B-EP values similar to the saline group's.

There is no information from previous research on the pharmakonetics of B-EP, its compartmental distribution or its half life during exercise. Nevertheless, when B-EP was infused at a continuous fashion and at a same dose as ours, plasma B-EP concentration increased 7-8 fold (Giugliano et al., 1987). In the present study, plasma B-EP concentration increased 7 fold above rest.

Possible mechanisms on how B-EP affects glucose regulation during exercise

Plasma glucose concentration reflects only the balance between the rate of glucose production or appearance and the rate of glucose clearance or uptake of glucose by peripheral tissues. Working skeletal muscle and the brain are the main tissues that utilize glucose during exercise (Vranic, 1975). Therefore, the hyperglycemic effect of B-EP during exercise observed in this study and at rest in previous studies could be attributed to either an increase in its appearance, a decrease in its disappearance or both. It has been shown that glucose production rather glucose utilization is more likely to be stimulated by either a central (Radosevich et al., 1989; Nush et al., 1989) or peripheral B-EP infusion (Matsumura et al., 1984; Allan et al., 1983;

Leach et al., 1985). However, two other studies could not confirm these results (El Tayeb et al., 1985; Brubaker et al., 1987).

If B-EP stimulates glucose production, then this could be achieved through only two pathways; hepatic glycogenolysis and/or gluconeogenesis from various substrates such as lactate, amino acids and glycerol. Opioid peptides have been shown to induce glycogenolysis in the liver and this effect was cAMP related (Allan et al., 1983; Matsumura et al., 1984). However, no changes in glycogen phosphorylase-a activity after B-EP addition in rat hepatocyte medium was noted (Brubaker et al., 1987).

B-EP was able to stimulate gluconeogenesis as well in liver from L-lactate (Matsumura et al., 1984). Lactate concentration in plasma was significantly increased in the plasma in the B-EP group in the present study. The glycogenolytic control process during exercise is multifactorial. It appears to be primarily regulated by the combined drop in insulin and increase in glucagon (Farrell, 1992; Galbo et al., 1992; Kjaer et al., 1987; Wasserman et al., 1992). Wasserman et al. (1992), have shown that the effect of the fall in insulin is to relieve the inhibition of hepatic glycogenolysis, and that this can account for as much as 65% of the increased hepatic glucose output. In addition, the fall in insulin sensitizes hepatic tissue to glucagon, such that the same absolute level of this hormone elicits a more potent response (Wasserman et al, 1992). It has been found that the elevation in glucagon primarily increases hepatic gluconeogenic efficiency, and that this accounts for almost 40% of the rise in hepatic glucose output (Farrell, 1992; Galbo, 1992; Kjaer et al., 1987). Our results confirm

these observations. Plasma glucose increased significantly in the B-EP group at a time when plasma insulin and C-P decreased and glucagon increased compared to the other two groups. This also agrees with the significant shift towards glucagon in the B-EP group at 60 and 90 minutes based on the glucagon to insulin molar ratio in plasma (GLU/INS). Other counteregulatory hormones such as catecholamines (CATS), cortisol and growth hormone can contribute to hepatic glycogenolysis and gluconeogenesis (Vranic et al., 1979) but their measurement was beyond the scope of this study. B-EP has been shown to stimulate (Feldberg, 1974; Van Loon et al., 1985) and inhibit CATS (Tannenbaum et al., 1979). Naloxone studies with exercise increased CATS (Farrell et al., 1991; Hickey et al., 1994; Grossman et al., 1984). Data concerning cortisol and growth hormone responses to naloxone and not B-EP administration during exercise is equivocal. The majority of the investigations suffer from the limited duration problem indicated previously. Cortisol and growth hormone have been shown to exert metabolic actions after 90 minutes of exercise (Vranic et al., 1979).

Another possibility for the B-EP-induced hyperglycemia during exercise, is an decreased utilization of glucose by the working muscles. Eventhough our data can not support this claim, it has been shown that B-EP can have a lipolytic effect on adipose tissue at rest (Richter et al., 1983) and during exercise (Vettor et al., 1987). Increased utilization of fatty acids during prolonged exercise can spare glucose utilization (Vranic et al., 1979). However, the increased plasma lactate concentrations with B-EP infusion observed in this

study, support a higher glycolytic flow.

After 90 minutes of exercise plasma glucose declined at a higher degree in the B-EP group (35%) compared to the other two groups (14% each). At the same time, C-P/INS and GLU/INS decrease was significantly attenuated in the B-EP group compared to the other groups, indicating a lower insulin clearance. A secondary rise in insulin can result in hypoglycemia at basal conditions (Reid et al., 1981). Chronic opioid administration has been associated with a paradoxical hypoglycemia and hyperinsulinism (Schmid et al., 1953; Reed et al., 1973). This could contribute to a higher plasma glucose uptake by the muscle after 90 minutes. Vranic et al. (1975) showed that even limited amounts of circulating insulin are enough to stimulate muscle glucose uptake due to the increased blood flow with exercise. Another explanation could be that higher liver glycogenolysis occurred in the B-EP group. However, liver glycogen was not measured in this study.

In summary, B-EP was found to cause an elevation of plasma glucose at 60 and 90 minutes of a prolonged bout of exercise lasting 120 minutes. This effect seems to be due to B-EP impact on the function of the endocrine pancreas by reducing insulin secretion and stimulating glucagon release. These results suggest that B-EP could be involved in the glucoregulatory mechanism during prolonged exercise. However, further research is needed in order to determine the mechanism(s) of its action.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

Our initial null hypothesis stated that B-EP infusion will not affect glucose homeostasis during exercise and the underying changes in the counterregulatory hormones. This hypothesis was rejected after analysis of the data obtained in this study. B-EP has been shown to yield elevations in plasma glucose concentration during a bout of prolonged exercise. Specifically, B-EP maintained higher plasma glucose levels than during a control situation at 60 and 90 minutes of a prolonged run which lasted 120 minutes. This effect of B-EP was seen at a time when plasma insulin and glucagon responses were significantly modified by B-EP. The infusion of the opioid resulted in a substantial decline of insulin release and a synchronous significant increase of plasma glucagon at 60 and 90 minutes of exercise. Thus, it appears that the hyperglycemia manifested by B-EP infusion was mediated by the B-EP effects on insulin and glucagon metabolism. In addition, a higher plasma lactate concentration was seen with B-EP infusion, possibly due to a higher glycolytic flow in the working muscle. Nevertheless, this hyperglycemic effect of B-EP during the first 90 minutes of running was followed by a larger drop of plasma glucose in the last 30

minutes of exercise. This effect was accompanied by a simultaneous reduction of plasma insulin clearance.

Naloxone did not influence either glucose homeostasis or the counteregulatory hormones during exercise at all times. It might be that the effects of B-EP on glucose metabolism and substrate utilization are expressed through cellular pathways that can not be blocked by naloxone.

Our results confirm previous research findings from studies performed at rest that B-EP elicits hyperglycemia through its effects on major glucoregulatory hormones such as insulin and glucagon. However, the exact cellular mechanism(s) of such action by B-EP are not currently known and they were beyond the scope of this study. Therefore, the investigators can offer the following recommendations for future research in this field:

1. Substrate mobilization was assessed indirectly in this study by looking at plasma glucose and lactate. The effects of B-EP on the utilization of liver and muscle glycogen, the oxidation of fats can be measured in the future in order to determine fuel interelationship and how it is affected by B-EP. This could account for the observed hyperglycemic effect of B-EP. Tracer methodology could be extremely valuable in this case.

2. The effects of B-EP on CATS, cortisol and growth hormone were not exemined in this study. These hormones and especially CATS are known to influence glucose homeostasis during prolonged exercise. CATS interaction with the pancreatic hormones and their effects on muscle glycogenolysis and adipose tissue lipolysis could

help to answer questions on the mechanisms of B-EP actions.

3. Investigation of B-EP infusion effects during a pancreatic clamp at exercise with somatostatin. This manipulation would allow to examine if B-EP effects on glucose homeostasis during exercise are secondary to changes in the pancreatic hormones.

4. The effects of B-EP in humans should be assessed in order to examine if this B-EP effects on glucose metabolism are species related phenomena.

5. Work with animal models suggests that central and peripheral levels opioid systems are indepedently regulated, and that elevations in peripheral levels can not be taken as an indication of concomitant central responses (Sforzo et al., 1985). Therefore, it is recommended that future research should examine how a central B-EP infusion during exercise affects glucose metabolism peripherally.

6. Exercise constitutes a stress condition where glucose homeostasis can be drastically disturbed. It is important to investigate if B-EP is involved in the counterregulatory mechanism during other situations where disturbance of glucose metabolism is also observed; namely, diabetes and obesity.

7. Different dosages of B-EP and naloxone should be used. Very little is known about the pharmacokinetic behavior of these agents during exercise.

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APPENDIX A

(Raw Data and Descriptive Statistics of the Depedent Variables)

GROUP	TIME	ID	GLUC	LAC	INS	C-P	C-P/IN	GLU	B-EP
1	1	1	133.6	1.27	32	483	2.26	250	7.0
1	1	2	142.7	1.18	33	400	1.81	185	9.0
1	1	3	139.8	1.05	40	520	1.95	180	11.0
1	1	4	123.8	1.66	29	480	2.48	170	5.0
1	1	5	133.5	1.19	31	360	1.74	140	6.0
1	1	6	128.0	1.26	27	500	2.77	180	8.0
1	2	1	116.7	1.89	23	240	1.57	300	18.0
1	2	2	97.6	2.40	25	210	1.26	260	21.0
1	2	3	94.2	2.65	23	180	1.17	320	22.0
1	2	4	125.8	2.88	25	210	1.26	320	22.0
1	2	5	130.9	3.36	25	190	1.14	300	24.0
1	2	6	99.8	2.51	26	270	1.55	330	19.0
1	3	1	95.5	1.72	20	120	0.90	460	27.0
1	3	2	80.6	1.31	21	140	1.00	480	29.0
1	3	3	68.5	4.03	21	140	0.99	390	29.0
1	3	4	79.8	1.59	22	115	0.78	330	26.0
1	3	5	96.5	1.52	22	105	0.71	400	31.0
1	3	6	69.4	1.68	18	160	1.33	410	28.0
1	4	1	69.7	2.11	16	115	0.89	1100	38.8
1	4	2	76.6	2.26	16	100	0.93	740	41.0
1	4	3	83.3	2.03	14	105	1.12	820	34.0
1	4	4	54.9	1.71	15	110	1.10	420	34.0
1	4	5	55.9	1.32	17	80	0.70	720	39.0
1	4	6	78.5	1.36	17	75	0.66	580	43.0

2	1	1	145.4	1.02	31	500	2.07	210	15.0
2	1	2	126.3	1.67	36	400	1.67	260	21.0
2	1	3	161.2	1.82	32	500	2.34	250	17.0
2	1	4	152.0	1.88	32	460	2.15	250	20.0
2	1	5	157.5	1.48	27	400	2.22	440	12.0
2	1	6	142.1	1.55	32	480	2.25	150	22.0
2	2	1	139.8	2.64	24	115	1.22	420	36.0
2	2	2	167.5	3.22	19	150	1.18	500	37.0
2	2	3	121.8	4.06	20	110	1.22	460	39.0
2	2	4	164.1	3.86	24	100	1.02	300	34.0
2	2	5	128.6	2.83	20	125	0.93	480	36.0
2	2	6	131.6	3.35	22	100	1.18	440	38.0
2	3	1	99.3	2.59	18	70	0.58	520	40.0
2	3	2	86.9	2.81	20	50	0.37	700	43.0
2	3	3	112.6	3.18	18	60	0.50	640	45.0
2	3	4	79.3	3.09	20	30	0.22	640	47.0
2	3	5	124.6	3.19	17	60	0.53	600	40.0
2	3	6	96.1	3.32	17	50	0.44	600	47.0
2	4	1	56.2	2.09	17	40	0.35	1100	58.0
2	4	2	50.5	2.90	15	55	0.55	942	60.0
2	4	3	61.2	3.65	17	30	0.26	1100	82.0
2	4	4	62.3	2.57	13	80	0.92	850	46.0
2	4	5	75.8	3.56	14	60	0.64	840	43.0
2	4	6	81.4	2.89	18	30	0.25	820	57.0
2	5	1	-	3.42	-	350	-	270	-
2	5	2	147.4	2.56	33	380	2.03	240	43.0

2	5	3	127.4	3.13	29	340	1.75	250	45.0
2	5	4	148.4	3.80	34	350	2.18	-	40.0
3	1	1	132.2	1.13	32	460	2.13	200	11.0
3	1	2	133.0	1.21	34	440	1.94	210	5.0
3	1	3	120.9	1.55	38	460	1.81	230	5.0
3	1	4	136.2	1.09	32	400	1.87	200	7.0
3	1	5	130.1	1.42	40	600	2.23	150	10.0
3	1	6	143.5	1.74	27	460	2.55	150	5.0
3	2	1	84.5	1.55	25	250	1.50	200	18.0
3	2	2	140.2	3.01	24	260	1.62	400	18.0
3	2	3	126.7	2.89	24	220	1.37	420	23.0
3	2	4	143.3	3.22	23	190	1.24	280	20.0
3	2	5	102.3	2.23	25	210	1.26	280	21.0
3	2	6	118.1	1.54	26	180	1.04	390	21.0
3	3	1	68.6	2.07	23	110	0.72	500	28.0
3	3	2	82.7	3.56	21	135	0.96	560	27.0
3	3	3	99.2	1.91	19	160	1.26	550	27.0
3	3	4	80.4	1.81	21	150	1.07	270	28.0
3	3	5	93.7	2.12	24	120	0.75	400	30.0
3	3	6	70.0	1.49	22	120	0.81	560	26.0
3	4	1	71.2	2.90	16	60	0.56	820	37.0
3	4	2	78.3	2.10	12	110	1.37	950	37.0
3	4	3	60.0	1.78	15	87	1.20	750	42.0
3	4	4	85.7	2.47	17	90	0.66	1100	35.0
3	4	5	79.9	2.64	14	89	0.64	800	35.0
3	4	6	58.3	1.81	13	85	1.04	760	33.0

3	5	1	163.8	1.65	27	82	2.55	220	12.0
3	5	2	143.9	1.71	37	78	2.22	390	5.0
3	5	3	151.1	1.76	34	72	1.60	220	11.0

- Group 1 is the saline group, group 2 is the B-EP group, and group 3 is the naloxone group.

- Times 1, 2, 3, 4 denote 0, 60, 90, 120 minutes of exercise. Time 5 represents the 120 minute infusion of B-EP and naloxone at rest.

- GLUC is the plasma glucose concentration (mg/dl).

- LAC is the plasma lactate concentration (mM).

- INS is the plasma insulin concentration (uIU/ml).

- C-P is the plasma C-peptide concentration (pM).

- C-P/INS is the C-peptide to insulin molar ratio in the plasma.

- GLU is the plasma glucagon concentration (pmol/l).

- B-EP is the plasma beta-endorphin concentration (pmol/l).

Means + S.E. for Plasma Glucose Concentrations in all Three Groups Across Time.

TIME	Saline Group	B-EP Group	Naloxone Group
O Min.	133.6 ± 3.5 (N=6)	147.4 ± 5.6 (N=6)	132.6 ± 3.1 (N=6)
60 Min.	110.8^{c} ± 6.4 (N=6)	142.2^{a} ± 7.7 (N=6)	119.2^{b} ± 9.2 (N=6)
90 Min.	81.7^{c,d} ± 4.9 (N=6)	99.8^{a,c,d} ± 6.8 (N=6)	82.4^{b,c,d} ± 5.9 (N=6)
120 Min.	69.8^{c} ± 4.9 (N=6)	64.6^{c,e} ± 4.8 (N=6)	72.2^{c} ± 4.5 (N=6)

(Glucose concentration is in mg/ dl, a denotes a significant difference between the saline and B-EP groups, b denotes a significant difference between the B-EP and naloxone groups, c denotes values significantly different from rest, d denotes a significant difference between 60 and 90 minutes, e denotes a significant difference between 90 min. and 120 minutes)

Means + S.E. for Plasma Lactate Concentrations in all Three Groups Over Time.

TIME	Saline Group	B-EP Group	Naloxone Group
O Min.	1.26 ± 0.09 (N=6)	1.57 ± 0.12 (N=6)	1.35 ± 0.10 (N=6)
60 Min.	2.61^{c} ± 0.20 (N=6)	3.32^{a,c} ± 0.22 (N=6)	2.40^{b,c} ± 0.30 (N=6)
90 Min.	1.97^{c,d} ± 0.41 (N=6)	3.03^{a,c} ± 0.11 (N=6)	2.16^{b,c} ± 0.29 (N=6)
120 Min.	1.72^{c} ± 0.13 (N=6)	2.94^{a,c} ± 0.24 (N=6)	2.28^{b,c} ± 0.18 (N=6)

(Lactate concentration is in mM, a denotes significant difference between the saline and B-EP groups, b denotes significant difference between the B-EP and naloxone groups, c denotes values significantly different from rest, d denotes significant difference between 60 and 90 min.)

Means + S.E. for Plasma Insulin Concentrations in all Three Groups Over Time.

TIME	Saline Group	B-EP Group	Naloxone Group
O Min.	32.0 ± 1.71 (N=6)	31.6 ± 1.16 (N=6)	33.8 ± 1.73 (N=6)
60 Min.	24.5^{c} ± 0.49 (N=6)	$21.5^{a,c}$ ± 0.88 (N=6)	$24.5^{b,c}$ ± 0.42 (N=6)
90 Min.	$20.6^{c,d}$ ± 0.61 (N=6)	$18.3^{c,d}$ ± 0.55 (N=6)	$21.6^{b,c,d}$ ± 0.71 (N=6)
120 Min.	$15.8^{c,e}$ ± 0.43 (N=6)	15.6^{c} ± 0.80 (N=6)	$14.5^{c,e}$ ± 0.76 (N=6)

(Insulin concentration is in mIU/ ml, a denotes significant difference between the saline and B-EP group, b denotes significant difference between the B-EP and naloxone group, c denotes significant different from rest, d denotes significant difference between 60 and 90 min., e denotes significant difference between 90 and 120 min.)

Means + S.E. for Plasma C-Peptide Concentrations in all Three Groups Over Time.

TIME	Saline Group	B-EP Group	Naloxone Group
O Min.	457.2 ± 40.1 (N=6)	456.7 ± 22.1 (N=6)	470.0 ± 24.4 (N=6)
60 Min.	216.6[c] ± 13.5 (N=6)	116.7[a,c] ± 7.7 (N=6)	218.3[b,c] ± 12.9 (N=6)
90 Min.	130.0[c,d] ± 8.2 (N=6)	53.3[a,c,d] ± 5.5 (N=6)	132.5[b,c,d] ± 7.9 (N=6)
120 Min.	97.5[c] ± 5.7 (N=6)	49.2[c] ± 8.0 (N=6)	85.8[c] ± 10.3 (N=6)

(C-P concentration is in pM, a denotes significant difference between the saline and B-EP group, b denotes significant difference between the B-EP and naloxone group, c denotes means significant different from rest, d denotes significant difference between 60 and 90 min)

Means + S.E. for C-P/INS in all Three Groups Over Time.

TIME	Saline Group	B-EP Group	Naloxone Group
O Min.	2.16 ± 0.19 (N=6)	2.11 ± 0.11 (N=6)	2.09 ± 0.11 (N=6)
60 Min.	1.32[c] ± 0.07 (N=6)	1.12[c] ± 0.04 (N=6)	1.33[c] ± 0.08 (N=6)
90 Min.	0.95[c,d] ± 0.08 (N=6)	0.44[a,c,d] ± 0.04 (N=6)	0.93[b,c,d] ± 0.08 (N=6)
120 Min.	0.90[c] ± 0.07 (N=6)	0.49[a,c] ± 0.10 (N=6)	0.91[b,c] ± 0.13 (N=6)

(a denotes significant difference between the saline and B-EP group, b denotes significant difference between the B-EP and naloxone group, c denotes means significant different from rest, d denotes significant difference between 90 and 120 min)

Means + S.E. for Plasma Glucagon Concentrations in all Three Groups Over time.

TIME	Saline Group	B-EP Group	Naloxone Group
O Min.	184.2 ± 12.9 (N=6)	243.3 ± 33.4 (N=6)	190.0 ± 11.3 (N=6)
60 Min.	305.0[c] ± 10.2 (N=6)	433.3[a,c] ± 29.0 (N=6)	308.3[b,c] ± 35.8 (N=6)
90 Min.	411.6[c] ± 21.8 (N=6)	616.6[a,c,d] ± 24.4 (N=6)	473.3[b,c,d] ± 56.6 (N=6)
120 Min.	730.0[c,e] ± 87.5 (N=6)	942.0[a,c,e] ± 59.0 (N=6)	863.3[c,e] ± 55.6 (N=6)

(Plasma glucagon concentration is in pg/ ml, a denotes significant difference between the B-EP and saline groups, b denotes significant difference between the B-EP and naloxone groups, c denotes values significantly different from rest, d denotes significant difference between 60 and 90 min. times, e denotes significant difference between 90 and 120 min. times)

Means + S.E. for Plasma B-EP Concentrations in the Three Groups Over Time.

TIME	Saline Group	B-EP Group	Naloxone Group
O Min.	7.7 ± 0.89 (N=6)	17.8[a] ± 0.99 (N=6)	7.2[b] ± 1.03 (N=6)
60 Min.	21.0[c] ± 0.89 (N=6)	36.7[a,c] ± 0.71 (N=6)	20.2[b,c] ± 0.79 (N=6)
90 Min.	28.4[c,d] ± 0.79 (N=6)	43.6[a,c,d] ± 1.30 (N=6)	27.6[b,c,d] ± 0.61 (N=6)
120 Min.	38.3[c,e] ± 1.50 (N=6)	57.6[a,c,e] ± 6.28 (N=6)	36.5[b,c,e] ± 1.25 (N=6)

(Plasma B-EP concentration is in pmol/ ml, a denotes significant difference between the B-EP and saline groups, b denotes significant difference between the B-EP and naloxone groups, c denotes values significantly different from rest, d denotes significant difference between 60 and 90 minutes, e denotes significant difference between 90 and 120 minutes)

APPENDIX B

(Raw Data and Statistics of Animals' Weights)

GROUP	TIME	I.D.	I.W.	O.W.	F.W.	W.G.
1	1	1	201	254	260	59
1	1	2	214	311	323	109
1	1	3	204	288	306	102
1	1	4	211	308	308	97
1	1	5	221	295	318	97
1	1	6	218	291	303	85
1	1	7	212	278	288	76
1	2	1	228	270	280	52
1	2	2	216	290	298	82
1	2	3	208	290	305	97
1	2	4	208	283	293	85
1	2	5	209	271	295	86
1	2	6	215	278	301	86
1	3	1	201	275	299	98
1	3	2	208	295	300	92
1	3	3	204	318	328	124
1	3	4	219	294	294	75
1	3	5	205	277	295	90
1	3	6	210	292	299	89
1	4	1	220	239	254	34
1	4	2	201	269	278	77
1	4	3	205	271	291	86
1	4	4	210	276	291	81
1	4	5	212	284	292	80

1	4	6	214	286	298	84
1	4	7	218	265	283	65
2	1	1	210	225	262	52
2	1	2	217	300	318	101
2	1	3	201	277	311	110
2	1	4	206	274	288	82
2	1	5	213	295	305	92
2	1	6	204	275	305	101
2	1	7	221	282	301	80
2	2	1	212	270	295	83
2	2	2	208	306	328	120
2	2	3	220	278	290	70
2	2	4	203	282	295	92
2	2	5	210	283	300	90
2	2	6	220	301	315	95
2	3	1	207	314	315	108
2	3	2	201	285	294	93
2	3	3	210	282	290	80
2	3	4	220	290	306	86
2	3	5	211	269	289	78
2	3	6	221	277	302	81
2	4	1	205	290	308	103
2	4	2	211	278	299	88
2	4	3	204	305	324	120
2	4	4	221	279	310	89
2	4	5	212	277	304	92

2	4	6	212	280	301	89
2	5	1	217	256	256	39
2	5	2	210	313	323	113
2	5	3	213	213	305	92
2	5	4	208	208	291	83
3	1	1	224	242	250	26
3	1	2	206	306	308	102
3	1	3	202	285	314	103
3	1	4	211	285	302	94
3	1	5	208	297	310	103
3	1	6	207	306	320	118
3	1	7	215	275	286	71
3	2	1	206	265	290	84
3	2	2	209	275	294	85
3	2	3	204	288	299	95
3	2	4	204	301	309	105
3	2	5	224	285	300	76
3	2	6	208	286	309	101
3	3	1	210	293	308	98
3	3	2	200	295	298	98
3	3	3	215	233	266	51
3	3	4	208	290	302	94
3	3	5	209	285	302	93
3	3	6	211	291	309	98
3	4	1	218	314	343	125
3	4	2	216	286	301	85

3	4	3	204	273	291	87
3	4	4	209	286	299	90
3	4	5	210	293	299	89
3	4	6	218	292	303	85
3	5	1	218	293	300	82
3	5	2	229	306	307	78
3	5	3	209	265	281	72

- Group 1 is the saline group, group 2 is the B-EP group, and group 3 is the naloxone group.

- IW is the animals' initial weight.

- 0W is the animals' operation weight.

- FW is the animals' final weight.

- WG is the animals' weight gain during the experiment.

Group means of Body Weights and Weight Gain During the Experimental Period (Means + S.E)

TIME		Saline Group	B-EP Group	Naloxone Group
0 Min.	IW (g)	211.6 ± 2.6	210.3 ± 2.7	210.4 ± 2.7
	OW (g)	289.3 ± 7.3	275.4 ± 9.2	285.1 ± 8.4
	FW (g)	300.9 ± 8.1	298.5 ± 7.0	298.5 ± 9.0
	WG (g)	89.3 ± 6.5	88.3 ± 7.3	88.1 ± 11.6
		(N=7)	(N=7)	(N=7)
60 Min.	IW (g)	214.0 ± 3.1	212.2 ± 2.7	209.2 ± 3.0
	OW (g)	280.3 ± 3.6	286.7 ± 5.6	283.3 ± 4.9
	FW (g)	295.3 ± 3.5	303.8 ± 5.9	300.2 ± 3.1
	WG (g)	81.3 ± 5.7	91.7 ± 6.7	91.0 ± 12.6
		(N=6)	(N=6)	(N=6)
90 Min.	IW (g)	207.8 ± 2.5	211.7 ± 3.1	208.8 ± 2.0
	OW (g)	291.8 ± 6.3	286.2 ± 6.2	281.2 ± 9.7
	FW (g)	302.5 ± 5.1	299.3 ± 4.1	297.5 ± 6.5
	WG (g)	94.7 ± 6.6	87.7 ± 4.6	88.7 ± 7.5
		(N=6)	(N=6)	(N=6)
120 Min.	IW (g)	211.4 + 2.5	210.8 ± 2.4	212.5 ± 2.3
	OW (g)	270.0 ± 5.9	284.8 ± 4.4	290.7 ± 5.5
	FW (g)	283.8 ± 5.5	307.7 ± 3.6	306.0 ± 7.5
	WG (g)	72.4 ± 6.9	96.8 ± 5.1	93.5 ± 6.3
		(N=7)	(N=6)	(N=6)

(IW is initial weight, OW is operation weight, FW is final weight, WG is weight gain)

Summary Statistics of Animals Initial Weight (IW)

Source	DF	SS	MS	F	Pr > F
Treatment	2	15.9	7.9	0.17	0.84
Time	3	60.7	20.2	0.43	0.72
Treat*Time	6	118.8	19.8	0.42	0.85
Error	64	2985.5	46.6		

Summary Statistics of Animals' Final Weight (FW)

Source	DF	SS	MS	F	Pr > F
Treatment	2	643.6	321.8	1.29	0.28
Time	3	17.9	5.9	0.02	0.99
Treat*Time	6	2024.6	337.4	1.35	0.24
Error	64	15989.8	46.6		

Summary Statistics on Animals Weight Gain (WG)

Source	DF	SS	MS	F	Pr > F
Treatment	2	89.9	44.9	0.15	0.86
Time	3	246.5	82.1	0.28	0.84
Treat*Time	6	2579.7	429.9	1.44	0.21
Error	64	22022.8	298.5		