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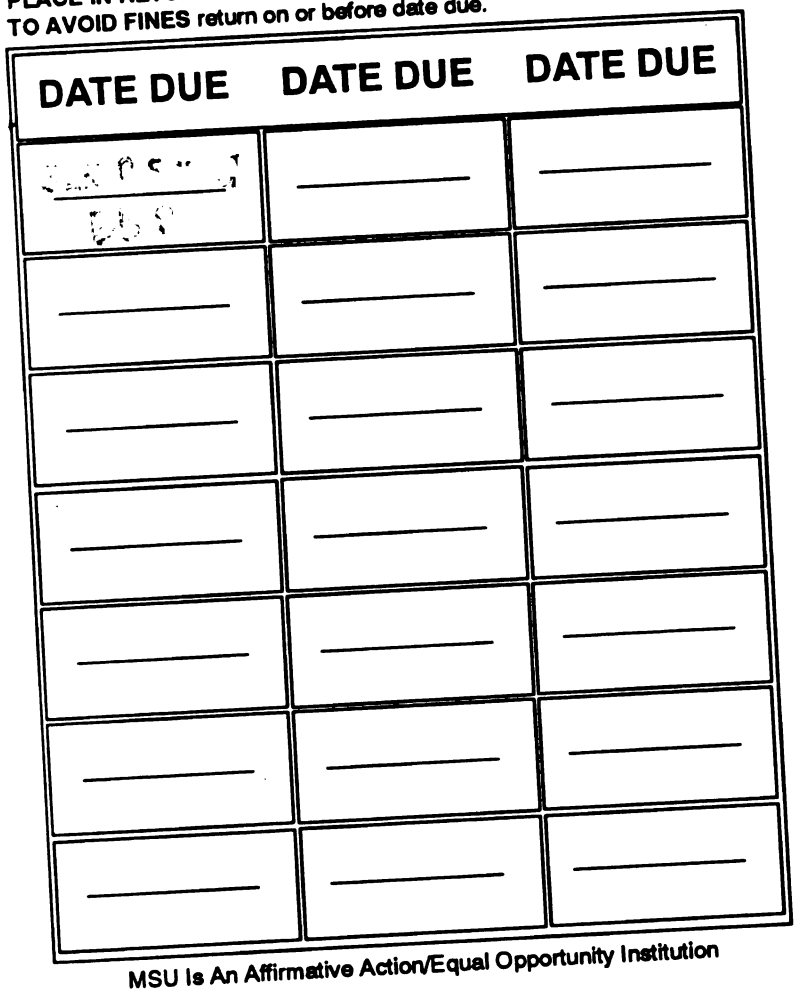

THE EFFECTS OF CENTRAL AND PERIPHERAL OPIATE BLOCKADE ON EXERCISE PERFORMANCE IN THE RAT

by

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ABSTRACT

THE EFFECTS OF CENTRAL AND PERIPHERAL OPIATE BLOCKADE ON EXERCISE PERFORMANCE IN THE RAT

By

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Exercise and various other forms of physical and psychological stress stimulate the release of endogenous opiates. The extent to which this non-specific stress-adaptive mechanism affects exercise is unknown. The purpose of this study is to determine if opioid blockade alters exercise performance.

Male, albino, Sprague-Dawley rats were trained on a program of low-intensity, long-duration exercise. Eighteen of the best performers were selected to participate in this exercise performance study. The test conditions included one run prior to which the rats were administered 0.5 ml/kg body weight of naltrexone and a control run where they received an injection of saline. On test days the rats run for 45 minutes at an expected speed of 2 ft/sec. Their performance was measured as percentage of expected meters run and as percentage of shock-free time.

The results of this study demonstrated that opiate blockade did not alter exercise performance. The rats' running performance diminished with time.

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CHAPTER I

INTRODUCTION

The therapeutic value of opium extracts and their remarkable ability to abolish pain have been used medically for centuries. Despite the toxicity and addictiveness of these drugs, the medical world has yet to find a pharmacological replacement for the profound analgesic action of the opioid. Out of the search for a comparable, non-addictive opioid analgesic has come a basic understanding of the powerful opioid mechanisms. Recent studies have demonstrated the existence of opioid-specific receptors in the central nervous system (CNS) and the inherent ability of the brain to produce its own opium-like substances.

The exact role that the opioid system plays in the body has not been clearly defined. Primary opioid action, however, is believed to take place within the spinoreticular pain pathways of the CNS (1,2). Endogenous opioids, through action on specific receptors, inhibit or alter neurotransmission to produce the same effects as their exogenous opium-based counterparts (3,4). Substances producing morphine-like effects are collectively referred to as opioid agonists. Similarly structured substances with high affinity to the opioid receptors that block or reverse morphine or other opioid effects are referred to as opioid antagonists.

The three families of opioid peptides are endorphins, enkephalins, and dynorphins. They are classified on the basis of their distinct precursor origins, pro-opiomelanocortin, proenkephalin, and prodynorphin, respectively (5). Individual opioid peptides have affinities for different types of opioid receptors and demonstrate a variety of roles in many biological processes. The opioids behave as hormones, neurohormones, and neurotransmitters and have both CNS and peripheral effects in the body.

The existence of the opioids and their receptors throughout the pain pathways and in the emotional centers of the brain has directed much study towards the role of the endogenous peptides in pain suppression. The complex association between stress, opioids, and analgesia is well-documented. Primary focus has been placed on the involvement of pituitary β-endorphin (β-END) in stress response. Both β-END and adrenal corticotrophic hormone (ACTH) are fragments of the larger polypeptide, beta lipotropin. These two neuropeptides are released concomitantly from secretory granules within the pituitary corticotropic cells in response to endogenous or environmental stimuli (6). In the rat, shock, cold-water swims, insulin-induced hyperglycemia, exercise, restraint, and forms of psychological stress have been reported to produce a morphine-like response that can be reversed by the opioid antagonists naloxone and naltrexone (5-9).

Strenuous activity is frequently associated with pain, and training at high intensity has been proven to increase pain tolerance (10). During exercise, there is an increase in plasma β-END (11-13). This stress-dependent relationship could be explained if it is assumed

that the major stimulus for pain during exercise arises from metabolic changes, such as decreased pH, within the muscle cell.

When deprived of daily exercise, those who train regularly have reported symptoms similar to opioid withdrawal, such as anxiety and irritability (14). An opioid interaction within the CNS is strongly indicated. However, the frequently reported elevation of plasma β-END seen during exercise cannot be held responsible for any such psychological alterations as the claimed euphoria of the "runner's high." Peripheral β-END does not cross the blood-brain barrier (BBB) and, therefore, is unable to interact with opioid receptors in the brain (15).

Specific interactions between peripheral and central opioid systems cannot be assumed. However, exercise may stimulate CNS opioid release or the peripheral opioid pool may activate central opioid mechanisms. There are a number of other questions that remain unanswered regarding the function of endogenous opioids and their production. The majority of studies involving exercise-induced opioid production have measured increases in β-END. These findings reveal little about opioid function or the degree to which the opioids influence exercise performance.

Four separate strategies currently are being used to study opioids (5,15,16). One method is the study of the physiological, biochemical, and behavioral changes produced by administration of endogenous opioids in humans and laboratory animals. A second involves stimulated release of endogenous opioids through pain, stress, or electrical activation of areas in the brain rich in opioid receptors. A third method entails use

of radio-immunoassay for the measurement of opioid substances in body fluids and in ground tissue homogenates. Finally, narcotic antagonists are used to displace both exogenous and endogenous opioids from their receptors. The current study incorporated a combination of two of the above methods. Exercise was used to provide a stimulus for the release of endogenous opioids, and the opioid antagonist naltrexone was used to block any effects that the endogenous opioids produce in the rat during exercise.

Literature pertaining to opioid blockade during exercise is sparse and presents an array of confusing and conflicting evidence. The nature of any effects produced by opioid blockade during exercise in the human have not been successfully documented. Exercise produces an increase in plasma β-END levels above levels found at rest in both man and laboratory animals (11-13), but the effect this documented response has on exercise performance has not yet been determined. In the rat, various forms of exercise produce analgesia of either an opioid or a non-opioid nature (5). These findings generally have not been replicated in human subjects with the exception of one study (17). The results of that study showed a post-exercise elevation in pain threshold that was significantly diminished by naltrexone. These findings also are inconclusive, however, as different doses of naltrexone produced contrasting results. Other studies have failed to report naloxone induced changes in humans, demonstrating no significant physiologic or psychologic alterations during exercise following the administration of naloxone (18).

The results of several studies suggest that opioids influence hormonal release during exercise as demonstrated by the administration of the opioid antagonist naltrexone. These results also are inconclusive, and this area requires further investigation. If opioids mediate some form of endocrine modulation during exercise, the degree to which this influence affects exercise performance is unknown.

Opioid release during exercise may simply be a non-specific reaction of the body to stress. The release of β-END, along with a host of other hormones, may be a basic component of the stress adaptive axis which has no direct influence on exercise performance. Evaluation of the specific opioid action during exercise is secondary to the actual determination of whether the opioids affect exercise performance at all.

Purpose

The purpose of this study is to evaluate the effects of opioid blockade on exercise performance in the rat. Exercise performance was measured as percentage of expected revolutions run (PER) and as percentage of shock-free time (PSF) in controlled running wheels (19). These dependent variables were examined during a 45-minute test period of running.

Rationale

Aerobic running was chosen over swimming for two primary reasons: (1) running allows the investigator greater control over exercise intensity and; (2) running eliminates the risk of complications secondary to murine pneumonia associated with swimming. Opioid

antagonists were used to abolish the effects of endogenous opioids because there is currently no known method of inhibiting endogenous opioid release.

Significance of the Problem

Research showing that the endogenous opiate system plays a role in exercise performance in the rat could promote rigorous investigation into whether similar results might be found in humans. Such information would provide many fields of research with valuable insight into the mechanisms of motivation, pain threshold, human energetics, and stress adaptation.

Limitations

The results of this investigation are limited to the male, albino, Sprague-Dawley rat. Special care must be taken in the evaluation of these experimental results due to the wide variety of biological processes including behavior, analgesia, and hormonal responses that are associated with the opioid system These results only demonstrate whether or not opioid blockade affects exercise performance. The distinct mechanisms responsible for any such effect(s) cannot be inferred. The results of this study also are limited to aerobic exercise performance and cannot be generalized to include anaerobic exercise.

Special consideration must be given to the fact that mild shock stimulus was used to motivate continued running in the rat. Shock in itself may cause release of endogenous opioids. The rats, when treated

with opioid antagonists, might exhibit increased sensitivity to the shock stimulus and, therefore, might be more prone to continue running as demonstrated by decreased PSF and increased PER values. This confounding factor could have considerably skewed the performance data.

CHAPTER II

REVIEW OF THE LITERATURE

Historical discussion of the endogenous opioid system would be incomplete without brief mention of the exogenous ligand, opium. Knowledge of opium use dates back to classical Greece. The ancient Greeks found opium valuable for two basic reasons: it deadens pain and it gives rise to euphoria. The word opion means poppy juice. Opium is extracted from the milky juice of the unripe seed capsules of the poppy plant, papaver somniferum. Raw opium is composed of organic acids, alkaloids, resins, sugars, albumin, and water. More than 20 extractable alkaloids constitute the active substances in opium. By the mid-nineteenth century, medicinal science began to replace the use of crude opium preparations with those of the pure alkaloids (morphine, codeine, and papaverine). Morphine, named after the Greek god of dreams, Morpheus, is by far the most potent opium derivative and to this day remains the universal standard against which all new analgesics are measured.

Research dealing with alternative analgesics has promoted a far greater understanding of opioid action. Such study has introduced substances similar in structure to that of morphine with the capability of abolishing the effects of morphine or heroin. Drugs capable of binding to opioid receptor sites, but lacking narcotic activity, are

known as opioid antagonists. Opioid antagonists can be synthesized from opioid alkaloids through manipulation of methyl groups on the nitrogen in the phenyliperidine structure with either an alkyl group (as in naloxone) or a cyclopropylmethyl group (as in naltrexone). Morphine and substances producing opioid (narcotic) effects are referred to as opioid agonists.

The extreme chemical specificity demonstrated by morphine and related agonists, along with those of opioid antagonists, has generated interesting speculation. By 1967, researchers had constructed a theoretical model proposing the existence of a specifically sculpted tissue site or receptor location within the brain (20). In 1973, the discovery of the opiate receptor marked the beginning of a new era in the field of opioid research. Almost simultaneously, several independent research laboratories identified a stereospecific binding site in the mammalian CNS (21,22). This was demonstrated by the use of radioactively tagged agonists and antagonists.

Actual isolation of the long hypothesized opioid receptor led to speculation regarding why such sites exist. The opioid receptors in the brain appear to be targets for endogenously produced, morphine-like substances. Consequently, laboratories all over the world attempted to isolate this mysterious endogenous opioid peptide. In 1975, the search for "the body's own morphine" culminated when Hughes published his discovery of a morphine-like substance in the brain he named enkephalin (23). These findings were confirmed by several others.

Over a decade has passed since the isolation of the first opioid receptor and endogenous ligand. The existence of a single opioid

receptor and occupying endogenous ligand was a neat and simple concept, but the clarity of this relationship was soon clouded by the fact that at least eight different endogenous opioid peptides were identified, along with a system of multiple opioid receptors. This extreme heterogenity significantly complicated experimentation and the functional interpretation of such studies.

The use of recombinant DNA techniques beginning in 1982 has shed new light on the subject. The DNA studies gave each peptide a separate home and, in the process, erected a complex structure of opioid nomenclature (5).

The Opioid Receptors

Morphine and related opiates exert their analgesic effects by interacting with specifically sculpted tissue sites on pre- and post-synaptic neurons. Studies involving the binding of opioid drugs demonstrate that opioid receptor binding is mediated by Na^+ and GTP. Opioids function through directly or indirectly opening K^+ channels, modulation of Ca^{++} channels, and reduction of C-AMP levels. These interrelationships may differ in various locations of the body, further complicating such analysis.

Four basic categories of opioid receptors have been identified within the human CNS. These are called the Mu (μ), Kappa (κ), Delta (δ), and Sigma (σ) receptors (24). There is evidence that additional receptors exist independently or as subtypes of the others.

Within the CNS, dense clusters of opioid receptors line areas of the paleospinothalamic and spinoreticular pain pathways including the

substancia gelatinosa of the spinal cord, the perqueductal grey, and the intralaminar nucleus of the thalamus (25). The amygdala, the region in the human and primate brain directly associated with emotion, contains the densest population of opiate receptors (26). Such neurochemical specificity provides the basis for the profoundness of the opioid pain suppression system.

The wide distribution of opiate receptors in regions of the brain not associated with analgesia suggest that the endogenous opioids have a variety of physiological roles in addition to pain suppression. The pituitary gland and the retina both contain dense regions of opioid receptors (27). Smaller populations of opioid receptors have been isolated throughout the autonomic nervous system, the adrenal medulla, and the gastrointestinal tract.

The Opioid Peptides

All opioid peptides contain an amino sequence of either tyrosine-glycine-glycine-phenylalanine-methionine (tyr-gly-gly-phe-met) or tyrosine-glycine-glycine-phenylalanine-leucine (tyr-gly-gly-phe-leu) which appears to be the active prerequisite for opioid receptor occupancy (28). Despite the replication of this active terminal sequence in each peptide, the peptides have three distinct origins. Classification of the opioid peptides into the primary groups enkephalin, endorphin, and dynorphin is based on these origins. Known opioid peptides come from three separate precursors: (a) proenkephalin or proenkephalin A; (b) pro-opiomelanocortin (POMC); and (c) prodynorphin. The cells producing these precursors release different

mixtures of opioid peptides. The ratio of peptides released varies from one region of the CNS to another depending on the cellular complement of peptidases or the post translational events which modify these products through acetylation, amidation, phosphorylation, methylation, glycosylation, or cleavage (5).

The endogenous opioid peptides display a variety of relative affinities for different types of receptors. In general, peptides derived from proenkephalin show marked preference for δ sites. All prodynorphin peptides bind predominantly to κ receptors with the exception of several derivatives that bind to μ and σ sites. Endorphins or POMC-derived peptides bind to both μ and δ sites, with greatest affinity being to the μ receptors (5).

Proenkephalin

The 28K protein termed proenkephalin or proenkephalin A is the precursor for enkephalins. All peptides processed from this precursor have active opioid properties. Seven peptides are cleaved from proenkephalin. These include four peptides containing ty-gly-gly-phe-met; two extended versions of met enkephalin, one with either a carboxyl addition or argine and phenylalanine (tyr-gly-gly-phe-met-arg-phe), the other with additional argenine, glycine, and leucine (tyr-gly-gly-phe-met-arg-gly-leu); and finally one copy of leu enkephalin (tyr-gly-gly-phe-leu) (29,30).

Peripheral proenkephalin is found primarily within the chromafin cells of the adrenal medulla and to a lesser extent in the gastrointestinal tract, sympathetic ganglion, vagus nerve, retina, and

phaeochromocytoma. In the brain, enkephalins are produced within interneurons throughout every level of the neural axis (31). The proenkephalin by-products in the brain are highly processed and have little resistance to proteolytic enzymes. Their simple structure and size also enable them to cross the blood-brain barrier. Whether or not the adrenal enkephalin precursor is identical to that in the brain is still debated (32).

The proenkephalin by-products, the enkephalins, function primarily as neurotransmitters and are rapidly inactivated by proteolytic enzymes. The substance responsible for this degradation has been named enkephalinase. Enkephalinase is located at enkephalinergic synapses and cleaves the peptide between glycerine and phenyalaline (33).

In vivo study of the effects of enkephalins has identified a wide variety of actions, including modulation of sensory input, mood alteration, decreased respiration, gut motility, and release of pituitary hormones. Enkephalinergic effects are mediated through activation of the δ receptor for which enkephalins have the greatest affinity (29). Pharmacological studies have shown that the enkephalins inhibit neuronal firing and that this inhibition is mediated through at least three modes of action in the autonomic and central nervous systems: (a) presynaptic inhibition, (b) direct inhibition, and (c) indirect disinhibition (34,35).

In the autonomic nervous system, enkephalins have influence over both acetylcholine and catecholamine release. Inhibition of acetylcholine release in the mysenteric plexus appears to alter the peristaltic actions of the gut (36). Enkephalins are present in the

sympathetic ganglia and nerve cells in the adrenal medulla (37,38). Enkephalins released from axon terminals in the splanch nerve interact with acetylcholine receptors located in the catecholamine releasing chromafin cells of the medulla, thus modulating the release of adrenalin (37,40).

Within the CNS, enkephalins modulate hormone and neurotransmitter release. Hypothalmic enkephalins indirectly stimulate the release of prolactin (41) and depress that of thyrotropin through inhibition of dopamine release (42). Enkephalins also stimulate release of growth hormone from the pituitary (43). The exact mechanism by which this takes place is still unknown.

Pro-opiomelanocortin

The huge polypeptide pro-opiomelanocortin (POMC) is the precursor of adrenocorticotrophic hormones (ACTH), beta-lipotropin (β-LPH), three types of melanocyte-stimulating hormone (MSH), and the opiate peptides referred to as endorphins. The hormone β-LPH contains 91 amino acids from which α-MSH and β-END are cleaved. β-END contains the last 31 amino acids in the β-LPH sequence and includes the familiar met-enkephalin terminal (try-gly-gly-phe-met) (44). The POMC precursor is processed differently in different cells. Unlike proenkephalin, the POMC by-products have been demonstrated to have both active and inactive analgesic actions (5).

Active β-END is produced primarily in the non-neural pituitary tissue of the arcuate and periarcuate regions of the hypothalamus (45). There are endorphin fibers from the hypothalamus to the thalamus,

throughout the limbic system, and in the mesencephalon and telencephalon (46). In a general response to stress, ACTH and β-END are concomitantly released from the pituitary into the bloodstream (47). Further processing of POMC takes place in the separate lobes of the pituitary and in various regions of the brain. Several modifications of active $\beta\text{-END}_{1\text{-}31}$ have been isolated, namely the C_1 fragment $\beta\text{-END}_{1\text{-}27}$, the dehistidine C_1 fragment and α-N-acetyl derivatives of the three endorphins. Of these peptides, only $\beta\text{-END}_{1\text{-}31}$, and to a lesser degree $\beta\text{-END}_{1\text{-}27}$, demonstrate analgesic activity (48,49). Immunoreactive studies show that the inactive forms of β-END are found primarily in the hippocampus, brainstem, and pars intermedia (50).

The endorphins are generally regarded as neuromodulators, rather than transmitters, due to their long <u>in vivo</u> half-life (24). Intracerebral administration of β-END leads to prolonged and profound analgesia. Endorphin in the active form has 30 times the potency of morphine (4). Morphine and β-END both have high affinity for the μ receptor. β-END has an equally strong affinity for the σ receptor (51).

The presence of β-END within the hypothalamus indicates its possible role in control of pituitary hormone release. Opioids in general act in the hypothalamus to inhibit the release of specific releasing factors. This effect is not mediated through direct inhibition of the releasing factors but through indirect inhibition or disinhibition of the dopaminergic system which directly regulates hypothalamic releasing factors (52). β-END has been found to produce an increase in plasma growth hormone and prolactin levels (53-55). Lutenizing hormone also has been found to be influenced by β-END (15).

Prodynorphin

Prodynorphin is the 28K precursor of the peptide containing the leu-enkephalin dynorphin A_{1-17}, which can be further processed to dynorphin A_{1-8}, dynorphin, dynorphin B_{113}, and α and β-neoendorphins which differ by only one amino acid (56). The processing of this precursor into its end-products has not been completely defined. Prodynorphin is found in the gut, posterior pituitary, and brain. In the brain, prodynorphin is found in the vasopressin-producing cells of the hypothalamus, brainstem, and supraoptic nucleus pathway to the posterior pituitary (57).

The dynorphin precursor family shows a preference for the κ receptor (58). Ligands interacting with κ receptors cause a general "apathetic" sedation which is distinct from that of other opioids. They also cause CNS activation including pupillary dilation, tachypnea, and tachycardia (5,59). Kappa receptors located in the cortex may mediate their sedative qualities through regulation of cortical sensory input from the thalamus. The role of the dynorphins in this process is controversial, and injection of dynorphin into various regions of the brain and spinal cord have yielded varying degrees of analgesia. Further studies have shown that modulation of leutenizing hormone from the hypothalamus is mediated through the κ receptor and that β-END and dynorphin antibodies cause an increase in serum lutenizing hormone. The localized presence of the κ receptor and dynorphin in neurons containing vasopressin and corticotropin releasing factor suggests the importance of dynorphin in regulating the release of vasopressin and ACTH (60).

Dynorphin$_{1-17}$ is the most active of all opioid peptides. In vitro studies have shown this peptide to be 700 times as active as leu-enkephalin and 50 times as active as β-END. Dynorphin$_{1-8}$ has only 3% the potency of dynorphin$_{1-17}$ and is far less resistant to proteolytic degradation. Thus, dynorphin$_{1-8}$ displays the short duration of action common to neurotransmitters. The larger dynorphin$_{1-17}$ peptide, in contrast, displays hormonal qualities similar to β-END. In general, the dynorphins demonstrate comparable potency to enkephalins in contracting gut smooth muscle (61).

Methods of Study

The combined actions of the opioid peptides produce clinical manifestations similar to those of morphine. Although the exact mechanisms by which the opioids interact with various neurotransmitters and hormones is not yet clear, the opioids influence various regulatory processes throughout the body. Their actions, whether they be similar to those of neurohormones or those of neurotransmitters, have been shown experimentally to play a major role in analgesia, pain modulation, sleep, sexual activity, feeding behavior, endocrine regulation, thermo-regulation, ventilatory response, and the mechanisms of tolerance and addiction (62-64).

Clinical study of the endogenous opioids reveals an array of complex variables that are quite difficult to control. Thus, results from many studies provide conflicting evidence or findings which are non-conclusive.

Currently, four basic experimental approaches are used in opioid research. Each strategy is experimentally specific and yields exclusive information. The first approach to a hormonal study is to measure the amount of substance in the plasma, urine, or cerebralspinal fluid. The use of radio-immunological assays has been useful for this purpose. However, the structural similarity of the opioids complicates such procedures and yields a high incidence of cross reaction between different peptides. Recent procedures have remedied much of this problem by increasing the specificity of reacting antiserums. The use of gel exclusion chromatography has further enabled researchers to identify the activities of distinct peptides. Radioimmunoassay of the short-lived enkephalins have been made possible through the use of a proteolytic inhibitor, aprotinin (33).

A second research procedure, the administration of various opioids to human subjects, attempts to examine any behavioral, physiologic, or biochemical changes that the opioid may produce (66-69). Such studies lack adequate behavioral control and produce data that are largely subjective.

A third experimental approach is based on the use of opioid antagonists, enabling the researcher to partially reverse or abolish the effects of endogenous or exogenous opioids. Opioid antagonists, such as naltrexone and naloxone, have a high affinity for opioid receptors. This property enables them to competitively displace opioid effects. There is much dispute over the nature of pure antagonists, and whether or not the drugs themselves have any pharmacological actions is debated (70-72). Further debate exists over the proper dosage of these drugs.

There is some question as to whether or not small doses provide adequate blockade of all the opioid receptors as well as whether or not large doses interact with other neurosystems.

Finally, attempts have been made to activate the endogenous opioid system. Exposing experimental subjects to various forms of pain or stress stimulates the release of β-END from the pituitary (73-75). Electrical stimulation of the brain also causes release of opioids (76).

The Opioids in Stress and Pain

Strong evidence indicates an opioid role in the stress-adaptive mechanisms of the body. Opioid peptides are present in the hypothalamus, the three lobes of the pituitary, and the adrenal medulla. The adrenal cortex is highly sensitive to POMC products. The entire autonomic nervous system, including the central regulating nuclei and the nucleus tractus solitaris, is rich in both opioid receptors and opioid peptides (77). In combined effort, the autonomic nervous system and the adrenal cortex and medulla orchestrate a complex sequence of interactions which protect the organism against stress. The presence of the opioid system within the anatomical structures of this adaptive axis suggests another important role in the physiologic response to both environmental and endogenous stress.

Pain and the Opioids

Pain is an abstract term describing a cognitive awareness of bodily discomfort whether acute or chronic. Pain serves as a protective mechanism in the body signaling the organism of tissue damage. Stimuli

from endangered areas in the body are transmitted through specialized neural pathways and manifest themselves in an array of distinct signals. These pain signals include sensations of pricking, burning, aching, throbbing, nausea, stabbing, and cramping. In clinical practice, pain is classified as either "organic," representing reaction to a somatic lesion, or "psychogenic," representing a mental manifestation of discomfort. All pain syndromes have both a psychogenic and an organic component, and distinction between them is difficult.

It has become evident that the opioid system is anatomically located within specific pain pathways and plays a major role in the powerful mechanism of endogenous pain control.

Stress and Analgesia

A logical association exists between the stress activation of the opioid system and the production of analgesia. Such an association implies that it may be adaptive for the organism to become analgesic in the face of stress. Exposure to various forms of stress activates the endogenous opioid system in both man and laboratory animals (7,11,13). A general response to stress appears to be the co-secretion of pituitary ACTH and β-END in the peripheral circulation (7,11). In the rat, acute stress, induced by periods of intermittent foot shock or cold water swims, leads reproducibly to an insensitivity to pain (7,11,78,79). This reduction of pain is referred to as stress-induced analgesia (SIA). The potency of SIA is comparable to that of large doses of morphine, and the effect can be reversed by the opioid antagonist naloxone (78,79).

Madden and Akil (5) correlated increased opioid levels in the rat brain to the analgesia produced by the stress of sporadic foot shock. However, chronic daily exposure to such stress led to eventual adaptation of the animal. As stress exposures were repeated, much less analgesia was exhibited. In both man and the rat, direct electrical stimulation also produces analgesia (80). This form of analgesia is referred to as stimulation-produced analgesia (SPA) which also can be reversed by naloxone. The results of these studies strongly indicate some form of opioid involvement in the production of SIA.

The concomitant secretion of ACTH and β-END from the pituitary gland in response to stress presents what appears to be a direct association between β-END and SIA (81). However, β-END levels increase only in the peripheral circulation and not in the brain (82). Furthermore, ACTH and β-END do not cross the blood-brain barrier (BBB) (83). The inability of β-END to enter the brain prevents it from interacting with opioid receptors in the CNS. This fact has been demonstrated by the observation that peripheral injections of β-END do not alter spinal fluid endorphin levels, nor do they affect pain sensitivity or mood in humans (84). In contrast, intrathecal and cerebral injections of β-END produce profound analgesia (85). These results indicate that although endorphins alter pain sensitivity within the CNS, the stress-induced release of pituitary β-END does not appear to be directly involved in the production of SIA.

As previously mentioned, several studies have shown that chronic exposure to stress leads to loss of SIA. The loss of SIA has been found to accompany an enhanced release of β-END (86). This loss of analgesia

may be the result of various adaptations. For example, the pituitary has been shown to make several specific modifications in peptide release during prolonged exposure to stress. These changes include an increase in the production and release of N-acetylated forms of β-END which characteristically are inactive (87-89). Therefore, it is possible that a large percentage of the enhanced β-END release, following periods of chronic stress, is inactive. Such modifications may explain why an increase in β-END release accompanies loss of SIA.

Enkephalins and dynorphins are located in area of the CNS associated with pain perception and the stress axis. Hypothalamic dynorphin is elevated following acute exposure to foot shock (90). The use of an enkephalinase inhibitor, thiorphan, has been shown to enhance SIA (33). This effect can be reversed by naloxone and demonstrates a possible role of enkephalins in SIA. However, the selectivity of thiorphan to inhibit only enkephalin breakdown has not been established. Thus, there is no conclusive evidence linking SIA to a specific opioid family.

Opioid antagonists have been used in determining the nature of analgesia produced by stress. The literature contains conflicting accounts of naloxone's ability to reverse SIA. Such discrepancy reflects the fact that SIA contains both an opioid and a non-opioid component. Lewis demonstrated that the duration of stress exposure determines the type of analgesia produced (91,92). His experiments showed that short durations of stress produce analgesia of a non-opioid nature, while chronic stress exposures activated opioid analgesia. Lewis confirmed these findings by demonstrating complete naloxone

reversal of SIA and morphine cross-tolerance with SIA of an opioid nature (92).

The concept of a dual system of analgesia has been supported by several other studies. For example, a single stressor has been shown to produce either opioid or non-opioid analgesia, depending on where the stimulus is applied to the body (93). The dualistic nature of SIA has been demonstrated by other findings. Hypophysectomy abolishes opioid SIA but has no effect on non-opioid SIA (94). Additionally, corticosterone has the ability to replenish the opioid analgesia that is abolished by hypophysectomy (95). Such evidence suggests that there may be synergistic interaction between peripheral and CNS opioid pools in the production of SIA.

Peripheral Opioid Influence

The fact that pituitary β-END does not enter the brain suggests that this peptide has an alternative role which is limited to the periphery. Opioid receptors have been found in the adrenal cortex (29,96) and have been associated with corticosterone synthesis (97). Such evidence supports the speculation that pituitary β-END functions as a tropic hormone with direct action on various peripheral target organs.

The presence of enkephalins in the sympathetic ganglia and in the adrenal chromafin strongly suggests an intrinsic opioid role in the peripheral stress axis (98-100). Catecholamine release from the adrenal medulla is under primary control of neural input from the splanic nerve within the sympathetic nervous system. Morphine has direct influence over the release and synthesis of adrenal catecholamines (101-102).

This is apparent even after complete sympathetic denervation of the adrenal medulla.

Several studies suggest that there is an interaction between the peripheral stress axis and the activity of the opioid system in the CNS. Enkephalin-like peptides are co-stored with catecholamines in the adrenal medulla and are concomittantly released in response to stress. Lewis and co-workers (5) demonstrated that adrenal opioids influence SIA. In several experiments, they examined the effects that various adrenal manipulations have on SIA. Their findings showed that total adrenalectomy and emedulation blocked SIA, while removal of the adrenal cortex had no effect.

Stress activates both the hormonal and neural components of the stress-adaptive areas in release of opioid peptides into peripheral circulation. β-END is released through stimulation of the hypothalamic pituitary axis, while catecholamines and enkephalins are released under the primary influence of the peripheral sympathetic nervous system. The presence of the opioid system in the stress axis demonstrates that the opioids have an inherent physiologic role in the trophic defense mechanism of the organism. The function and regulation of these dual components appear to be distinctly independent; however, they are intricately related and are prophylactic in the face of stress.

Exercise and the Opioids

Stress-mediated hormonal responses have been studied through the use of exercise in both humans and the rat. The lower centers of the brain cannot differentiate one form of stress from another and,

therefore, react in a non-specific manner. Exercise is simply a general stress on the body which stimulates the secretion of a host of hormones from the pituitary. As do other forms of stress, exercise stimulates the concomittant release of pituitary β-END and ACTH into the plasma (12,13,104). Elevated levels of β-END have been linked to several psychological and physiological changes occurring during exercise. These include mood shifts, altered perception, menstrual disturbances in female athletes, and modulation of stress-related hormones (GH, ACTH, prolactin, catecholamines, and cortisol).

Hormonal Responses

The presence of opioid receptors and ligands in the hypothalamic-pituitary axis indicates the critical role of opioids in governing the release of pituitary hormones during exercise. By producing a condition absent of opioid influence, opioid antagonists alter hormonal release during exercise.

Exercise has been shown repeatedly to stimulate significant increases in growth hormone (GH) (104,105). The effect of the opiate antagonist naloxone on this response is not clear. Generally, low doses of naloxone have been shown to produce an effect, while higher doses have produced contrary results. For example, the use of .03 mg/min of naloxone produced a complete inhibition of GH secretion in professional cyclists (106); whereas, higher doses of naloxone during intense exercise yielded either no change in GH response or an enhanced response (107-109). Such discrepancy may be related either to the selection of subjects or to the intensity of exercise.

Exercise also produces significant increases in prolactin secretion. Low doses of naloxone have been shown to have no effect on prolactin response during running (107). However, higher doses of naloxone yield conflicting evidence. Moretti (106) demonstrated that naloxone inhibited the normal prolactin response in professional cyclists, but other investigators have not shown the same results (106,110).

Exercise produces an elevation of cortisol and ACTH (104,105). The opioids may influence the secretion of cortisol and ACTH by modulating the secretion of corticotrophic releasing factor (CRF) from the hypothalamus (110,111). Naloxone alone produces elevations in cortisol and ACTH (112). ACTH stimulates the adrenal cortex to release glucocorticoids such as cortisol which are important to gluconeogenesis and the availability of glucose during long-term exercise (113). Grossman (109) showed that naloxone produces a significant increase in cortisol levels during exercise; however, he did not cite any alteration in glucose or lactate levels.

The increased levels of leutenizing hormone (LH) and follicle stimulating hormone (FSH) seen in exercising males are enhanced by an infusion of naloxone (109,113-115). This response may vary in the female. Several animal and human studies report that opioid control fluctuates throughout the menstrual cycle (116,117). Many female athletes have reported various forms of menstrual disturbances (118-121). Such findings parallel those seen with morphine abuse in young women (122). Female athletes who have chronically elevated levels of opioids may alter the intricate hormonal balance required to stimulate

ovulation. If chronic training leads to opioid-mediated suppression of LH and perhaps FSH, these reductions could be responsible for such disturbances. Hyposecretion of estrogen has been cited consistently in amenorrheic athletes (118,123). MacArthur reports that the depressed levels of gonadotropin in female athletes with amenorrhea are reversed by naloxone (124).

Vasopressin is released under stressful conditions in both humans and rats (125,126). Opioid inhibition of this reponse is suppressed by naloxone. That is, when naloxone is administered under stressful conditions, an increased release of vasopressin has been reported (127-129). Dynorphin is located primarily in the CNS, particularly in the hypothalamus and posterior piutitary, and this opioid may play a major role in the release of vasopressin (130). Opioid influence on vasopressin release during exercise has not been examined. However, renin and aldosterone levels are significantly elevated by naloxone during exercise (109). Possible opioid regulation of osmotic conditions during exercise is indicated.

Opioid influence over catecholamine release is suggested by the fact that opioid receptors exist in the adrenal medulla and the autonomic nervous system (38,39). The normal rise in circulating catecholamines during exercise is augmented by naloxone (109). An interesting relationship exists between catecholamine and opioid responses following training. Physical training, while enhancing the endorphin response at a given workload (131), tends to diminish the catecholamine response (105). Levels of perceived exertion have been associated with catecholamine levels (132). These findings suggest that

enhanced opioid release following training may be responsible for the suppression of catecholamine release. The trained individual, therefore, may be able to perform at higher intensities of work while perceiving less exertion than the untrained subject.

Physiologic Responses

Athletes demonstrate a different endorphin response during exercise than that seen in sedentary individuals. Not only is the endorphin response enhanced with training, but peak endorphin levels occur at different points during exercise. Berk et al. (133) reported that athletes exhibit an enhanced endorphin response which peaks prior to maximal performance. In contrast, peak endorphin levels in untrained individuals occur 15 minutes post-exercise. Unlike the response of other stress-related hormones during exercise, an intensity-dependent relationship between workload and endorphin release has not been consistantly demonstrated. Colt et al. (12) studied 26 well-trained runners and found evidence supporting an intensity-dependent release of β-END (12). Farrell (13), in contrast, observed little if any correlation between exercise intensity and endorphin response. In 30-min treadmill runs at 60% and 80% of VO_2 max, six trained runners exhibited significantly elevated opioid levels only during runs of 60% VO_2. However, a relationship between endorphin levels and perceived exertion was noted in the study.

Morphine has long been known to drastically suppress respiration, and large doses produce lethal results. Endogenous opioids also appear to influence ventilation (135-137). Human studies have demonstrated

that naloxone produces a marked increase in ventilation during maximal exercise (17,109). Opioid influences on ventilatory responses to exercise are strongly indicated by the presence of opioids in two areas that are critical in the regulation of breathing, the brainstem (138) and carotid body type I glomus cells (139). Experiments have shown that naloxone reverses the opioid inhibition of chemoreceptor discharge in carotid body cells (140). This phenomenon was demonstrated in the presence of both hypoxic and non-hypoxic conditions.

Opioids have been reported to produce a variety of effects on the cardiovascular system. Individual opioid receptors mediate distinctly different vasoregulatory responses. These include basic depressions of heart rate and blood pressure as mediated by the μ and δ receptors (5). Exercise studies have failed to show any opioid effects on heart rate, blood pressure, cardiac output, or maximum oxygen uptake (17,109).

Behavior and Analgesia

A specious association has been made between exercise and the reported euphoric "runner's high" that has promoted the endorphins as prime mediators. Exercise has been reported to produce a "high," an increase in pain tolerance, and a behavioral addiction (71). All of these effects mimmick the effects of opioid ingestion. Strong and stimulating personal accounts, although devoid of scientific basis, have captured the fancy of many enthusiastic exercisers.

Exercise has been established as being therapeutic by effecting positive mood shifts in both normal and depressed subjects (141-146). There are several explanations for such mood alterations; however, few

studies have successfully correlated the opioids with such modulations. Markoff et al. (145) did not demonstrate mood shifts following exercise with the administration of naloxone. Haier (17) examined an opioid basis for pain modulation during exercise in human subjects. According to his findings, exercise decreases pain sensitivity as demonstrated by an increase in the time required to report pain produced by a 1.2 g weight placed on the subject's fingertip. However, further study yielded different results. While 10 mg of naloxone abolished post-exercise analgesia, doses of 2 mg appeared to enhance insensitivity to pain (17). In other studies, naloxone has been reported to produce mildly elevated perceptions of fatigue and exertion during and following exercise (137). In summary, the effect that the opioid system has on pain sensitivity during exercise in humans is unknown and requires further study.

In contrast to the sparse and unclear documentation of opioid involvement in pain modulation during exercise in human subjects, animal studies have shown much more definitive findings. In the rat, prolonged periods of voluntary running produce significant analgesia as discerned by the squeak threshold. The exercise-induced analgesia demonstrated was totally reversed by 1-2 mg of naloxone (10). These findings suggest that prolonged muscular exercise, acupuncture, and low frequency electrical stimulation all produce discharges in muscle afferents which stimulate the opioid mechanism to produce analgesia. Pert and Bowie (150) reported reduced binding of [^{3}H]-D-Ala^{2}-Met^{5} enkephalinamide in the brain of exercising rats. From these findings the investigators suggest that the decreased binding of enkephalinamide was the result of

the increased binding of endogenous opioid ligands. Bodnar et al. (79) reported that cold water swimming produces significant tolerance to flinch jump tests. However, in separate studies, treatments with morphine and swimming failed to produce cross-resistance (151,152). As a result, Bodnar surmised that SIA has a non-opiate component.

Christie et al. (153) demonstrated an association between the increased binding of endogenous opiates in mouse brain homogenates and antinociception produced by exercise. Further results demonstrated that the discontinuation of chronic exposure to warm water swimming produces behavioral changes similar to those occurring from withdrawal from morphine (154,155). Collectively such evidence supports an opioid role in pain modulation during exercise.

How significant the production of analgesia during exercise is to actual performance is not clear. Many studies have demonstrated elevated pain threshold in the rat following exercise (5-9,79,153). A direct explanation for this occurrence has not been established. The investigators strongly suggest that the opioids are at least partially responsible for modulating behavior in the rat. Naloxone is presumed to produce an alteration in such behavior. However, other opioid influences should not be ruled out. Opioid blockade in the rat may intensify the animal's sense of fatigue or exertion, as is noted in human subjects during exercise. Therefore, the activity produced by naloxone may have been related simply to the animal's heightened awareness of fatigue or exertion. The assessment of behavioral and perceptual characteristics in the laboratory animal is difficult and should not be hastily presumed.

The established fact that exercise stimulates the release of pituitary β-END into the peripheral circulation does not indicate opioid involvement in the production of analgesia or euphoria. The blood-brain barrier separates the periphery from the CNS, creating two physiologic compartments, thus preventing β-END from producing these central effects. As mentioned before, large peptides such as ACTH and β-END are unable to enter the brain from peripheral circulation under normal conditions. However, it is unclear whether conditions of severe stress alter BBB permeability. Tryphan blue, a substance normally impermeable to the BBB, has been shown to enter the brain of the rat during severe exercise (156). Such findings do not necessarily hold true for endogenous substances under similar conditions.

Opioid mediated activity is not limited to only the stress activation of the bodies' endorphins. The opioid mechanisms are spread diffusely throughout the body and appear to have a definite role in the general response of the organism to stress. The involvement of this system in hormonal regulation and pain suppression has been previously mentioned. How much influence this opioid interaction exerts on exercise performance is still unclear.

CHAPTER III

RESEARCH METHODS

Twenty-four albino rats (Sprague-Dawley strain) were brought into the laboratory at 72 days of age. The rats remained in the laboratory for a ten-day adjustment period before the commencement of the training program. Lighting conditions were controlled in the animal quarters so that the lights were on daily between 10:00 p.m. and 10:00 a.m. The lights were completely off during the other twelve hours. Since rats are nocturnal and the investigators diurnal, these conditions allowed procedures to take place during the daily active period of all concerned.

Throughout the adjustment, training, and testing periods a relatively constant environment was maintained. Standard laboratory procedures were maintained such as regular handling of the animals, waste removal, and temperature control. The rats were housed in individual voluntary-activity cages, each equipped with a freely revolving activity wheel, throughout their stay in the laboratory. Water was available at all times, and they were permitted to eat commercial food pellets ad libitum.

Exercise Apparatus

The training and testing of the rats was performed in individual controlled-running wheels (CRW) developed at the Human Energy Research Laboratory of Michigan State University. The CRW is an animal-powered wheel which allows small laboratory animals to participate in a wide variety of specific controlled programs of reproducible exercise (19). The animals learn to run by shock-avoidance operant conditioning. Various exercise training programs are achieved by setting several parameters on a master control unit which regulates a battery of 12 CRW. These parameters include: acceleration time, work time, rest time, number of repetitions per exercise bout, number of bouts, time between bouts, shock level in amperes, and running speed.

A light above the wheel comes on at the beginning of each work interval (following automatic release of the wheel brake) and at any time during the work interval that the animal fails to maintain the pre-set speed. The light stays on for a specified acceleration period (0.5 - 5.0 seconds) allowing the animal to reach the pre-set running speed. If the animal fails to reach the set speed during the allotted time, a controlled shock current is applied through the running surface. The light and shock are discontinued once the animal achieves the set running speed and remain off for as long as this speed is maintained. Following the training period, most animals recognize that the light signals the shock and will run in response to the light.

Training Program

The rats were trained in preparation for three low-intensity, long-duration test runs. An aerobic training program of slow speed, long-duration work periods and short-duration rest periods was used. Throughout the training sessions, the velocity was pre-set at 2 ft/sec. For the first three and one-half weeks, the rats were exercised for two consecutive days, followed by a day of rest, for a total of 16 exercise sessions. Exercise duration was increased gradually until the daily exercise routine consisted of one bout of three repetitions. Each repetition was composed of a five-minute work interval and a 40-second rest interval.

One day prior to the first scheduled testing session, a viral infection was observed in the rats. Testing therefore was delayed for a ten-day period during which oral and ocular antibiotics were administered. The rats underwent a retraining program for two weeks consisting of a training session every other day for a total of eight exercise sessions. At the end of these sessions, the rats attained the same exercise level as they had before their illness.

Three inter-testing training sessions per week were conducted to maintain fitness in the rats between testing days. A complete training schedule is listed in Appendix A.

Rat Selection

A total of 18 rats were selected to participate in the testing sessions based on their previous training performance records. Daily performance data were expressed as a percentage of expected revolutions

run (PER) and as a percentage shock-free time (PSF). Total revolutions run (TRR) and total expected revolutions run (TER) were used to calculate PER as follows:

$$PER = 100(TRR/TER)$$

Cumulative duration of shock (CDS) and total work time (TWT) were used to calculate PSF as follows:

$$PSF = 100(TWT-CDS)/TWT$$

The rats that consistently performed the best with regard to PER and PSF over the entire training period were selected for final testing. A sample graph of daily training data is shown in Appendix B.

Test Sessions

The exercise performance of each rat was evaluated on each of three separate test days, exactly one week apart. Twenty minutes prior to the test session, each rat received a single subcutaneous injection of naltrexone, quaternary naltrexone, or saline. The treatments were distributed randomly over three test days so that each rat received each treatment once. The rats weighted between 325 and 425 grams each at the time of the test sessions.

Naltrexone

The opiate antagonist naltrexone hydrochloride crosses the blood-brain barrier when administered and thus blocks both central nervous system and peripheral opioid receptors. Naltrexone HCI (Sigma Chemical Co.) was dissolved in physiologic saline (0.15m) and administered in doses of 0.5 mg/kg body weight in volumes of 0.5 ml/kg of body weight.

Quaternary Naltrexone

The opiate antagonist quaternary naltrexone (naltrexone methobromide) does not cross the blood-brain barrier. When subcutaneously administered, this quaternary analog of naltrexone blocks only peripheral opiate receptors. Quaternary naltrexone is approximately 40 times less potent than naltrexone in the blockade of opiate receptors. Therefore, naltrexone methobromide (MRZ-2663, compliments of Dr. H. Merz, Boehringer Ingelheim KG) was dissolved in physiologic saline (0.15m) and administered in doses of 20.0 mg/kg body weight in volumes of 0.5 ml/kg body weight.

The quaternary naltrexone test condition was not an integral part of the original research design for this investigation. The quaternary naltrexone treatment was included because the animals were being used as subjects for a concurrent companion study. Careful analysis of all of the data have shown that the addition of the quaternary naltrexone treatment had no effect on the naltrexone-versus-saline comparisons that were planned for this experiment. Therefore, the quaternary naltrexone results have not been reported here.

Placebo

Physiologic saline (0.15m) was used as a placebo and was administered in volumes of 0.5 ml/kg body weight.

Data Collection

Following each drug injection, the rats were placed in individual controlled running wheels. The wheels were programmed for one continuous 45-minute run at a pre-set velocity of 2 ft/sec. One technician was assigned to watch one wheel per test. For every minute of the 45-minute test, the technician recorded the total revolutions run (TRR) and the cumulative duration of shock (CDS). A sample of the recording sheet is located in Appendix C.

Data from the 45-minute test sessions were grouped into six separate five-minute intervals as shown in Table 1. The data collected beyond 35 minutes were not used as some of the rats did not complete the total 45-minute run.

Table 1. Definition of the time intervals used during the test sessions.

Interval	Time Period (minutes)
1	5-10
2	10-15
3	15-20
4	20-25
5	25-30
6	30-35

Statistical Analysis

Performance data initially were analyzed using a two-way analysis of variance (ANOVA). The independent variables were drug, containing two levels, and time interval, containing six levels. If no interaction between independent variables was seen, individual one-way ANOVA's were run at each time interval. In addition, a nonparametric binomial test was used to perform time-series analysis between drug groups. An alpha of 0.05 was chosen as the level of statistical significance.

CHAPTER IV

RESULTS AND DISCUSSION

Mean percentages of expected revolutions run (PER) and mean percentages of shock-free time (PSF) were used for comparison of exercise performance between treatment groups.

PER Results

The performance data figured as PER are presented in Table 2. The results of the two-way ANOVA indicated no significant drug effect ($p = 0.481$), but there was a significant time interval effect ($p < 0.001$). No interaction existed between these two independent variables ($p = 0.944$); therefore, individual one-way ANOVA's were performed on the data collected during each time interval. These results indicated that naltrexone injections had no significant effect on PER, as compared to the placebo injections of saline, during any interval.

A graphical representation of the PER data (Figure 1) shows a significant ($p < 0.03$) time-related decrease in performance across both groups during the test sessions. As anticipated, the longer the rats ran, the more fatigued they became.

Table 2. Mean values for percentage of expected revolutions run (PER).

	Naltrexone		Saline	
Interval	N	Mean ± SEM	N	Mean ± SEM
1	18	109.00 ± 5.66	18	110.61 ± 5.17
2	18	106.39 % 3.91	18	107.28 % 3.41
3	18	98.33 ± 3.58	18	100.50 ± 3.00
4	18	96.33 ± 3.09	18	97.83 % 3.54
5	18	94.89 ± 4.23	18	94.11 ± 3.49
6	18	89.72 ± 4.43	18	94.17 ± 3.89
Means		92.39 ± 1.91		100.75 ± 1.64

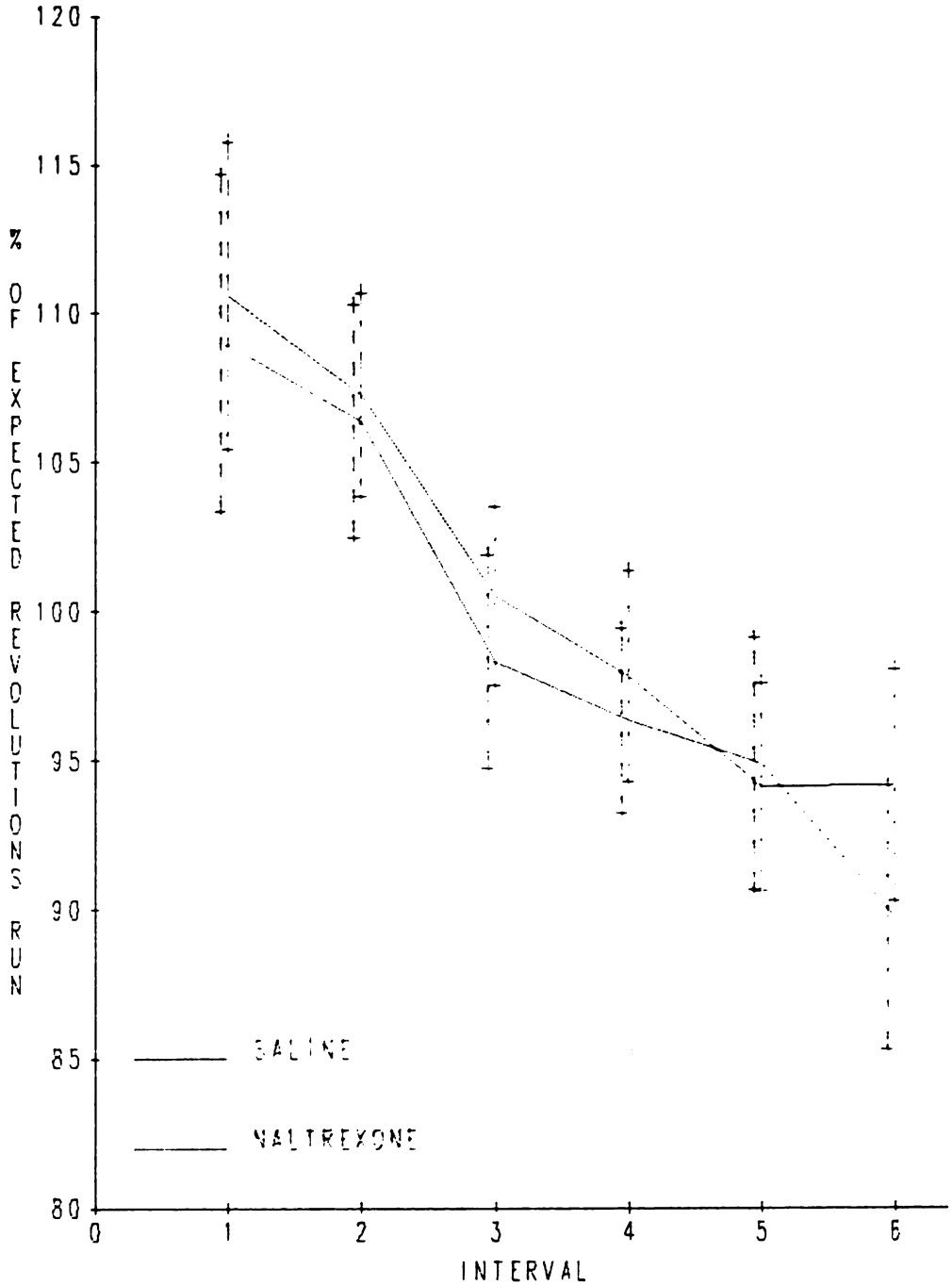

Figure 1. **Graphical representation of percentage of expected revolutions run (PER).**

PSF Results

The performance data figured as PSF are presented in Table 3. The results of the two-way ANOVA indicate no significant drug effect ($p = 0.379$) but a significant interval effect ($p = 0.001$). No interaction occurred between these two independent variables ($p = 0.998$); therefore, one-way ANOVA's were performed on the data obtained during each interval. No significant difference between PSF performance in naltrexone and saline injected rats was seen during any individual interval.

Table 3. Mean values for percentage of shock-free time (PSF).

	Naltrexone		Saline	
Interval	N	Mean ± SEM	N	Mean ± SEM
1	18	95.39 ± 1.83	18	96.50 ± 1.17
2	18	96.78 % 1.00	18	97.28 % 0.74
3	18	94.22 ± 1.76	18	96.06 ± 1.10
4	18	92.39 ± 1.90	18	92.44 ± 2.26
5	18	91.00 ± 2.50	18	92.39 ± 2.09
6	18	89.72 ± 2.41	18	90.50 ± 2.38
Means		91.30 ± 1.05		94.19 ± 0.74

A graphical representation of the PSF data (see Figure 2) shows a time-related trend which insignificantly ($p = 0.19$) parallels that of the PER data. This suggests that more shock was required to motivate the rats to run as the duration of exercise increased.

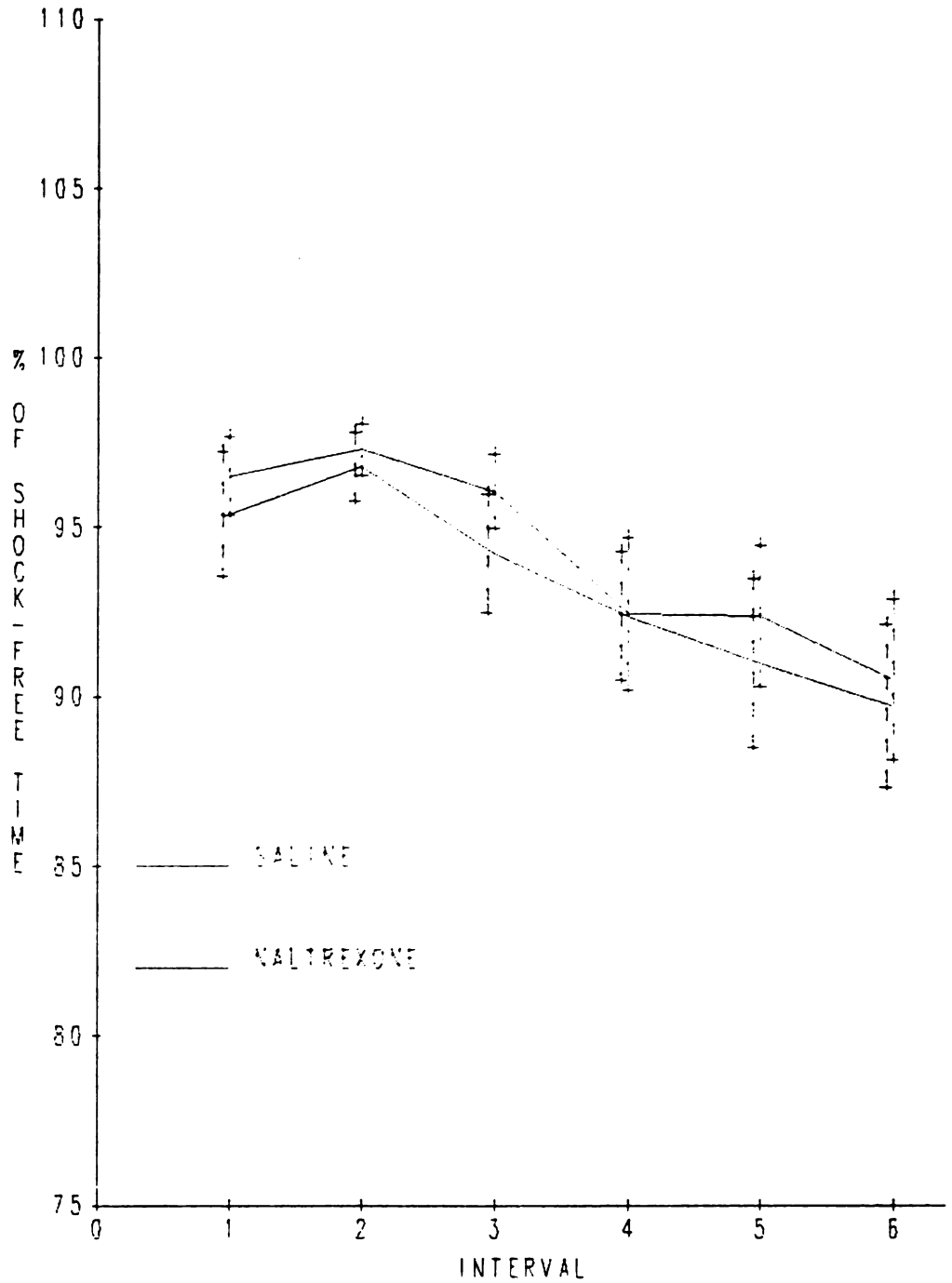

Figure 2. Graphical representation of percentage of shock-free time (PSF).

DISCUSSION

Exercise performance in the rat as measured by two separate criteria, PER and PSF, apparently is unaffected by the administration of the opioid antagonist naltrexone. As would be expected, running performance decreased in the rats as the duration of exercise increased.

The hypothesized results of this study proposed that blockade of opioid influence during exercise would diminish running performance in the rat. Such inference was made partially under the assumption that suppression of any analgesia produced by the exercise-stimulated activation of the opioid system would enhance the sensation of fatigue and produce a diminished running performance.

Several factors may have significantly affected the results of this study. The use of electrical shock as a method to stimulate continuous running in the rat in itself presents a problem. Electrical foot shock has been shown to produce analgesia in the rat. This analgesia can be reversed by the opioid antagonist naltrexone.

There is a strong possibility that naltrexone produced a hypersensitivity to foot shock in the rat. In such a case, the rats may have experienced a much greater urge to run. The rats' heightened awareness of the shock stimulus could have been of a magnitude significant enough to camouflage any subtle drug effect that naltrexone had in diminishing the rats' running performance. With the possibility that a state of generalized hypersensitivity existed, it is difficult to say which component provided the greatest incentive.

Another area of concern is the dosage of naltrexone. Several studies have cited a variety of results using different doses of

naltrexone. In this study, a dose of 0.5 ml/kg of naltrexone was used. This was chosen conservatively in an attempt to prevent cross-receptor blockade of another receptor system.

The results from a companion study using quaternary naltrexone, a form of naltrexone which does not cross the blood-brain barrier, demonstrated different results. The companion study was completed using a protocol identical to the one used in this study. A comparable dose of quaternary naltrexone was injected into the peripheral circulation of the rat. The results showed a significant decrease in the rats' running performance following injections of quaternary naltrexone.

The doses of naltrexone and quaternary naltrexone were estimated to be of equal potency; however, the receptor coverage may have differed significantly. The highest density of opioid recepters lies within the CNS. Injection of quaternary naltrexone into only the peripheral circulation may have provided a much greater ratio of opioid antagonist to receptors. The effect of the drug may have been more pronounced or may have provided cross-coverage into another receptor system. Optimal drug dosage remains an area requiring further investigation.

CHAPTER V

CONCLUSIONS

The following conclusions pertain to aerobic exercise in the rat using electric foot shock as the running incentive:

1. A 0.5 mg/kg body weight dose of naltrexone providing both central and peripheral opiate blockade does not significantly affect running performance in the rat.

2. During a continuous aerobic run, the rats' performance decreases as the length of the run increases. Rats demonstrate a fatigue curve similar to that seen in human subjects.

Recommendations

1. This study should be repeated using a different running incentive. The development of such a technique may be difficult due to the fact that any method producing enough stimulus to provoke running may in itself produce analgesia.

2. A study with a design similar to this study using human subjects could provide much clearer results. Human subjects would not require an external incentive to run. Motivation in the human to continue to run would depend on internal factors, including perceptions of pain and fatigue, all of which may be affected significantly by

opioid blockade. These subjective factors would be much easier to isolate through a well-controlled study using human subjects.

3. Additional studies should be performed using a variety of exercise intensities and durations. Such studies would help to determine if the opioid response is present in an acute versus chronic situation.

4. Finally, dose-response studies using larger doses of naltrexone would help determine if there is a level of opiate blockade capable of diminishing running performance in the rat.

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APPENDICES

APPENDIX A

TRAINING PROGRAM

Table A1. Aerobic training program of slow-speed, long-duration

Week of Study	Day of Week	Day of Training	Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Reps per Bout	Run Speed (ft/sec)	Total Work Time (sec)	Total Expected Revolutions
0	Fri	-2	---	---	900	---	---	---	---
	Sat	-1	---	---	900	---	---	---	---
1	Sun	1	3.0	0:10	0:10	45	2.0	450	225
	Mon	2	3.0	0:10	0:10	45	2.0	450	225
	Wed	3	3.0	0:20	0:10	30	2.0	600	300
	Thurs	4	2.0	0:20	0:10	30	2.0	600	300
	Sat	5	2.0	0:30	0:15	20	2.0	600	300
2	Sun	6	2.0	0:40	0:20	15	2.0	600	300
	Tues	7	2.0	0:50	0:25	12	2.0	600	300
	Wed	8	1.5	1:00	0:30	10	2.0	600	300
	Fri	9	1.5	1:00	0:30	10	2.0	600	300
	Sat	10	1.5	1:00	0:30	10	2.0	600	300
3	Mon	11	1.5	2:30	0:40	5	2.0	750	375
	Tues	12	1.5	2:30	0:40	5	2.0	750	375
	Thurs	13	1.5	2:30	0:30	6	2.0	900	450
	Fri	14	1.5	2:30	0:30	6	2.0	900	450
4	Sun	15	1.5	5:00	0:40	3	2.0	900	450
	Mon	16	1.5	5:00	0:40	3	2.0	900	450
5	Thurs	17	1.5	0:40	0:20	15	2.0	600	300
	Sat	18	1.5	0:50	0:25	12	2.0	600	300
6	Mon	19	1.5	1:00	0:30	10	2.0	600	300
	Wed	20	1.5	1:00	0:30	10	2.0	600	300
	Fri	21	1.5	2:30	0:40	5	2.0	750	375
	Sun	22	1.5	2:30	0:30	6	2.0	900	450
7	Tues	23	1.5	5:00	0:40	3	2.0	900	450
	Thurs	24	1.5	5:00	0:40	3	2.0	900	450
	SAT TEST DAY		1.5	45:00	0:00	1	2.0	---	---
8	Mon	25	1.5	15:00	0:00	1	2.0	900	450
	Tues	26	1.5	15:00	0:00	1	2.0	900	450
	Thurs	27	1.5	15:00	0:00	1	2.0	900	450
	SAT TEST DAY		1.5	45:00	0:00	1	2.0	---	---
9	Mon	28	1.5	15:00	0:00	1	2.0	900	450
	Tues	29	1.5	15:00	0:00	1	2.0	900	450
	Thurs	30	1.5	15:00	0:00	1	2.0	900	450
	SAT TEST DAY		1.5	45:00	0:00	1	2.0	---	---

APPENDIX B

SAMPLE TRAINING PERFORMANCE DATA

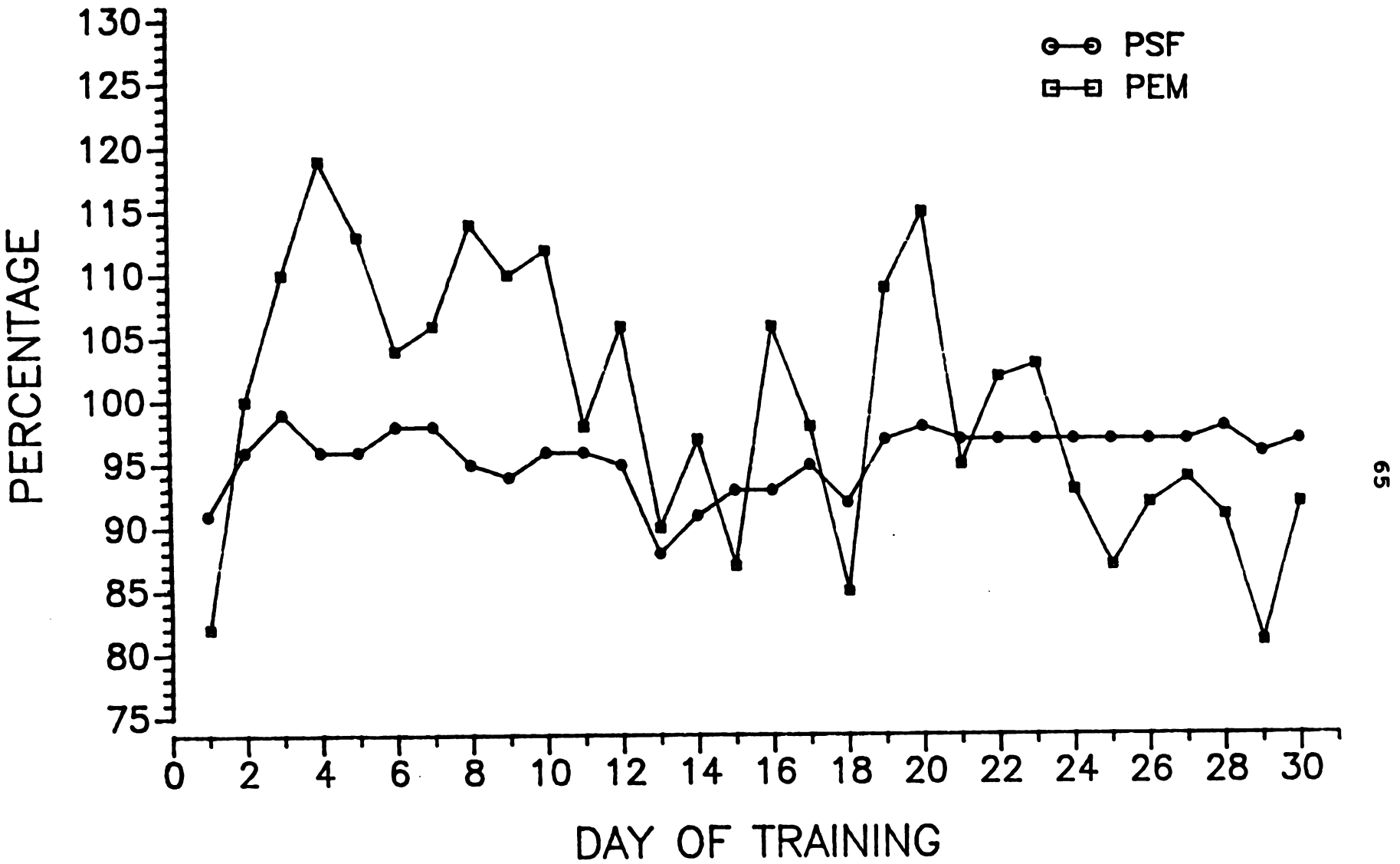

Figure B1. Training performance data for rat #4.

APPENDIX C

SAMPLE TEST RECORDING SHEET

Rat Number _____ Wheel Number _____ Test Date ___/___/___

Test Time _____ Treatment _____ Wheel Operator _____

	TRR[1]	CDS[2]		TRR	CDS
1.			23.		
2.			24.		
3.			25.		
4.			26.		
5.			27.		
6.			28.		
7.			29.		
8.			30.		
9.			31.		
10.			32.		
11.			33.		
12.			34.		
13.			35.		
14.			36.		
15.			37.		
16.			38.		
17.			39.		
18.			40.		
19.			41.		
20.			42.		
21.			43.		
22.			44.		
			45.		

[1]TRR = total revolutions run.

[2]CDS = cumulative duration shock.

Figure C1. Sample recording sheet used on test days.