# THE RELATIVE EFFECTS OF TIZANIDINE AND BACLOFEN ON SENSORIMOTOR FUNCTION IN RATS

By Suzanne De Haney

A Thesis Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Physiology

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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## Abstract

INTRODUCTION: Tizanidine, an α2-noradrenergic/imidazoline agonist, and baclofen, a GABAb agonist, have been shown to have both antinociceptive and myorelaxant effects. In this study, the relative effect of tizanidine and baclofen on motor performance was tested at comparable analgesic dosages in rats. METHODS: Analgesia was measured using the tail-flick test and an analgesic index was calculated based on the cumulative increase in tail-flick latency. Motor performance was measured from kinematic analysis of swimming and a performance index was calculated based on the cumulative decrement in swim speed. Drugs, tizanidine and baclofen, were administered intraperitoneally following three baseline measurements at twenty minute intervals and subsequently monitored at regular twenty minute intervals for a period of two hours. Three comparable dosages were identified based on analgesia: low dose (0.5mg/kg tizanidine and 2.0 mg/kg baclofen), mid dose (1.5 mg/kg tizanidine and 5.0 mg/kg baclofen), and high dose (3.0 mg/kg tizanidine and 6.0 mg/kg baclofen). RESULTS: Tizanidine and baclofen produced a dose-dependent increase in the duration of response to a thermal stimulus in the tail-flick test at all doses, relative to the pre-injection baseline and the saline control. Similarly, both drugs at all doses produced positive analgesic indices. Tizanidine and baclofen produced comparable analgesia that both differed significantly from the placebo (saline control) at low, mid and high dosages, based on an analysis of variance (ANOVA) and the Tukey-Kramer Honestly Significant Difference (HSD) test. The myorelaxant effects of tizanidine and baclofen produced a decrement in motor performance, as reflected by a decrease in swim speed in the swim test, relative to the pre-injection baseline and the saline control. Similarly, both drugs at all doses produced negative performance indices; however, mid dose baclofen produced the lowest performance index relative to all doses. At low dosages, there was no significant difference between the analgesic response ($p=.9312$) or the decrement in swim speed produced by baclofen and tizanidine ($p=.4257$). At mid dosages, the myorelaxant effects

of tizanidine and baclofen differed significantly ($p=.0007$) with comparable analgesia ($p=.0616$). In addition, motor performance with baclofen differed significantly from the saline control while motor performance with tizanidine did not differ significantly from the control treatment. At high dosages, there was no significant difference between the analgesic response ($p=.6199$) or the decrement in swim speed produced by baclofen and tizanidine ($p=.6575$). A significant time effect was observed at mid and high doses ($p<0.0001$) and low dose analgesia ($p=.0334$). CONCLUSION: The present study describes the use of an integrated sensorimotor model to compare two drugs used as antispasticity medications. Results showed that at selected dosages, tizanidine produced less decrement in motor strength relative to baclofen in rats, but with comparable analgesic effects for the two drugs. A potential application for tizanidine supported by this study is the reduction of the undesirable side effect of muscle weakness frequently associated with antispasticity medications.

## Dedications

This effort is dedicated to my mother and father, Violet and William, who I treasure as respected and generous teachers in many aspects of my life, and to friends, Wreatha and Jack Maw, whose motivation and perspective continues to be a source of inspiration.

## Acknowledgements

There are many people to whom I am truly grateful for the encouragement and support during this period of learning and growth. I would like to start by thanking Dr. Pat Nance for the opportunity to be a graduate student in a lab that deals with both clinical and basic science research. I am thankful to Orpha Shryvers for providing guidance on the hospital policies and procedures affecting the project. Thanks to Brian MacNeil for sharing insights and experience on all matters electrical and digital. I also appreciate Lei Quan for generous contributions of time and energy towards my scientific, medical and laboratory training. I sincerely appreciate the committee members, Dr. Jim Nagy, Dr. Ursula Tuor and Dr. Dwight Nance, for providing suggestions and feedback with such concern and consideration. I have to give thanks to Athena Neurosciences for providing the tizanidine hydrochloride as well as the financial support for the study. I also need to thank all the people who have continued to love and support me. To my family, thank you. A special thanks to all the friends made along the way, in classes and conferences, through tennis practices and volleyball games, from Winnipeg to Washington and back again. These cherished souls include, but are not limited to, Farah, Fay, Lisa, Sara, Sandra, Randy and Charles. The kindness demonstrated will not soon be forgotten and has provided me with many wonderful memories.

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## Abbreviations

GABA: gamma-aminobutyric acid
NE: norepinephrine
DA: dopamine
5-HT: 5-hydroxytryptamine (serotonin)
6-OHDA : 6-hydoxydopamine
I: imidazoline
AR: adrenergic receptor
IR: imidazoline receptor
GAD: glutamate decarboxylase
SP: substance P
LC: locus ceruleus
DS 103 282: prior name for tizanidine
T: tizanidine
B: baclofen
HRP: horseradish peroxidase
DBH: dopamine-B-hydroxylase
EPSP: excitatory postsynaptic potential
IPSP: inhibitory postsynaptic potential
PAD: primary afferent depolarization
MS: multiple sclerosis
SCI: spinal cord injury
CNS: central nervous system
i.p.: intraperitoneal
i.v: intravenous
i.th.: intrathecal
TF test: tail flick test
PBC test: phenyl-p-benzoquinone test
EMG: electromyogram
ANOVA: analysis of variance
MANOVA: multi-way analysis of variance
p: probability
HSD: Honestly Significant Difference

# Chapter One: Introduction

## 1.1 Introduction

Many descending motor systems, such as corticospinal, reticulospinal, rubrospinal and vestibulospinal tracts, as well as ascending sensory systems, such as the spinothalamic tract and dorsal column-medial lemniscus, are involved in information flow between the brain and spinal cord. A spinal cord injury may result in an upper motor neuron syndrome, including spasticity and central pain. (164) A number of descending systems have been identified to act as monoaminergic inhibitory controls on both spinal motor and sensory systems; the neurotransmitters within these neurons often include dopamine, serotonin and norepinephrine. (114, 174) In addition, amino acid-mediated neurotransmission by aspartate, glycine and GABA has been identified in the spinal cord level. (42) This is a study of two pharmacologic agents, tizanidine and baclofen, which interact with neurotransmitter mechanisms underlying spasticity, at cerebral or segmental spinal levels.

## 1.2 Statement of the Problem

Various disorders of the central nervous system produce increased skeletal muscle tone, such as multiple sclerosis or spinal cord injury, and are treated with a variety of antispasticity medications, including Baclofen (Lioresal), Diazepam (Valium), Dantrolene Sodium (Dantrium), Clonidine (Catapres/Dixirit) and Tizanidine (Zanaflex). While baclofen in widely prescribed in North America, tizanidine has become more recently available and it is suggested that tizanidine and baclofen differ with respect to the undesireable effect of muscle weakness. (8, 57, 63). For example, tizanidine was reported to produce antispastic effects in patients without adversely affecting muscle power. (91) Thus, the opportunity for decreasing muscle tone without increasing muscle weakness

is relevant to pharmacologic treatment selection. In contrast, other studies have not demonstrated a clear advantage of tizanidine relative to baclofen (109, 157). This variability in results, and between patients, is a function of the dependent measure such as spasm frequency, muscle tone improvement, or efficacy as well as the complexity of the spasticity syndrome. In this study, a novel measure of motor function, the swim test, is combined with a standard measure of analgesia, the tail flick test, to determine the relative effects of tizanidine and baclofen in animals over a range of doses

1.3 Rationale for Hypothesis

Tizanidine and baclofen have demonstrated muscle relaxant and analgesic properties and function by distinct neuroanatomical pathways and receptor mechanisms. It was believed that motor effects would differ at doses that produce equivalent analgesic effects due to the preferential reduction of the excitation of spinal neurones at polysynaptic reflexes with tizanidine, rather than at monosynpatic reflexes with baclofen. Animal studies corroborate this suggestion where tizanidine demonstrated greater selectivity in depression of responses to noxious stimuli while baclofen produced less selective depression of responses to both noxious and innocuous stimuli. (30, 31) Similarly, the specificity of tizanidine relative to baclofen was demonstrated by the stong depression of group II afferents by tizanidine with negligible effects on group I afferents, while baclofen has produced inconsistent depression of group II afferents and and consistent depression of group I afferents. (152) Clinical studies of the comparative abilities of tizanidine relative to baclofen to suppress spasticity have shown that tizanidine has specific reflex effects separate from its effect on resting tone. (59) For example, in a study by Smolenski and colleagues to compare treatments for chronic spasticity in MS, muscle weakness was more commonly reported with baclofen while

tiredness was a more common side effect with tizanidine. (155) In another study, videomotion analysis of the pendulum test demonstrated significant improvements with tizandine relative to placebo in muscle tone in MS patients. (107) Thus, the dual antispastic and analgesic actions of tizanidine and baclofen will be considered simultaneously to determine dose effects on motor function at comparable sensory levels.

## 1.4 Hypothesis

We hypothesize that the two antispasticity drugs, tizanidine and baclofen, will produce a dose-dependent difference in motor performance, but comparable analgesic effects.

## 1.5 Purpose of the study

The purpose of the study was to test the dose-dependent effects of tizanidine and baclofen on swim speed and latency of the tail-flick response.

## 1.6 Experimental Objectives

1. To establish comparable analgesic dosages between tizanidine and baclofen.

2. To compare the relative effects of tizanidine versus baclofen on motor performance at doses that produce comparable analgesia.

## 1.7 Assumptions

It was assumed that the swim speed parameter reflected motor performance and segmental (spinal) reflex activity. Furthermore, it was assumed that the analgesic response measured by the

tail flick test was based on a spinal response and not as a result of learning or other higher center functions.

## 1.8 Scientific contribution

Results of this study will provide behavioral evidence for the relative contribution of tizanidine and baclofen to the undesirable side effect of muscle weakness using an animal model.

## Chapter Two: Review of the Literature

### 2.1 Norepineprine

#### 2.1.1 Anatomy of the Noradrenergic system

Classical studies by Dahlstrom and Fuxe to localize central noradrenergic neurons in the brainstem used histochemical fluorescence techniques to identify ten distinct catelcholamine-containing neurons, labelled from caudal to rostral in the brainstem. Noradrenergic neurons have been localized immunocytochemically by reaction to antiserum to dopamine-B-hydroxylase, the enzyme that converts dopamine to norepinephrine. Contrary to initial findings using the retrograde horseradish peroxidase (HRP) technique that all noradrenergic cell groups project to the spinal cord, newer methods using retrograde labelling of noradrenergic neurons by axonal transport of a specific indicator, the antibody to dopamine-B-hydroxylase, in conjunction with immunocytochemical staining, were used to trace descending noradrenergic pathways. Cell groups A6 (locus coeruleus) and A7 (subscoeruleus) were responsible for 91% of DBH labeled neurons, with the A5 cell group accountable for 5-10%; that is, nearly all spinally projecting neurons from groups A5, A6 and A7 are noradrenergic. Evaluation of the spinal projections from the nuclei locus coerulus and subcoerulus suggest that descending noradrenergic pathways may be divided into two distinct systems, the central and lateral tegmental systems.

The central noradrenergic system has widespread projections throughout the neuraxis. Norepinephrine-containing neurons contain both ascending and descending projections to the telencephalon, diencephalon, midbrain, cerebellum, pons, medulla and spinal cord. (171) Noradrenergic neurons in the locus coeruleus (the A6 cell group) are darkly pigmented small to medium-sized cells located in a compact nucleus at isthmus levels near cells of the mesencephalic

nucleus of the fifth cranial or trigeminal nerve. (13) Ascending projections from the LC are distributed widely in the cerebral cortex and hippocampal formation, as well as specifically to nuclear groups in the thalamus. Descending projections from caudal parts of the locus ceruleus project to nearly all levels of the spinal cord via the ventral and lateral funiculi. (171) Noradrenergic neurons in medial and lateral parabrachial nuclei surround medial and lateral regions of the superiour cerebellar peduncle and are associated with visceral sensations. Noradrenergic neurons of the A5 cell group are situated lateral to the facial and superiour olivary nuclei and innervate the intermediolateral cell column. (13) Despite some variability in the findings, spinal projections of the locus coeruleus and nucleus subcoeruleus terminate in both the dorsal and ventral horn.

2.1.2 Pharmacology of the Noradrenergic system

Norepinephrine (NE) is classified, with epinephrine and dopamine, as a catelcholamine. Catelcholamines contain a benzene ring with two adjacent hydroxyl substituents, and an amine group. NE has the following structure: 3, 4-(OH)-Ph-CHOH-$CH_2$-$NH_2$, which reflects its classification. It is synthesized (1) and degraded (2) metabolically as follows:

1)Tyrosine → DOPA → DA → Norepinephrine

2a) Norepinephrine (with MAO: monoamine oxidase) → 3, 4 - dihydroxyphenylglycoaldehyde

2b) Norepinephrine (with COMT: catechol-o-methyltransferase) → Normetanephrine

Adrenoceptors were traditionally subdivided based on anatomical localization, where α1-adrenoceptors were found in post-synaptic membranes in vascular smooth muscle while α2-adrenoceptors refers to presynaptic nerve terminals in peripheral sympathetic nervous tissue. They were further characterized into subtypes within each group: α1A, α1B, α2A, α2B and α2C. More

recently, adrenoceptors receptor subtypes have been classified by G-protein coupling: α1-adrenoceptors are linked to G proteins and stimulate phospholipase C action; α2-adrenoceptors are linked to inhibitory G proteins and decrease adenyl cyclase activity and β-adrenoceptors (β1 and β2) are linked to stimulatory G proteins which increase adenyl cyclase activity. (19)

Pharmacological specificities of the α-AR subtypes have shown that the α1A-AR is identified by oxymetazoline (agonist) and 5-methyluradipil or (+)niguldipine (antagonists) while the α1B-AR is antagonized by spiperone. Guanfacine and guanobenz preferentially stimulate the α2-AR while prazosin is a non-receptor specific antagonist of α2B-AR and rauwolscine has preferential affinity for α2C-AR over other sub types. Clonidine is structurally similar to tizanidine, is blocked by yohimbine and piperoxane and stimulates α2-autoreceptors at low doses and postsynaptic α-receptors at high doses. Tizanidine, an α2-adrenergic agonist, has demonstrated dose-dependent reduction of flexor reflexes at α2-adrenoceptors in intact rats and facilitated flexor reflexes at α1-adrenoceptors in spinal rats at high doses. (114) This could be explained by the dual role of NE on motorneurons, or by denervation supersensitivity due to NE depletion following spinal cord injury (17, 20, 74, 173).

### 2.1.3 Physiology of the Noradrenergic system

The noradrenergic system has widespread and complex effects, and its role as a modulator of spinal excitability influences motor, sensory and autonomic function of the sympathetic nervous sytsem. Specifically, NE responds to stress with the 'fight or flight response' to increase the heart rate and blood pressure, the rate of glyogenolysis and lipolysis as well as relaxing bronchial smooth

muscle to assist breathing. However, this discussion will focus on the analgesic and myorelaxant effects of NE. The gate control theory of pain explains stimulation-induced analgesia, where low-threshold myelinated afferent fibers reduce the response of dorsal horn neurons to pain whereas conduction block of myelinated fibers enhances the response of dorsal horn neurons. Thus, nociceptive control pathways modulated in the spinal cord function as diffuse noxious inhibitory controls (DNICs) to selectively inhibit neurons in the dorsal horn by noxious stimulation at sites distant from the neuron's excitatory receptive field. Both primary afferent nociceptors containing NE receptors on their surface membranes and structures at higher levels of the nervous system, modulate dorsal horn effects via 'descending analgesia pathways'. (175) These modulatory circuits on nociception utilize several different neurotransmitters, including opioids, serotonin and/or catelcholamines, and structures, including the locus coeruleus, nucleus subcoeruleus, periaqueductal gray and nucleus raphe magnus. (175) Animals studies have shown that iontophoretic NE and LC stimulation inhibit the nociceptive responses of laminae IV and V cells or lumbar interneurons, respectively, demonstrating that descending noradrenergic pathways have the ability to "facilitate the interneuronal output to produce presynaptic inhibition of fine afferent inputs." (45, 58, 62) Consequently, reduction in the response to a noxious stimulus (or pain) occurs by activation of inhibitory interneurons in which the dorsal horn acts like a gate to inhibit or facilitate neurotransmission. (45)

Electrical or chemical stimulation of spinal and supraspinal sites have demonstrated analgesic effects mediated by $\alpha$2-adrenergic receptors via the inhibition of nociceptive neurons in the deep dorsal horn and selective control of transmission in spinal neuronal pathways mediating the actions of group II afferents on motorneurons by presynaptic inhibition. (67, 138, 180) In studies

by Jankowska, a strong and selective depression of dorsal and intermediate field potentials for group II afferents on motor neurons was demonstrated by electrical conditioning stimulation of areas mediated by monoaminergic systems: the LC/SC and raphe nuclei. (67, 138) Specifically, tizanidine has been shown to suppress spontaneous activity of LC neurons. (21) Furthermore, the depressant effects of tizanidine on polysynaptic reflexes has been demonstrated in antagonist studies to be mediated by α2-adrenergic receptors and I-receptors. (20) Possible locations of tizanidine action on motor function include the noradrenergic LC neurons or its descending fibers, or interneurons (Renshaw, Group II or other). In a study by Corboz, tizanidine produced a decrease of the reflex response, with a less consistent depression of the background EMG level. (20) Similarly, it was reported by Curtis, Jankowska, Lacey and Riddell that tizanidine did not appear to "depolarize the terminals of group I muscle afferents in the motor nucleus of lower lumbar segments". (138) However, clinical studies have noted a more 'general depressive' action of tizanidine due to its decrease in background EMG produced, or the overall mean level of EMG activity. (57, 89) In summary, the noradrenergic system has been implicated in the action of tizanidine on polysynaptic reflexes by removing descending facilitation of the LC and noradrenergic pathways on spinal reflexes. (180)

## 2.2 Imidazoline: Non-adrenergic Pharmacology of Tizanidine

### 2.2.1 Definition

The imidazoline-binding site, or I-site, is a recently defined non-adrenergic binding site that refers to recognition sites for imidazolines, imidazoles, imidazolidines, guanidines, oxazolines and related structures. (16, 98) Though the natural ligand for I-sites has not yet been identified,

endogeneous and exogeneous compounds including agmatine, bromoxidine, rilmenidine, monoxidine, idazoxan and clonidine have demonstrated to have affinity for I-receptors distinct from α2-adrenergic receptors. (61) They have been identified in a number of tissues, including cardiovascular, brain, smooth muscle and kidney.

The clinical relevance of the I-site has been demonstrated with antihypertensive drugs, such as monoxidine and rilmenidine, which show greater selectivity for I1 sites than α2-adrenergic sites, as well as fewer side effects relative to clonidine. (98) The selectivity of tizanidine for the imidazoline receptor rather than the α2-adrenergic receptor was also greater than clonidine, which did not discriminate between the two receptor types. (102) It has been suggested that side effects from antispasticity medications, such as sedation and dry mouth, are linked to α2-adrenergic stimulation. (98) Recently, studies suggest that tizanidine interacts with imidazoline receptors to regulate its myorelaxant effects with fewer side effects, since "imidazoline-receptor-selective drugs such as tizanidine may cause unique pharmacological actions in addition to their actions on α2-adrenoceptors." (81, 102) Thus, the mechanism of tizanidine action has been associated with both noradrenergic and imidazoline receptor interaction.

### 2.2.2 Receptor classification

There are two broad classes of I-receptors, classified originally by Ernsberger as I1 (clonidine and idazoxan sensitive) and I2 (clonidine insensitive and idazoxan sensitive). (98) General imidazoline ligands include agmatine sulphate and harmane hydrochloride, both endogeneous ligands for the imidazoline binding site. I1 selective ligands include clonidine hydrochloride, an α2-receptor agonist, and cimetidine, an H2 histamine agonist. Amiloride chloride is an I2 selective

ligand used to identify I2-subtypes: I2A-amiloride sensitive and I2B-amiloride insensitive imidazoline binding sites. I-receptors preferentially bind drugs with an imidazoline, oxazoline or guanido structure. The physiological role of I1 receptor activation has been suggested to include: cardiovascular regulation, specifically a hypotensive effect, gastric and renal electrolyte secretion, or reduction of interocular pressure. (122, 129) The I2 binding sites have varying affinities for idazoxan, cirazoline, and monoxidine and the physiological role of I2 receptors has not been fully characterized.

A third class of receptors available for an imidazoline such as tizanidine to bind has been identified in the plasma and intracellular membranes of white fat cells as NAIBS, or non-adrenergic idazoxan binding sites. (77) NAIBS are distinct from adrenoceptors in that they have no affinity for catelcholamines, and are distinct from I-receptors in that they have no affinity for para-aminoclonidine while possessing affinity for some imidazoline and guanidine derivatives. (77, 79) However, it has been suggested that I-receptors and NAIBS are distinct protein complexes, and that they may be associated physically, while I-receptors and α2-adrenoceptors are distinct and act independently of one another. (36)

Clonidine is a mixed agonist of I-receptors and α2-adrenoceptors that has been extensively studied to differentiate these two binding sites. In a study by Liedtke, using the high affinity analogue p-[125I] iodoclonidine, "binding of clonidine to cells was determined to fit a two-site model, with one site of high specificity for α2-adrenergic receptors and the other with a high affinity for I1-imidazoline receptors." (81) A study of the affinity of tizanidine and NE in platelets showed the selectivity of these two compounds. Tizanidine produced a monophasic competition binding curve, displaying modest selectivity for I-sites over α2-adrenoceptors, whereas NE produced a

biphasic competition curve, indicating greater selectivity for the α2C-adrenoceptor than for I-sites. (123) It was noted that the colocalization of I1- and α2-sites remains a confounding factor in determining selectivity of pharmacological compounds.

2.2.3 Receptor distribution

Both imidazoline-preferring receptors and α2-adrenergic receptors were found to be distributed broadly and heterogeneously in the spinal cord and in all major brain areas. (71) However, in the CNS, I-receptors are uniquely distributed in a highly restricted network of neurons. (134) In one study, I-sites accounted for at least 50% of specific [3H] Rilmenidine binding in most spinal cord layers as well as some of the highest densities in spinal motor neurons, most cortical and hypothalamic nuclei, nucleus of the solitary tract and cranial motor nuclei (71). However, another study has identified low densities of I-receptors in the spinal cord. (36) Other studies have shown I-receptors to be heavily represented in sensory processing centres, in particular the superficial laminae I and II of the dorsal horn, lateral-cervical and lateral-spinal nuclei and the sympathetic cell column. (134) In addition, I-receptors may be localized either presynaptically (60) or postsynaptically. It may be noted that presynaptic I-receptors have been shown to modulate NE release. (47)

2.3 Tizanidine, 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiodiazole hydrochloride

Tizanidine hydrochloride has been described as a fine, white, odourless crystalline powder. It is slightly soluble in water and methanol and its molecular weight is 291.2 grams per mole. Its molecular formula is C9H8ClN5S*HCl, its chemical name is 5-chloro-4-(2-imidazolin-2-ylamino)-

2,1,3,-benzothiodiazole hydrochloride and its trade name is Zanaflex in North America; in Europe, South and Central America and Asia, the trade name is Sirdalud.

The clinical applications of tizanidine include the suppression of muscle spasms and a treatment for spasticity with the principal side effects of drowsiness, dizziness and nausea. (52) Other commonly reported side effects include dry mouth, sedation, asthenia, hypotension and bradycardia. (158) Similar to clonidine, its structural analogue, it is contraindicated with antihypertensive drugs, but it may be co-administered with baclofen to produce additive effects. (57) Tizanidine has been an approved short-term muscle relaxant in Europe and Japan for over a decade, and more recently as an antispasticity treatment in the U.S. (1996) and Canada (1999).

### 2.3.1 Analgesic Effects

Antinociceptive activity associated with tizanidine appears to be primarily spinally-mediated, through depression of excitatory responses of spinal neurones. (30) In a study by Davies in 1989, ionophoretically administered tizanidine produced a "profound, long-lasting and selective depression of the responses to noxious stimuli."(29, 32) That is, in response to noxious heat stimuli, 100% of responses from laminae 1, 4 and 5 dorsal horn neurones were depressed, while the responses to puffs of air, a non-noxious stimulus, 0% of laminae 1, 4 and 5 dorsal horn neuron responses were depressed. In addition, administration of baclofen near lamina 4 and 5 neurons reduced responses indiscriminately. (30) In a study by McCarthy and colleagues in 1991, an antinociceptive effect from intrathecal tizanidine was found to dose-dependent and lasting at least 90 minutes with a 25ug (high) dose. However, in the latter study, tolerance to tizanidine was observed , as the analgesic efficacy of tizanidine decreased with repeated dosing. (93) Furthermore, in a study by Leiphart, et. al. in

1995, in a model of neuropathic pain, intrathecal tizanidine did produce an antinociceptive effect, or an increase in paw pinch withdrawal latency in the affected hindpaw, but did not produce an antinociceptive effect in a model of normal (reflexive) pain, where paw withdrawal latency from a noxious heat stimulus did not change. (80)

2.3.2 Myorelaxant Effects

Tizanidine has myorelaxant properties, as shown through the suppression of electromyographic (EMG) activity in both extensor and flexor muscles in patients with spasticity due to spinal cord injury. (167) In a study by Kaneko, Ono and Fukuda in 1987, tizanidine depressed both segmental reflexes as well as descending modulators of reflexes in rats. In this study, tizanidine reduced the ventral root reflexes in unconditioned responses and slightly reduced the monosynaptic reflex in response to conditioning, whereas baclofen had no effect on conditioned responses. (69) These results suggest both spinal and supraspinal sites of action associated with tizanidine. Another study by Ono, Fukuda, et. al. in 1986 showed the ability of tizanidine to reduce muscle activity in rat rigidity models, including reduction of intercollicular decerebrate rigidity and gamma-activity, inhibition of alpha-rigidity and depression of MSR, PSR and the crossed extensor reflexes. (69,112, 113, 114)

2.4 GABA-Gammaaminobutyric acid

2.4.1 Anatomy of the GABAergic system

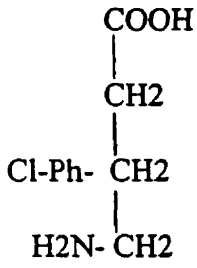
GABA is found in high concentrations in the brain, including the cerebellum, the substantia nigra and the olfactory bulb, and the spinal cord in the mammalian CNS. (19) GABAergic neurons

localized using immunocytochemical methods generally assume the presence of GABA is an indication of transmitter function, based on an antibody reaction to glutamate decarboxylase, GAD, the GABA synthetic enzyme. While a consistent direct relationship has been shown between GAD immunoreactivity and GABAergic activity, a similar consistent inverse relationship with the GABA degradative enzyme, transaminase, has not been shown. (19) In the spinal cord, immunoreactive GABA-containing cells were concentrated, using antisera raised against conjugates of GABA itself, in lamina I-III in the dorsal horn, and present in smaller numbers in deep laminae. (7, 161, 162) One study demonstrated GABA-like immunoreactivity in 28% of cells in laminae I, 31% of cells in laminae II, and 46% of cells in laminae III. (163) Another study found that "(1) the distribution of GAD-positive reaction product appeared equally on (right and left) sides of the spinal cord, and (2) an intense band of GAD-positive reaction product appeared within laminae II and III." (7) In a histochemical study of GABA distribution in the cat spinal cord, "the highest concentration of GABA (2-20mmol/L) were found in the dorsolateral part of the dorsal horn." (97) It has also been reported that the distribution of GABA is similar within species, including rats, cats, monkeys and humans.

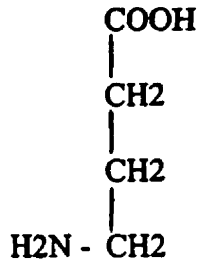
### 2.4.2 Pharmacology of the GABAergic system

GABA and baclofen are structural analogues, differing only in the presence of a chorophenyl group: (52)

Baclofen (Lioresal):




**Gamma-aminobutyric acid (GABA):**




GABA is synthesized (1) and degraded (2) as follows:

(1) Glutamate (with GAD - glutamate decarboxylase) → GABA

(2) GABA (with GABA-transaminase and alpha-oxoglutarate) → Succinic semialdehyde (and Glutamate)

'True' GABA receptors have been defined as postsynaptic sites which produce a change in membrane permeability to inorganic ions through binding with GABA or an agonist. (19) The two classes of GABA receptors, GABAa and GABAb, have been distinguished pharmacologically through their affinities for different compounds. (11, 40, 107, 108) GABAa is bicuculline-sensitive and binds muscimol, whereas GABAb is bicuculline-insensitive and binds baclofen. A selective GABAa antagonist is picrotoxin, whereas a selective GABAb antagonist is phaclofen. A change in chloride permeability with binding to the GABAa receptor "results in hyperpolarization of the receptive neuron in the case of postsynaptic inhibition or depolarization in the case of presynaptic inhibition." (19) In contrast, the GABAb receptor has an inhibitory action mediated by increases in potassium or decreases in calcium permeability or conductance. Furthermore, GABAb receptors have been shown to mediate slow inhibitory synaptic potentials associated with an increase in potassium conductance, where receptors are activated only by strong afferent inputs. (40) The synaptic activation of these two inhibitory receptors is suggested to depend on the strength of the

afferent input. In summary, baclofen is classified as a GABAb agonist and modifies GABA release and metabolism via interaction with GABA heteroceptors, producing a decrease in calcium conductance and GABA release that results in presynaptic inhibition. (19, 40)

### 2.4.3 Physiology of the GABAergic system

In the spinal cord, interneuronal GABA has been associated with the mediation of pre- and postsynaptic forms of inhibition and may also be involved in Renshaw cell-mediated recurrent inhibition of motor neurons, as shown through electrophysiological studies. (42) The inhibitory role of GABA was first suggested in studies by Eccles and colleagues in 1963 and has been supported by subsequent studies. A study of inhibitory (Renshaw) interneurons in mice was performed by activating Ia afferent fibers from stretch receptors of antagonistic muscles and recording from single chemically gated Cl- inhibitory channels in spinal neurons. The result was an inhibitory current that was activated by GABA, and associated with an increase in chloride permeability. (141) The role of GABA in synaptic transmission includes a number of phenomena such as: (a) presynaptic inhibition, (b) presynaptic facilitation, (c) primary afferent depolarization, (d) the dorsal root reflex, and (e) primary afferent hyperpolarization. (7) Evidence for these synaptic relationships to primary afferents is further supported by the anatomical studies of the distribution of GABA axon terminals and pharmacological studies of the role of GABA in presynaptic inhibition of primary afferents within the spinal cord. (7, 85)

## 2.5 Baclofen, (RS)-4-amino-3-(4-chlorophenyl)butanoic acid

The clinical application of baclofen as an antispasticity treatment has been effective "in

patients with spasticity secondary to spinal cord lesions and ... particularly effective in patients with severe flexor spasms." (52) Its effectiveness, however, "may be limited by its adverse effects, which include drowsiness, insomnia, dizziness, weakness and mental confusion." (52) While weakness is the most commonly reported side effect, in at least 33% of patients, other side effects include "sedation, somnolescence, ataxia and respiratory depression." (130)

### 2.5.1 Analgesic Effects

Baclofen has demonstrated its antinociceptive effect in the mouse formalin test, the tail flick test and the hot plate method. (5, 136, 137, 144, 147, 179) The results have demonstrated a dose-dependent reduction in response to a nociceptive stimulus with baclofen, which is inhibited by GABAb receptor antagonists, such as CGP35348. Thus, baclofen has been classified predominantly as a GABAb agonist, though it may have additional sites of action. Specifically, both baclofen and GABA have been shown to produce a dose-dependent reduction in the release of substance P, a neuromodulator of primary afferent neurons, from spinal cord slices in the rat. This effect was antagonized by GABAb antagonists, CGP 35348 and CGP 36742 but not by a GABAa antagonist, bicuculline, and provides evidence that the antinociceptive effect of baclofen is mediated by GABAb receptors on primary afferent terminals containing substance P at the level of the dorsal horn in the spinal cord. (90) Furthermore, interaction between baclofen and substance P has revealed that "desensitization to SP alters the spinal analgesic effect of baclofen." (146) There is also evidence that α2-adrenergic stimulation, with clonidine in reserpine-pretreated animals (136), or chemical lesioning of the LC, with 6-OHDA (145), increases baclofen-induced nociception and reduces NA levels at both spinal and supraspinal levels associated with LC lesions.

Baclofen analgesia was potentiated by reserpine (an NA depleter), phentolamine (an α-blocker), ergotamine (a DOPA precursor), haloperidol (a false dopa agonist) and chlorpromazine indicating the importance of catechol mediators, whereas it was insensitive to naloxone, an opioid antagonist, bicuculline and picrotoxin, GABAa antagonists, indicating no interaction of baclofen with opiate and GABAa systems. (146) Specifically, ascending and descending noradrenergic pathways, and nicotinic mechanisms have been implicated in baclofen analgesia, while the role of serotonergic mechanisms remains less understood. (138, 144) Other suggested mechanisms of GABA-independent afferent depolarization involve potassium transients. (75)

Baclofen has been shown to modulate spinal afferent processing by at least two mechanisms (70) and has been shown to modulate analgesia supraspinally by multiple mechanisms. (146, 160) The spinal effects of baclofen are illustrated through intrathecal studies in rats that produced a dose-dependent stereospecific antinociceptive effect. (176) In a study by Roberts, et. al. in 1986, results suggested a mechanism of action of baclofen in which "GABAergic systems act directly at the spinal cord to modulate both sensory and motor activity and influence motor function." (133) In a study by Yaksh and Dirig in 1995 of spinally-mediated nociception, it was shown that both GABAa and GABAb agonists were antinociceptive at the spinal cord level and provide evidence for the involvement of both types of receptors in antinociceptive processing. (38) It was further suggested that "GABAergic neurons maintain a tonic suppression of activity in nociceptive afferent pathways." (133) More recently, in a study by Sawynok et. al. in 1987, it was also suggested that the spinal mechanism of baclofen analgesia may involve an interaction with substance P, since baclofen is blocked by this agent. (145) More recently, the spinal site of action of baclofen has been illustrated through intrathecal studies to produce a dose-dependent stereospecific antinociceptive effect.

A study by Thomas and colleagues in 1995 showed that the analgesic effects of baclofen, as determined by the tail flick test, were both stereoselective and stereospecific, when administered by microinjection to the ventromedial medulla over a wide range of doses. Supporting previous findings by Sawynok, it was shown that (-)BAC was more potent than the (+) BAC isomer, and the R(-)BAC isomer was more active than the S(-)BAC isomer. (160) It may be noted that in the measurement of sensory and/or motor function, there is an inevitable confounding effect in which a subject's response to a sensory stimulus may not reflect inability to perceive the stimulus but inability to elicit a motor response. (160)

2.5.2 Myorelaxant Effects

As a chemotherapeutic agent that reduces the contractility of muscle fibers to relieve muscle spasms, baclofen is well known for its myorelaxant properties. Its effects on muscle relaxation have been demonstrated by depression of spinal transmission from primary afferent nerves. (151) In a study by Klockgether and colleagues in genetically spastic rats, the effect of baclofen on the spinal transmission in (monosynaptic) Hoffman reflexes and (polysynaptic) flexor reflexes was recorded. It was observed that baclofen produced "a rapid and dose-dependent suppression of EMG activity" which was antagonized by gamma-aminovalerate, a presumed GABAb antagonist. This study confirmed the potent, long-lasting myorelaxant effects of intrathecal baclofen. (73) Similar results have been shown *in vitro* in the immature rat spinal cord, where baclofen abolished all components of the ventral root reflex (DR-VRP) (151) as well as in clinical studies, where intramuscular baclofen in 13 spastic patients produced significant changes in the H-reflex recovery curve following stimulation of the ipsilateral tibial nerve at the ankle. (35)

## 2.6 Comparison of Antinociceptive Actions of Tizanidine vs. Baclofen

In 1980 Sayers et al. showed the relative antinociceptive activity of tizanidine compared with baclofen was shown using two models of pain (Table 1). The effects of the drugs in the phenyl-p-benzoquinone (PBC)-writhing and tail-flick tests at the 50% effective dose, within 95% confidence limits, are shown below. In both the PBC- test and the tail-flick test, both drugs demonstrated an analgesic effect. However, the response to tizanidine occurred at a much lower dose, where it was approximately 10 times more potent in the PBC-writhing mouse and 2.5 times more potent in the tail-flick rat. (148)

**TABLE 1: Antinociceptive activity of Tizanidine and Baclofen** (*148*)

| DRUG | PBC-writhing mouse ED 50 (mg/kg, p.o.) | Tail-flick rat ED 50 (mg/kg, p.o.) |
|---|---|---|
| Tizanidine | 0.21 | 4.8 |
| Baclofen | 2 | 12 |

Davies and Johnston compared the antinociceptive activity of tizanidine and baclofen and found that tizanidine produces selective depression of excitation of laminae II and III, or IV and V, in response to noxious stimulation, while baclofen and GABA produced non-selective depression in response to noxious and innocuous stimulation. (30)

More recently, the specificity of depressive actions of tizanidine and baclofen on sensory transmission was demonstrated via its effects on dorsal and intermediate zone (monosynaptic) field potentials. (152) It was originally observed that group II afferents also contribute to the (monosynaptic) stretch reflex using a tonic vibratory stimulus to inactivate Ia afferents, though the

stretch reflex continued to be elicited. With ionophoretically administered tizanidine, the late component of the monosynaptic field potential from group II afferents was depressed at 5 minutes and returned to control levels by 30 minutes, with no change or slight facilitation of potentials from group I afferents. In contrast, with ionophoretically baclofen, the early component of the monosynaptic field potential from group I afferents was depressed at 2 minutes and returned to control levels by 30 minutes with inconsistent effects on group II potentials. Similarly, intravenously administered tizanidine produced a dose-dependent depressive effect on group II afferents but no significant effect on group I afferents and intravenously administered baclofen produced stronger depression of the group I component than the group II component. Thus, local and systemic application of tizanidine or baclofen selectively depressed group II or I afferents, respectively.

Thus, there is evidence that antinociceptive activity of tizanidine and baclofen may be distinguished by their different selectivity and specific effects of tizanidine for group II afferents and baclofen for group I afferents. However, it may be noted that both drugs have analgesic effects at doses below those that produce muscle relaxation.

2.7 Comparison of Myorelaxant Actions of Tizanidine vs. Baclofen

The relative myorelaxant effects of tizanidine and baclofen are summarized in Table 2 that follows. Novack investigated the relative myotonolytic and CNS-depressant effects of tizanidine and baclofen, as agents that reduce skeletal muscle tone and facilitate muscle relaxation. (111) The specificity ratio was derived to illustrate the extent of incoordination relative to muscle relaxation based on motor performance in the rotarod test relative to the morphine-induced Straub tail test, respectively. In the Straub tail test, mice were injected subcutaneously with 30 mg/kg of morphine

following the test drug and observed for the presence an elevated tail to at least 90 degrees above the horizontal, known as the Straub tail. In the rotarod test, trained mice were judged for their ability to remain on a rod rotating at 15 rpm for 90 seconds. (111) Thus, a ratio of less than one would indicate poor specificity, and greater than one would indicate good specificity. Results showed the specificity ratios of both tizanidine and baclofen were statistically significantly greater than 1.0, where tizanidine (5.71) was more effective than baclofen (2.15). (111) In a study by Sayers, the effects of tizanidine relative to baclofen on different models of muscle relaxant activity, including thalamonal rigidity and decerebrate rigidity in the rat and the hindlimb extensor reflex in the rabbit, were measured. (148) These tests demonstrated the increased activity of tizanidine relative to baclofen, where ED50 was the 50% effective dose. Results indicated that while both drugs "reduced EMG activity recorded in the gastrocnemius muscle in response to an involuntary, 3-second flexion of the foot," the response to tizanidine occurred at a much lower dose, where it was approximately "15 times more active than baclofen in this test procedure" following i.v. drug administration. (21) Thus, in these studies, tizanidine demonstrated greater specificity in myorelaxant activity than baclofen. (Table 2)

**TABLE 2: Myorelaxant Activity of Tizanidine and Baclofen (*111, 148*)**

| Drug | Straub Tail ED50 (mg/kg, i.p.) | Rotarod ED50 (mg/kg, i.p.) | Specificity Ratio (RR ED50/ST ED50 | Thalamonal rigor ED block (mg/kg, i.v.); rat | Decerebrate rigidity ED block (mg/kg, i.v.); rat | Hindlimb extensor reflex ED 50 (mg/kg, i.p.);rabbit |
|---|---|---|---|---|---|---|
| Tizanidine | 0.4 | 2.3 | 5.71 | 0.06 | 0.49 | 0.02 |
| Baclofen | 3.6 | 7.8 | 2.15 | 4.5 | 1.3 | 0.3 |

The relative depressive effects of tizanidine and baclofen on monosynaptic (Hoffmann) reflexes at various doses were summarized from a number of studies in Table 3 that follows. In a study by Sayers in spinal cats, results show an insignificant or weak effect of tizanidine on MSR-inhibition at all doses, from 0.2 to 5.0 mg/kg, i.v. In contrast, baclofen showed a dose-dependent inhibition of monosynaptic reflexes in the spinalized cat, over the same range of doses. (148). Similar reflex studies by Davies and colleagues in 1982 in cats showed that monosynaptically evoked excitatory responses were more sensitive to the depressant action of baclofen (94% of cells) than to tizanidine (65% of cells). This was also reflected by the greater number of neurones that depressed monosynaptic excitation by smaller ejection currents of baclofen (7nA relative to 16nA for polysynaptic responses) or the increased potency of baclofen. (27) In addition, in a study by Schwarz and colleagues the relative effects of tizanidine and baclofen on MSR in anesthetized rats following intrathecal administration results showed that while baclofen reduced the magnitude of the MSR ($p<0.001$ versus the solvent), tizanidine did not have a significant effect relative to the solvent. (139) Thus, with systemic, ionotophoretic or intrathecal administration, baclofen produced significantly greater depression of MSR than tizanidine. (Table 3)

| **TABLE 3: Monosynaptic (Hoffman) Reflex Inhibition with Tizanidine or Baclofen** *(27, 139, 148)* | | |
|---|---|---|
| DRUG | DOSE | Depressed MSR |
| Tizanidine | 0.2 mg/kg, i.v. | -12 |
| Baclofen | 0.2 mg/kg, i.v. | -62 |
| Tizanidine | 1.0 mg/kg, i.v. | -4 |
| Baclofen | 1.0 mg/kg, i.v. | -90 |
| Tizanidine | 5.0 mg/kg, i.v. | -1 |
| Baclofen | 5.0 mg/kg, i.v. | -100 |
| Tizanidine | 36.5+/- 6.7 nA | 5/14, or 65 (+/- 21.7) % of cells |
| Baclofen | 7 +/- 1.2 nA | 7/7, or 94 (+/- 4) % of cells |
| Tizanidine | 100 nmol, i.th. | 93 (+/- 7) % |
| Baclofen | 2 nmol, i.th. | 28 (+/- 11) % |

The relative depressive effects of tizanidine and baclofen on polysynaptic (flexor) reflexes at various doses were summarized from a number of studies in Table 4 that follows. In a study by Sayers in spinal cats, results show an insignificant or weak effect of tizanidine on PSR-inhibition at all doses, from 0.2 to 5.0 mg/kg, i.v. In contrast, baclofen showed a dose-dependent inhibition of spinal reflexes in the spinal cat, with the same doses (148). Similar reflex studies by Davies and colleagues in 1982 in cats showed that polysynaptically evoked excitatory responses were more sensitive to the depressant action of tizanidine (67% of cells) than to baclofen (48% of cells). This

was also reflected by the greater number of neurones that depressed polysynaptic excitation by smaller ejection currents of tizanidine (21nA relative to 36.5nA in monosynaptic responses) and the longer duration of depression in the PSR (20.6 minutes) relative to the MSR (5 minutes). (27) In addition, the study by Schwarz in anesthetized rats showed that while intrathecal baclofen reduced the magnitude of the PSR ($p<0.001$ versus the solvent), intrathecal tizanidine did not have a significant effect on the PSR relative to the solvent. It was further shown, however, that tizanidine "selectively influenced the flexor system after systemic application." (139) Thus, results from systemic administration showed that baclofen produced greater depression of PSR than tizanidine, which was relatively weak, while ionotophoretic or intrathecal administration produced greater depression of PSR with tizanidine than with baclofen. (Table 4)

**TABLE 4: Polysynaptic (Flexor) Reflex Inhibition with Tizanidine or Baclofen** *(27, 139, 148)*

| DRUG | DOSE | DEPRESSED PSR |
|---|---|---|
| Tizanidine | 0.2 mg/kg, i.v. | -16 |
| Baclofen | 0.2 mg/kg, i.v. | -39 |
| Tizanidine | 1.0 mg/kg, i.v. | -7 |
| Baclofen | 1.0 mg/kg, i.v. | -84 |
| Tizanidine | 5.0 mg/kg, i.v. | -3 |
| Baclofen | 5.0 mg/kg, i.v. | -97 |
| Tizanidine | 21 +/- 2.7 nA | 15/16, or 67 (+/- 10.1) % of cells |
| Baclofen | 16 +/- 2.4 nA | 6/6,or 48 (+/- 11.4) % of cells |
| Tizanidine | 100 nmol, i.th. | 98 (+/- 10) % |
| Baclofen | 2 nmol, i.th. | 27 (+/- 6) % |

2.8 Comparative Clinical Studies

Preliminary clinical studies by Hassan and McLellan comparing tizanidine and baclofen, with respect to muscle strength, found that "stretch responses were suppressed more effectively by (tizanidine) than by baclofen", but the drugs had a similar effect on the shortening activity or resting tone based on EMG recordings. (57) It was suggested that tizanidine "could reduce stretch reflexes without necessarily weakening the limb." (57) In subsequent studies, it was shown that tizanidine "(increased) the isometric torque relative to joint velocity generated by spastic quadriceps and hamstring muscles."(92) In contrast, baclofen demonstrated weaker and less frequent flexor and extensor spasms, in addition to the side effects of drowsiness and accentuation of limb weakness, particularly of leg extensor muscles. (92)

A comparison of results from clinical studies in patients with spasticity due to cerebral or spinal cord injuries or MS are summarized in Table 5 extracted from Wagstaff. (167) The associated drug dosages and duration of evaluation are listed, where the duration includes that of titration, generally two to three weeks, and maintenance phases. When considering the improvement in muscle tone, three studies indicated no difference between tizanidine (T) and baclofen (B), while one found B >=T and another found T>=B. That is, baclofen was shown by Bass (53) to be equal to or better than tizanidine with regard to quantitative improvement in muscle tone based on Ashworth scores, while, tizanidine was assessed by Newman to be equal to or better than baclofen with regard to both quantitative improvement in muscle tone based on Ashworth scores and frequency of muscle spasms. In the objective improvement in muscle strength, four studies found no difference between T and B, while two showed T>=B. That is, in studies by Medici and Smolenski (155), tizanidine had a tendency towards greater improvement relative to baclofen. Similarly, in the evaluation global

efficacy, three studies indicated no difference between B and T, while one study indicated B>=T, and despite inconsistent differences, the global efficacy of tizanidine and baclofen was almost consistently equivalent. However, in the subjective reporting of muscle weakness, baclofen was reported in a higher percentage of patients in all but one study, where the extent of reporting was equal to tizanidine. The reported difference of significantly higher muscle weakness with baclofen than with tizanidine was demonstrated in only two studies, by both Hoogstraten ($p<=0.05$) and Bass ($p<=0.01$) In addition, it may be noted that more patients withdrew from studies due to intolerable side effects when receiving baclofen than tizanidine, as follows: 11 compared to 4, in the study by Bass et. al.; 25 compared to 0, in the study by Hoogstraten et. al.; 21 compared to 0, in the study by Medici et. al. (167) Further evidence for the occurrence of muscle weakness, from a review by Wallace, reported that "tizanidine was found to reduce symptoms of spasticity in patients with MS or SCI without increasing muscle weakness and to be at least as effective as the comparative agents." (169) The suitability of tizanidine as a therapeutic alternative for treating spasticity was also demonstrated in a combined analysis of clinical data. (78) It was revealed that when compared with baclofen, tizanidine had similar benefits with the main difference being a higher frequency of muscle weakness reported with baclofen.

**TABLE 5: Comparative efficacy of Tizanidine and Baclofen in Clinical Trials (*12, 167*), *extracted from Wagstaff***

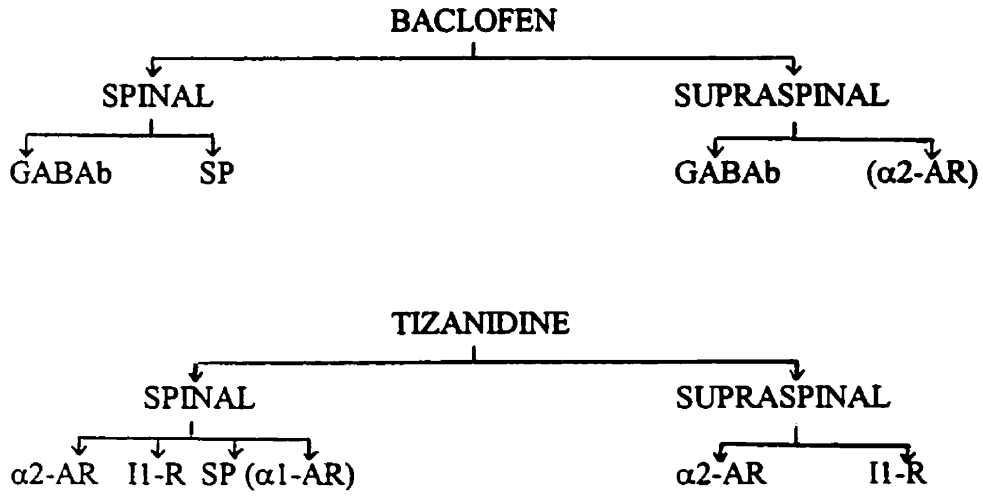
| STUDY | DRUG DOSE (mg/day) | DURATION (weeks) | IMPROVEMENT IN MUSCLE TONE | OBJECTIVE IMPROVEMENT IN MUSCLE STRENGTH | SUBJECTIVE REPORTS OF MUSCLE WEAKNESS (% patients) | GLOBAL EVALUATION OF EFFICACY |
|---|---|---|---|---|---|---|
| CV lesions (Medici, 1989) | T<=16-20 B<=50 | 50 | Not reported | T>=B | T=7 B=36 | Not reported |
| MS (Bass, 1988) | T<=32 B<=80 | 8 | B>=T | T=B | T=21 B=35 p<=0.01 | B>/>=T (Investigator and Patients) |
| MS (Eyssette, 1988) | T<=24 B<=60 | 8 | T=B | T=B | T<B B=23 | T=B (Investigator) |
| MS (Hoogstraten, 1988) | T<=12-24 B<=15-60 | 7 | Not reported | T=B | T=29 B=79 p<=0.05 | Not reported |
| MS;syringomyelia (Newman, 1982) | T<=16 B<=40 | 6 | T>=B | T=B | T=15 B=15 | T=B (Patients) |
| MS (Smolenski, 1981) | T<=36 B<=80 | 6 | T=B | T>=B | T=18 B=30 | T=B (Investigator and Patients) |
| MS; chronic myelopathy (Rinne, 1980) | T<=16 B<=80 | 4 | T=B | Not reported | T=37 B=56 | Not reported |

Thus it appears that the significant distinguishing features between tizanidine and baclofen are their side effects. Furthermore, it was shown that baclofen frequently caused severe muscle weakness, resulting in falling during walking or standing in patients with spasticity from multiple sclerosis (MS), while tizanidine had a beneficial effect on mobility. (63) The increase in voluntary muscle strength associated with tizanidine, as previously reported by Knutsson et. al. in which voluntary dynamic muscle strength was increased, was not replicated using different patient groups. (63) However, a multicentre study showed no significant differences between tizanidine and baclofen in terms of improvement of functional status, including walking distance, angle at which the stretch reflex occurred, muscular strength, efficacy or tolerability. (41) In contrast, in the comparison of side effects, muscular weakness was reported more frequently (ten cases) in the baclofen group while tizanidine "seemed to induce no undue muscle weakness in this study." (41) Further studies have demonstrated the beneficial effect of tizanidine on the reduction of lower limb spasticity, with production of fewer side effects, but perhaps only in a select minority of patients, and not significantly in the group.(109) Thus, while it appeared that the incidence of somnolescence or drowsiness was equivalent between both tizanidine and baclofen (15-67%), the incidence of muscle weakness appeared to be greater with baclofen (15-79%) than tizanidine (2-47%) while the incidence of dry mouth appears to be slightly greater with tizanidine (11-36%) than baclofen (3-20%) . (167)

A number of factors that have been considered in drug studies that compare tizanidine with baclofen. These include: 1) the type and degree of spasticity, such as with multiple sclerosis or spinal cord injury; 2) the measure of muscle strength, such as by the quantifiable improvement in function, by subjective reporting, or by the number of patients withdrawing from the study due intolerable side effects; 3) the effect of time and the duration of the treatment; 4) the definition of

'significance'; 5) the selection of comparable doses; 6) the route of drug administration, or effective concentration at the site of action; 7) the recording muscle, which may be either upper or lower limb; and 8) the level of measurement of nerve/muscle function, in either cellular, whole animal or patient studies. (12,35) In addition, since the causative neurologic impairment associated with spasticity is generally a non-remitting condition, an antispasticity agent that is well tolerated with minimal side effects is optimal. These factors are addressed in the methodology used to compare tizanidine with baclofen in this study.

Based on previous findings, it has been shown that baclofen acts predominantly at GABAb receptors at spinal and supraspinal sites, involving the release of SP in the spinal cord. In addition, baclofen may activate alternate mechanisms indirectly when GABAergic sites are completely activated, including noradrenergic (α2-AR) supraspinal sites. (136, 144, 145, 146) Conversely, tizanidine has demonstrated activity at α2-AR and I1-R at both spinal and supraspinal sites and also involves the release of SP in the spinal cord. In addition, the non-selective effects of tizanidine on α1-AR in the spinal cord have been shown by selective α1-AR antagonists in the spinal animal. (81, 102, 112-117, 129, 134) These receptor interactions are summarized in the following diagram:



# Chapter Three: Methods

## 3.1 Overview

The relative motor performance (MP) effects of tizanidine and baclofen at comparable i.p. analgesic doses were quantified. MP was assessed based on kinematic analysis of a swim test. Analgesic effects were assessed by the tail flick test. Using an integrated sensorimotor testing battery, each animal was tested first on the tail flick apparatus and subsequently recorded performing a trained swim task. The series of measurements was repeated at twenty intervals for three baseline and six post-injection measurements, for six to eight rats each weighing 400-450 grams.

## 3.2 Experiments

### 3.2.1 Animal Model

Sprague Dawley rats were the animal model of choice, due to their genetic homogeneity, characteristic docile behavior and availability. Male rats were used, where the hormonal fluctuations of the estrous cycle were not an intervening factor in monitoring a drug's effect. Rats were housed individually or in groups of two, in a room with a twelve hour light/dark cycle.

### 3.2.2 Sampling Procedure

Rats were selected based on consistent performance in the swim test with training. Consistent with recommendations of the Canadian Council on Animal Care, sample size was minimized, but large enough for statistical purposes.

### 3.2.3 Training

Sprague-Dawley rats were ordered from Charles-River, and within the first one to three days upon arrival, were acclimatized to the room and the handler by short visits to pick up, hold and weigh the animal. This was followed by a training period to learn the swimming task. Training involved placing the animal at the end or in the middle of a swim tank and allowing him to swim to a platform edge. The plexiglass tank had the dimensions 30x75x30 cm and a single narrow swimming lane was created using a removable divider. The level and temperature of the water in the swim tank were monitored and maintained at 7/8ths of a full tank and 37 degrees Celcius, respectively. Incentives were occasionally used, in the form of a small piece of apple or carrot on the platform, tapping the end of the tank or providing a guiding signal to the platform. Rats learned the task by repetition and were able to perform immediately, within two or three trials, or with practice, within two to five days when practicing approximately twenty trials per day per animal. Training was continued until a consistent level of performance was achieved and selection criterion was based on the ability to perform the swim task consistently.

3.2.4 Test Preparation

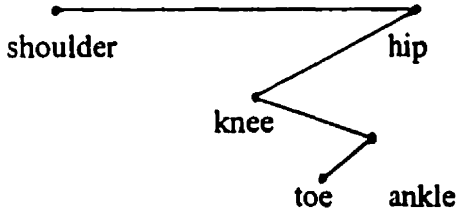
Animals were prepared for testing by shaving and marking their skin on the morning of the trial. Five data points on the rats body were identified, using a contrasting marker and included the shoulder, hip, knee, ankle and toe. The knee position did not accommodate a marker well, due to the excess of skin, and was digitized by visual estimation. The shoulder marker was used for reference and was not placed at the joint but at a constant position from the shoulder, where the movement of the skin would not influence its position. The entire procedure of shaving and marking the rats took approximately 1.5 to 2 hours for six animals, or 15 to 20 minutes per animal. Following

this, the rats were left undisturbed until testing.

3.2.5 Performance testing

Rats were videotaped through the clear glass while swimming across the length of the tank. At least three trials of the swim task were recorded, and the analog images of the best two, based on technical quality, were digitized using the Peak Performance or Peak Motus systems. A number of parameters were calculated using the above mentioned software, including swim speed, cycle time, as well as knee, hip and ankle joint angles.

Figure 3.2.5: Digital Rat Model




3.2.6 Nociceptive testing

The animal was placed onto the tail flick apparatus, with the mid portion of the tail placed over a high-intensity light source shining through a covered slit, with the whole apparatus being fan-cooled. (25, 176) Upon activation, the intensity of the light source increased automatically until the tail flick response was elicited. Reaction time between the onset of the stimulus and the flick of the tail out of the beam of light was recorded digitally by a timer synchronized with the activation of the bulb. A cut-off time of 20 seconds was established to prevent skin damage. Tail flick responses were measured at 20 minute intervals, in conjunction with the motor task and for the series of animals.

The total testing time lasted three hours, to obtain a stable baseline for three trials prior to drug injection, and to measure the drug effects six trials following drug injection.

3.2.7 Drugs

The source of the tizanidine hydrochloride was from Athena Neurosciences, San Francisco, California. The source of baclofen hydochloride was from Ciba-Geigy, Basal, Switzerland. Drugs were dissolved in physiological saline (0.15 M NaCl solution) and administered intraperitoneally.

3.3 Analytical methods

3.3.1 Dose Selection

A range of doses were tested to determine upper, moderate and lower limits of each drug effect, as well as to determine comparability between the tizanidine and baclofen. The low, or analgesic threshold, dose was defined as the first dose to produce a noticeable analgesic response above the saline control. The mid, or intermediate, dose was defined as a sustained analgesic response based on dose response curves. The high, or maximum, dose was defined as the maximum non-lethal dose without significant harmful effects. Preliminary doses of tizanidine were estimated from a study in mice in which doses of 0.5 mg/kg and 1mg/kg, i.p. showed a significant drug effect (on audiogenic seizure response) while large doses of tizanidine (2, 3 and 4 mg/kg, i.p.) produced sedation, reduction of locomotor activity, ataxia and splayed hind-limbs. (32) Previous literature values of i.p. doses of baclofen in mice were 2.5, 5 and 10 mg/kg, i.p. (138) In this study, doses for comparison were selected based on dose response curves, analgesic indices and ANOVA results.

### 3.3.2 Quantitation of analgesia

Dose-response curves were plotted using the mean tail flick latency for each time point at each dose, including standard error of the mean across all subjects. An antinociceptive index score was calculated from tail flick latencies based on the sum of the difference of each individual score from the average baseline at each dose.

### 3.3.3 Quantitation of motor performance

Swim speed was selected as the dependent measure of motor performance from a number of kinematic parameters, including cycle time and joint angles, due to its ability to respond to different doses. Dose-response curves were plotted using the mean swim speed for each time point at each dose, including standard error of the mean across all subjects. A performance index score was calculated from swim speed based on the sum of the difference of each individual score from the average baseline at each dose.

### 3.3.4 Statistical analysis techniques

#### 3.3.4.1 Descriptive statistics

Mean and standard error values were calculated for each drug, dose and timepoint to plot the time course of drug effects. Normality was assessed from frequency distributions of analgesic and motor scores for each drug at each dose. Statistical software used was JMP IN Version 3.12.

#### 3.3.4.2 Inferential statistics

A multi-way analysis of variance was used to compare the means from tail flick latencies and

swim speed, considering two factors at each dose: drug and time. Statistical software used was the SAS Version 7. A p-value of less than 0.05 was used to indicate significance between the two drugs.

3.4 Experimental Design

This study utilized a quantitative-experimental design, the Latin square design, in which each subject was exposed to all treatments in a counterbalanced order and with repeated measurements made before and after treatment, in a time series. That is, all animals received all doses of all drugs. In this experiment, the independent variables were drug, dosage and time, and the dependent variables were tail flick latency and swim speed. Using rats of the same gender (male) in the same weight range (400-450 grams) controlled the nuisance variables.

Measures were taken to control all potential sources of error. The primary sources of internal invalidity in this study were: 1) maturation, or the effect of subject growth, 2) testing, or a "test practice" effect, 3) instrumentation, or shifts in scoring standards, 4) regression, or score regression towards the mean, 5) selection, or subject recruitment without bias and 6) response to an injection. These were controlled by selecting animals within a limited age range and weight category, testing animals within a limited time frame, training animals to remove any training effect, calibrating video-motion analysis equipment and maintaining constant stimulus conditions in tail flick apparatus, selecting animals from a genetically homogenous population, Sprague-Dawley rats and using a saline control. In addition, the effect of a single treatment on subsequent treatments, or the potential for multiple-treatment interference, was minimized by allowing a day between testing for drug clearance from the body. Additional sources of internal invalidity were: 1) the time of day,

2) acclimatizing animals to the environment, diet and handler, 3) housing and 4) water temperature in the swim tank. These potential sources of invalidity were controlled by using a similar testing schedule each day, maintaining a consistent handler, housing the animals individually or in groups of two and maintaining the temperature of the water near body temperature.

Thus, the suitability of a Latin square design for this study was based on the moderate number of treatments to be tested, within the desired range of five to eight treatments, which included three tizanidine dosages, three baclofen dosages and a saline treatment. In addition, it was selected for its ability to control for sources of internal invalidity, and it minimized the number of animals tested and required to achieve a statistical significance.

# Chapter Four: Results

## 4.1 Dose Response

Both baclofen and tizanidine showed a dose-dependent increase in analgesic response and a dose-dependent decline in motor performance. The dose-response curves for each drug and each parameter, analgesia or motor performance, are discussed below.

### 4.1.1 Tizanidine Analgesia Dose Response

Means and standard errors are illustrated in Figure 4.1.1 and recorded in Tables 4.2.1, 4.2.3 and 4.2.5. All tizanidine doses and the saline control produced a consistent baseline, between 5 and 8 seconds. The onset of the drug effect appeared as an increase in tail flick latency following the drug injection. Tail flick latencies with tizanidine exceeded those of the saline control for all doses at all timepoints after drug injection. At the low dosage, 0.5 mg/kg, i.p., there was an elevation in tail flick latency above the saline control, but not significantly. At the mid dosage, 1.5 mg/kg, i.p., a dramatic increase in tail flick latency was recorded post-injection, which peaked at 20 minutes, exceeding the high dose analgesic response at this time ($p<=0.05$). A gradual decline to baseline followed this distinct peak. At the high dose, 3.0 mg/kg, i.p., an elevated tail flick latency was produced, which persisted from 20 to 120 minutes ($p<=0.05$). Thus, peak tail flick latencies averaged between 14 and 16 seconds. Notably, at the first recorded timepoint following drug injection, the mean tail flick latency with mid dose tizanidine was 15.3 (+/- 0.8) seconds, and with high dose tizanidine was 13.8 (+/- 0.9) seconds. Subsequently, at 40 minutes, the mean tail flick latency of mid dose tizanidine was 14.0 (+/- 0.6) seconds and of high dose tizanidine was 15.0 (+/- 0.78) seconds.

4.1.2 Baclofen Analgesia Dose Response

Means and standard errors are illustrated in Figure 4.1.2 and recorded in Tables 4.2.1, 4.2.3 and 4.2.5. All baclofen doses and the saline control produced a consistent baseline, between 6 and 8 seconds. The onset of the drug effect appeared as an increase in tail flick latency following the drug injection. Tail flick latencies with baclofen exceeded those of the saline control for all doses after drug injection. At the low dosage, 2 mg/kg, i.p., there was an elevation in tail flick latency above the saline control, but not significantly. At the mid dosage, 5 mg/kg, i.p., a gradual increase in tail flick latency which persisted from the point of injection to 60 minutes was observed, and differed significantly from saline only at 40 minutes ($p<=0.05$). At the high dosage, 6 mg/kg, i.p., a dramatic elevation in tail flick latency appeared at 20 minutes, followed by a continual increase which peaked from 40 to 60 minutes and plateaued from 80 to 100 minutes ($p<=0.05$). Thus, peak tail flick latencies averaged 17-18 seconds with high dose baclofen, 17.7 (+/- 0.6) seconds and 17.4 (+/- 0.5) seconds at 40 and 60 minutes, respectively. Similarly, plateau tail flick latencies show elevated levels of analgesia of 14.7 (+/- 0.5) seconds and 14.9 (+/- 0.6) seconds at 80 and 100 minutes, respectively.

4.1.3 Tizanidine Motor Performance Dose Response

Means and standard errors are illustrated in Figure 4.1.3 and recorded in Tables 4.2.2, 4.2.4 and 4.2.6. All tizanidine doses and the saline control produced a consistent baseline, between 0.30 and 0.33 m/sec. The onset of the drug effect appeared as a decrease in swim speed following drug injection. At the low dosage, 0.5 mg/kg, i.p., there was a small but insignificant decrement in motor

performance relative to the saline control. At the mid dosage, 1.5 mg/kg, i.p., there was a decrement in motor performance relative to the saline control overlapping the insignificant low dose effect. For example, at 40 minutes, mean swim speed of low dose tizanidine was 0.27 (+/- 0.02) m/seconds and of mid dose tizanidine was 0.28 (+/- 0.02) m/seconds. At the high dosage, 3.0 mg/kg, i.p., a significant decrement in motor performance relative to saline and other tizanidine doses was observed at 20 and 80 minutes. Trough swim speeds at this dose fell to 0.25 (+/- 0.02) m/seconds at 20 minutes and 0.25 (+/- 0.02) m/seconds at 80 minutes.

4.1.4 Baclofen Motor Performance Dose Response

Means and standard errors are illustrated in Figure 4.1.4 and recorded in Tables 4.2.2, 4.2.4 and 4.2.6. All baclofen doses and the saline control produced a consistent baseline, between 0.27 and 0.31 m/sec, at -20 minutes. The onset of the drug effect appeared as a decrease in swim speed following drug injection. Swim speed was reduced below the saline control and the pre-injection baseline values at all doses. At the low dosage, 2 mg/kg, i.p., a depressed swim speed was observed, though insignificantly relative to saline. At the mid dosage, 5 mg/kg, i.p., a dramatic decrease in swim speed was produced, which fell below both low and high dose values, and significantly below saline values ($p<=0.05$). The depressed motor performance with mid dose baclofen declined to its lowest level at 40 minutes, 0.15 (+/- 0.2) m/seconds, and began a gradual return to baseline from 60 to 120 minutes; however, the last recorded swim speed of mid dose baclofen, 0.19 (+/- 0.01) m/seconds, remained significantly below baseline and saline control values ($p<=0.05$). At the high dosage, 6 mg/kg, i.p., a significant decrease in swim speed was produced at 20, 60 and 100 minutes ($p<=0.05$) Trough swim speeds fell to 0.15 m/seconds with mid dose baclofen and to 0.20 - 0.25

m/seconds with high dose baclofen.

## 4.2 Descriptive Statistics

The mean and standard errors calculated for each drug, dose and time point are tabulated (Tables 4.2.1 - 4.2.6). Results are summarised according to the following tables: 1) low dose analgesia, 2) low dose motor, 3) mid dose analgesia, 4) mid dose motor, 5) high dose analgesia, 6) high dose motor.

## 4.3 Calculated Indices

The cumulative increase in tail flick latencies and decrement in swim speed for each drug and dose are represented graphically in Figures 4.3.1 and 4.3.2, respectively. Comparable analgesia at all three levels, low, mid and high, differed within a 10 second range. Performance comparisons were approximately equivalent at low doses, different at mid doses, and within a 0.1 m/second range at high doses.

### 4.3.1 Analgesic Indices

There was an increase in tail flick latency at all doses of all drugs, relative to the pre-injection baseline and to the saline control. This increase was reflected in positive analgesic indices. The analgesic indices of low dose tizanidine, 0.5 mg/kg, i.p., and low dose baclofen, 2 mg/kg, i.p., were 13.7 seconds and 8.8 seconds, respectively. The analgesic indices of mid dose tizanidine, 1.5 mg/kg, i.p., and mid dose baclofen, 5 mg/kg, i.p., were 29.9 seconds and 20.4 seconds, respectively. The analgesic indices of high dose tizanidine, 3 mg/kg, i.p., and high dose baclofen, 6 mg/kg, i.p., were

44.1 seconds and 55.1 seconds, respectively. The analgesic index of the saline control was 0.2 seconds.

4.3.2 Motor Performance Indices

There was a decrement in swim speed at all doses of all drugs, relative to the pre-injection baseline and to the saline control. This decrement was reflected in negative performance indices. The performance indices of low dose tizanidine, 0.5 mg/kg, i.p., and low dose baclofen, 2 mg/kg, i.p., were both -0.08 m/seconds. The performance indices of mid dose tizanidine, 1.5 mg/kg, i.p., and mid dose baclofen, 5 mg/kg, i.p., were -0.12 m/s and -0.46 m/s, respectively. The performance indices of high dose tizanidine, 3 mg/kg, i.p., and high dose baclofen, 6 mg/kg, i.p., were -0.24 m/s and -0.35 m/s, respectively. The performance index of the saline control was 0.07.

4.4 Frequency Distributions

4.4.1 Analgesia Distributions

At low and mid dosages, normal bell-shaped curves were characterised peaks at 6 seconds and ranges of 2-20 seconds. At high dosages, a bimodal distribution was observed, with peaks at 6 and 20 seconds, and a range of 2-20 seconds. (Figure 4.4.1)

4.4.2 Motor Performance Distributions

At low dosages, a normal bell-shaped curve was characterised by a peak at 0.30 m/seconds and a range of 0.14-0.46 m/seconds. At mid dosages, a normal bell-shaped curve was also characterised by a peak at 0.30 m/seconds and a range of 0.02-0.46 m/seconds. At high dosages, a

normal bell-shaped curve was further characterised by a peak at 0.30 m/seconds and a range of 0.10 - 0.52 m/seconds. (Figure 4.4.2)

## 4.5 Low Dose Comparisons

### 4.5.1 Analgesia Dose Response

Dose response curves for low dose tizanidine, 0.5 mg/kg, i.p., and baclofen, 2 mg/kg, i.p. overlap with the saline control prior to drug injection, and differ insignificantly from each other at all times. At 20 minutes, the analgesic response with tizanidine is further from the saline control than is baclofen; however, at 60 minutes, the dose response curves of tizanidine and baclofen cross and remain within close separation of one another. (Figure 4.5.1)

### 4.5.2 Motor Dose Response

Dose response curves for low dose tizanidine, 0.5 mg/kg, i.p., and baclofen, 2 mg/kg, i.p. overlap with the saline control prior to drug injection, and differ insignificantly from each other at all times. The motor response of tizanidine and baclofen from 20 to 60 minutes is within close separation and intersects at two points; subsequently, up to 100 minutes, the tizanidine motor curve is nearer to that of saline than it is to the baclofen motor curve. (Figure 4.5.2)

### 4.5.3 Analysis of Variance

Low dose analgesia comparison data showed an insignificant drug effect ($p=0.9312$) and drug*time interaction ($p=0.6566$). Thus, these are comparable doses. However, there was a significant time effect ($p=0.0334$), which provides further evidence of a dose response for these

doses over this time period. (Table 4.5.3)

Low dose motor comparison data also showed an insignificant drug effect (p=0.4257), time effect (p=0.0818) and drug*time interaction (p=0.3025). Thus, there was no significant difference in motor performance at selected low dosages of tizanidine and baclofen. (Table 4.5.3)

## 4.6 Mid Dose Comparisons

### 4.6.1 Analgesia Dose Response

Dose response curves for mid dose tizanidine, 1.5 mg/kg, i.p., and baclofen, 5 mg/kg, i.p. overlap with the saline control prior to drug injection, but differ significantly from each other at -20 and 120 minutes. The analgesic response with tizanidine does not differ significantly from that with baclofen from 20 to 100 minutes. (Figure 4.6.1)

### 4.6.2 Motor Dose Response

The dose response curve for mid dose tizanidine, 1.5 mg/kg, i.p., overlaps with the saline control prior to drug injection, while that of mid dose baclofen, 5 mg/kg, i.p., overlaps with the saline control only at -20 minutes. Specifically, mid dose tizanidine and baclofen differ significantly from each other at -60 and -40 minutes ($p<=0.05$). In the post-injection period, mid dose baclofen and tizanidine differ significantly from each other at all times. (Figure 4.6.2)

### 4.6.3 Analysis of Variance

Mid dose analgesia comparison data showed an insignificant drug effect (p=0.0616) and drug*time interaction (p=0.1954). Thus, these are comparable tizanidine and baclofen doses. As

at low dosages, there was a significant time effect ($p<0.0001$), which provides further evidence of a dose response for these doses over this time period. (Table 4.6.3)

Mid dose motor comparison data also showed a significant drug effect ($p=0.0007$), time effect ($p<0.0001$) and drug*time interaction ($p=0.0077$). Thus, this further supports the dose response associated with these two drugs, as well as a significant difference in motor performance at selected mid dosages of tizanidine and baclofen. (Table 4.6.3)

## 4.7 High Dose Comparisons

### 4.7.1 Analgesia Dose Response

Dose response curves for high dose tizanidine, 3 mg/kg, i.p., and baclofen, 6 mg/kg, i.p. overlap with the saline control prior to drug injection and differ insignificantly from each other at all times. The tizanidine and baclofen curves overlap at 20, 80, 100 and 120 minutes, where at 40 and 60 minutes, there is only a small separation in which the baclofen curve is further from the saline control than is the tizanidine curve. (Figure 4.7.1)

### 4.7.2 Motor Dose Response

Dose response curves for high dose tizanidine, 3 mg/kg, i.p., and baclofen, 6 mg/kg, i.p. overlap with the saline control prior to drug injection and differ insignificantly from each other at all times. The tizanidine and baclofen curves overlap at 20, 40, 80 and 120 minutes, where at 60 and 100 minutes, there is some separation in which the baclofen curve is further from the saline control than is the tizanidine curve. (Figure 4.7.2)

4.7.3 Analysis of Variance

High dose analgesia comparison data showed an insignificant drug effect ($p=0.6199$) and drug*time interaction ($p=0.4732$). Thus, these are comparable doses. However, there was a significant time effect ($p<0.0001$), which further supports a dose response. (Table 4.7.3)

High dose motor comparison data also showed an insignificant drug effect ($p=0.6575$), time effect and drug*time interaction ($p=0.6882$); however, there was a significant time effect. Thus, there was no significant difference in motor performance at selected high dosages of tizanidine and baclofen and dose response in motor performance was demonstrated at these dosages. (Table 4.7.3)

4.8 Dose comparability

Comparable low, mid and high doses from analgesia of tizanidine and baclofen were identified as follows: low doses were 2 mg/kg baclofen and 0.5 mg/kg tizanidine; mid doses were 5 mg/kg baclofen and 1.5 mg/kg tizanidine; high doses were 6 mg/kg baclofen and 3 mg/kg tizanidine. A saline control was used with each trial.

Comparable doses demonstrated an insignificant difference between pairs of means in response to the tail flick test.

4.9 Relative Motor Performance

In the low dose comparison of tizanidine (0.5 mg/kg, i.p.) with baclofen (2 mg/kg, i.p.), there was no significant difference between the two drugs on mean swim speed ($p=0.4257$).

In the mid dose comparison of tizanidine (1.5 mg/kg, i.p.) with baclofen (5 mg/kg, i.p.), there was a significant difference between the two drugs on mean swim speed ($p=0.0007$).

In the high dose comparison of tizanidine (3 mg/kg, i.p.) with baclofen (6 mg/kg, i.p.), swim speed means were similar at all time points and there was no significant difference between the two drugs on mean swim speed ($p=0.6575$).

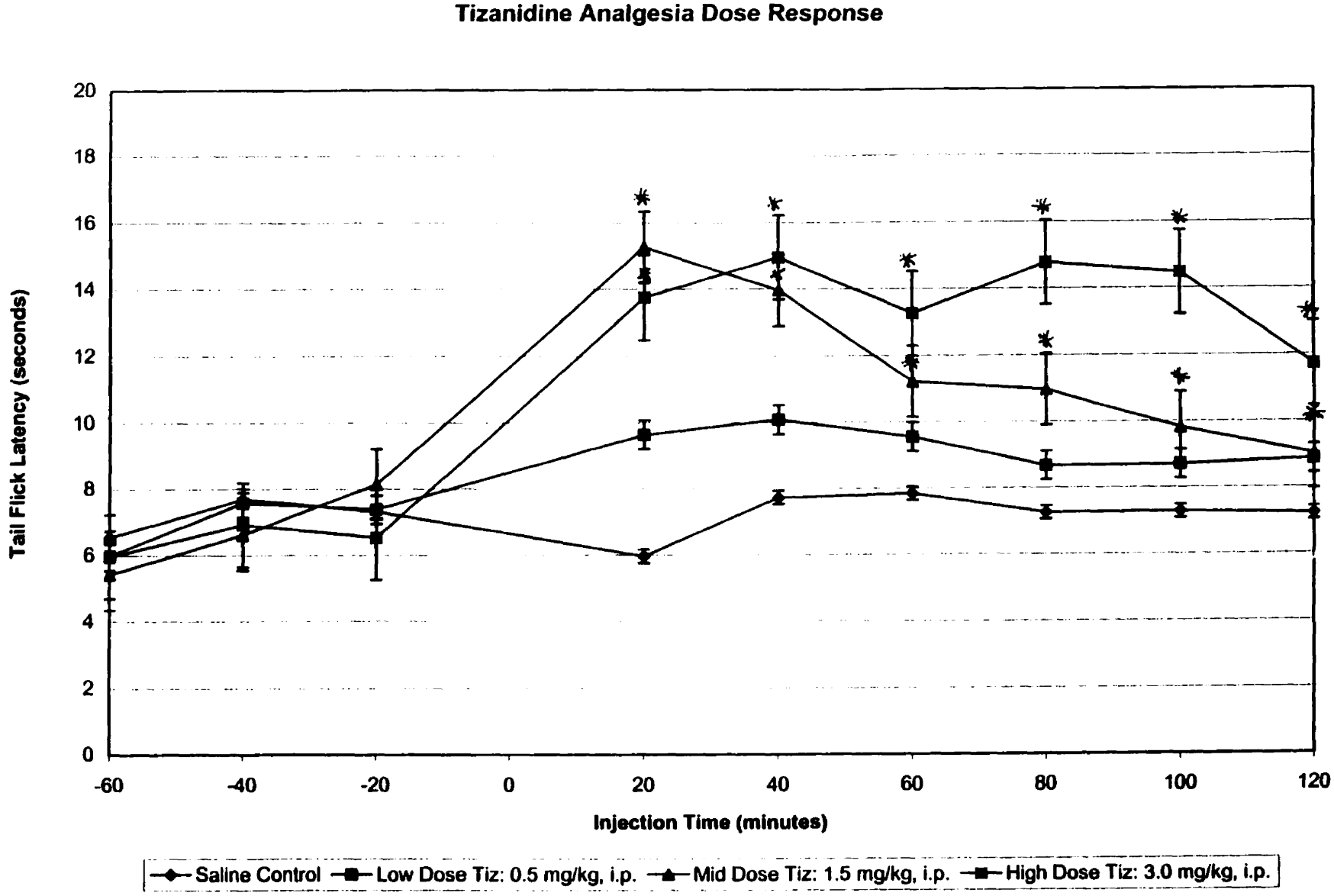

Tizanidine Analgesia Dose Response
Tail Flick Latency (seconds)
0
2
4
6
8
10
12
14
16
18
20
-60
-40
-20
0
20
40
60
80
100
120
Injection Time (minutes)
Saline Control
Low Dose Tiz: 0.5 mg/kg, i.p.
Mid Dose Tiz: 1.5 mg/kg, i.p.
High Dose Tiz: 3.0 mg/kg, i.p.

FIGURE 4.1.1

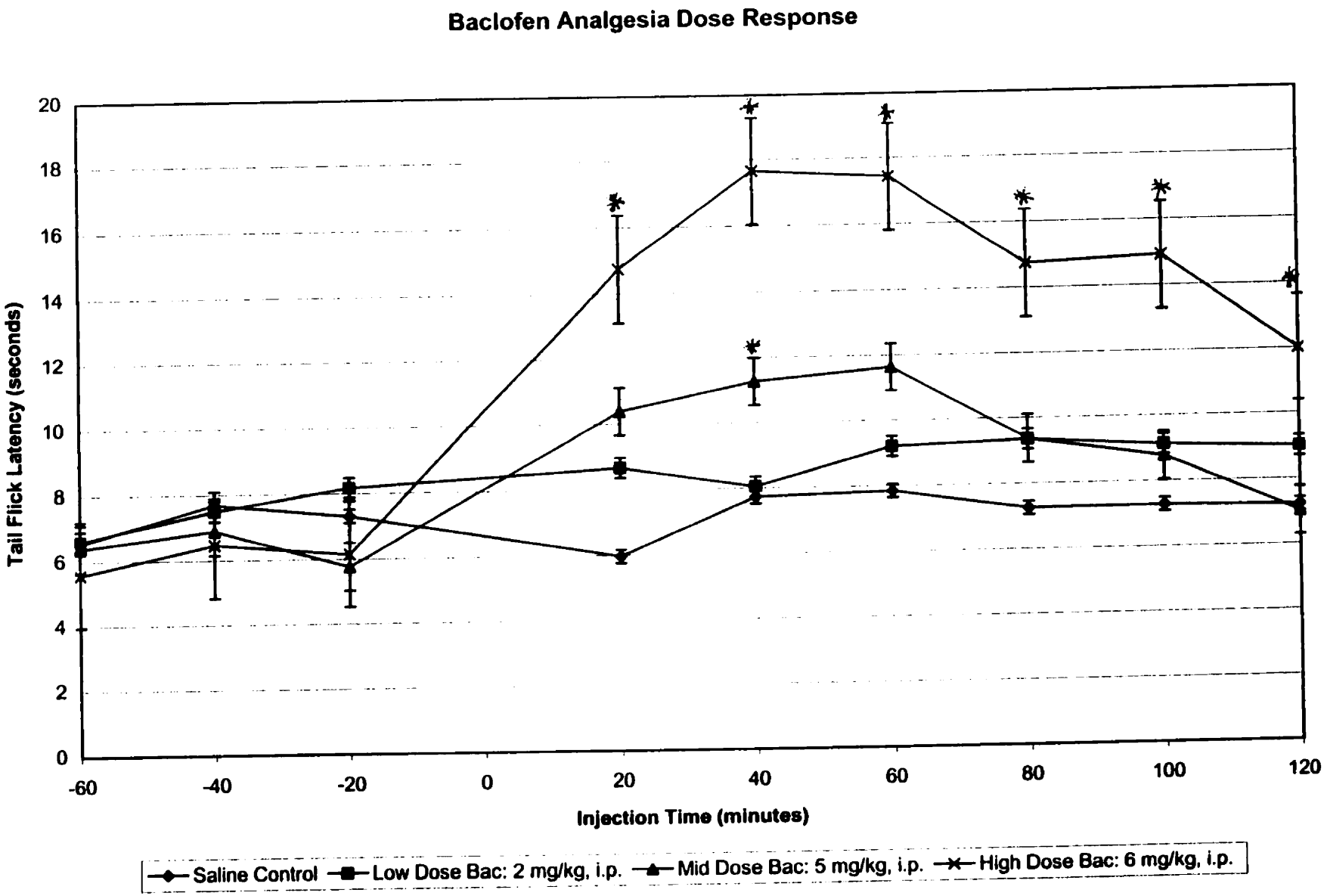

Baclofen Analgesia Dose Response
Tail Flick Latency (seconds)
0
2
4
6
8
10
12
14
16
18
20
-60
-40
-20
0
20
40
60
80
100
120
Injection Time (minutes)
Saline Control
Low Dose Bac: 2 mg/kg, i.p.
Mid Dose Bac: 5 mg/kg, i.p.
High Dose Bac: 6 mg/kg, i.p.

FIGURE 4.1.2

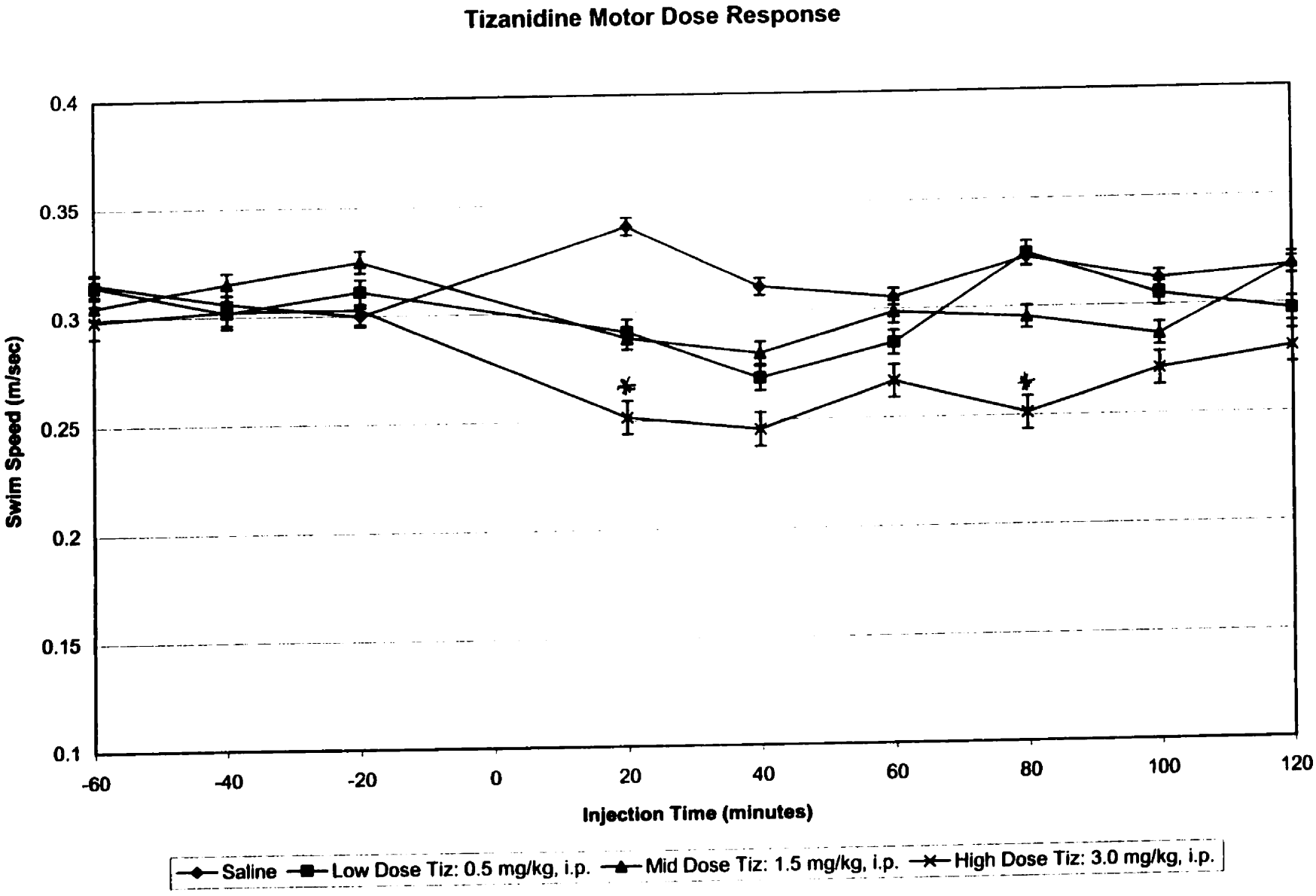

Tizanidine Motor Dose Response
Swim Speed (m/sec)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
-60
-40
-20
0
20
40
60
80
100
120
Injection Time (minutes)
Saline
Low Dose Tiz: 0.5 mg/kg, i.p.
Mid Dose Tiz: 1.5 mg/kg, i.p.
High Dose Tiz: 3.0 mg/kg, i.p.

FIGURE 4.1.3

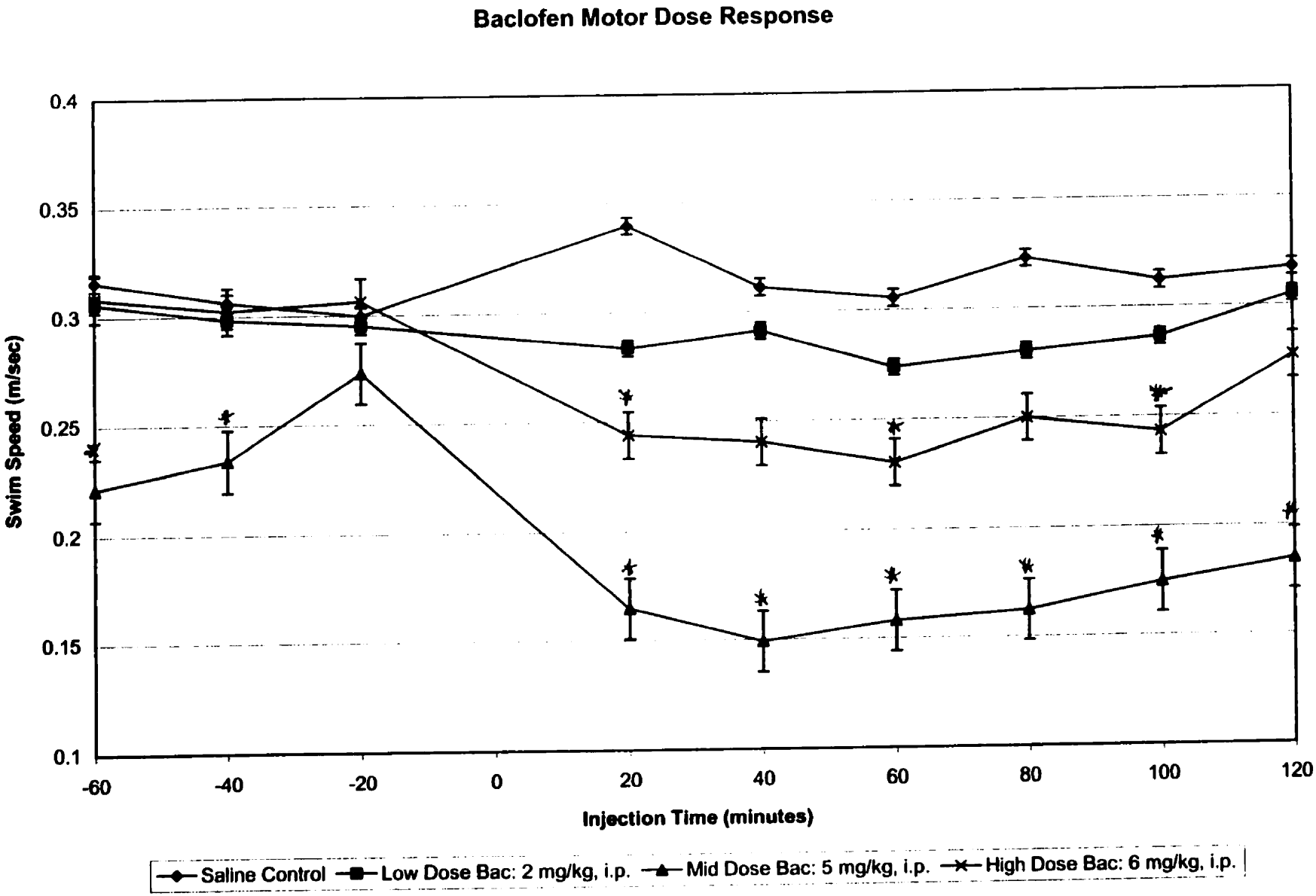

Baclofen Motor Dose Response
Swim Speed (m/sec)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
-60
-40
-20
0
20
40
60
80
100
120
Injection Time (minutes)
Saline Control
Low Dose Bac: 2 mg/kg, i.p.
Mid Dose Bac: 5 mg/kg, i.p.
High Dose Bac: 6 mg/kg, i.p.

FIGURE 4.1.4

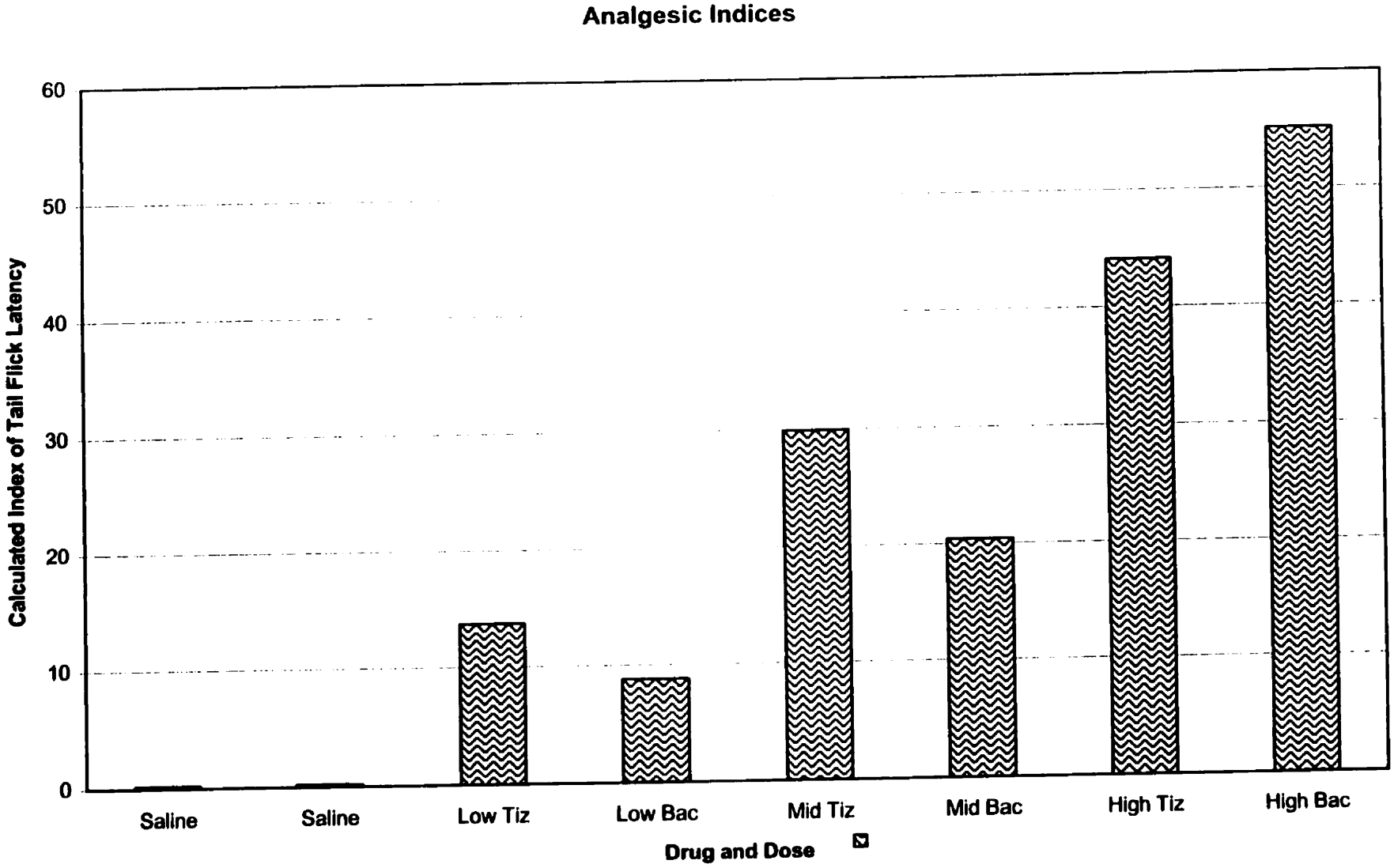

Analgesic Indices
Calculated Index of Tail Flick Latency
60
50
40
30
20
10
0
Saline
Saline
Low Tiz
Low Bac
Mid Tiz
Mid Bac
High Tiz
High Bac
Drug and Dose

FIGURE 4.3.1

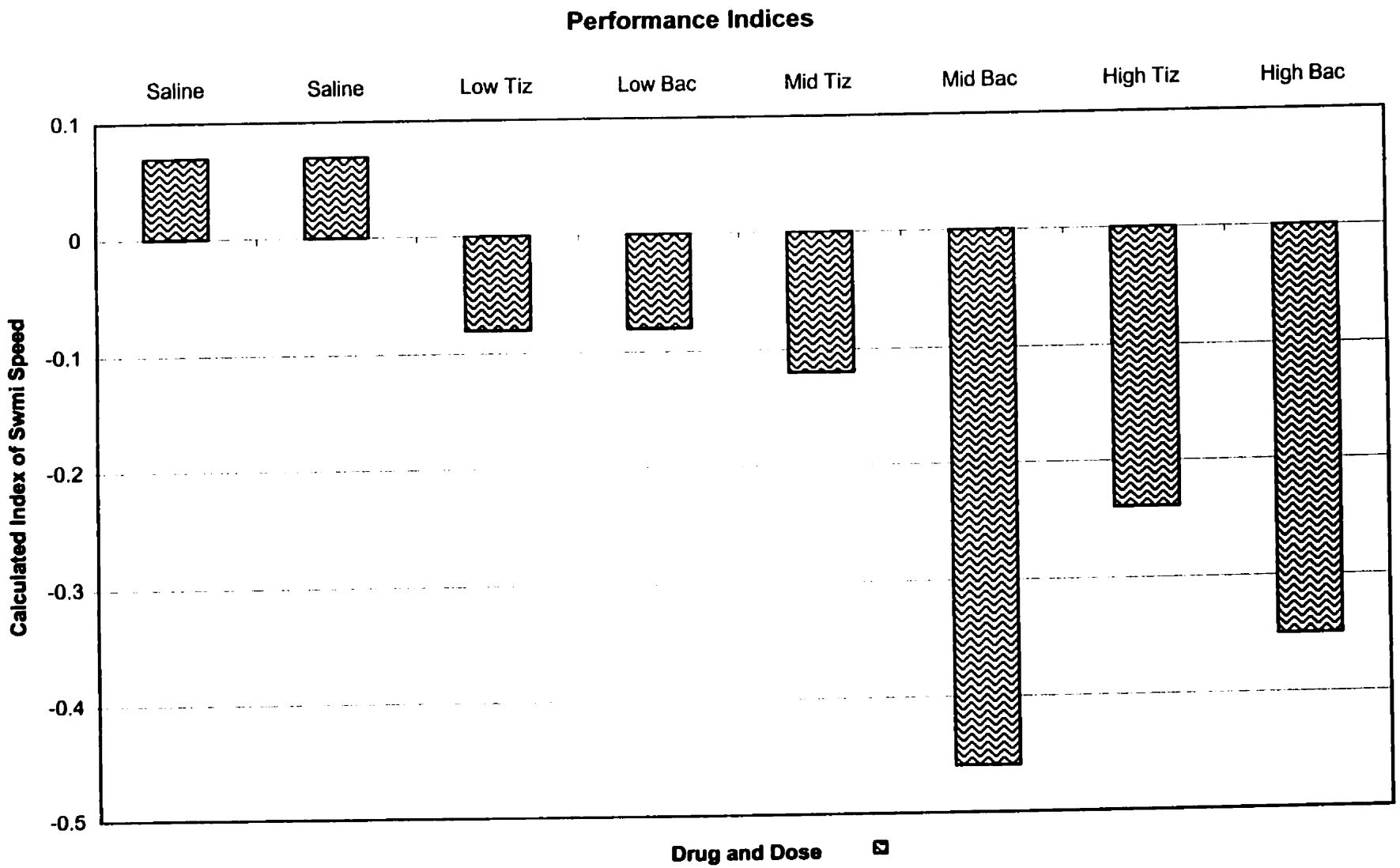

Performance Indices
Saline
Saline
Low Tiz
Low Bac
Mid Tiz
Mid Bac
High Tiz
High Bac
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
Calculated Index of Swmi Speed
Drug and Dose

FIGURE 4.3.2

# ANALGESIA DISTRIBUTIONS

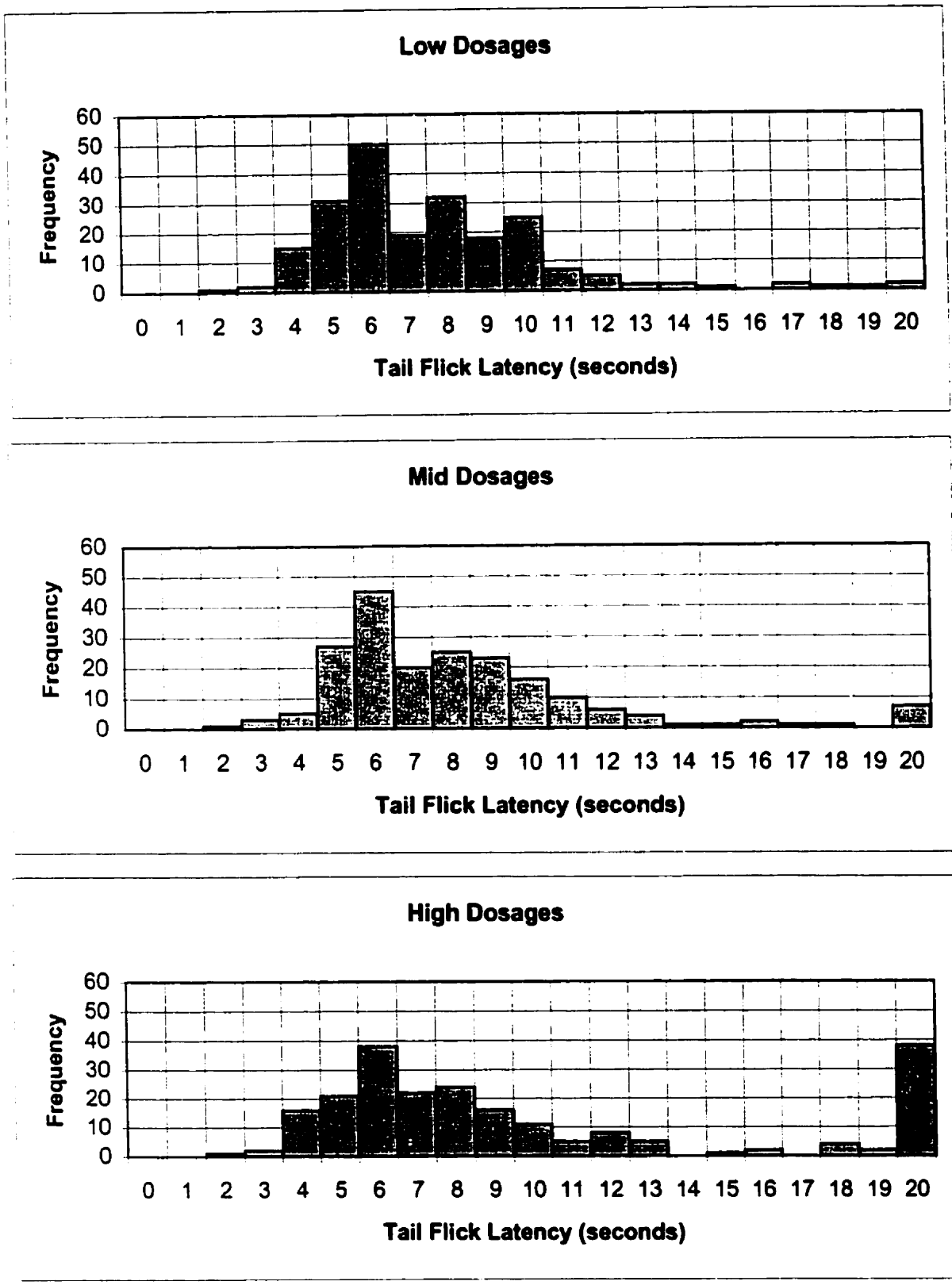



FIGURE 4.4.1

# PERFORMANCE DISTRIBUTIONS

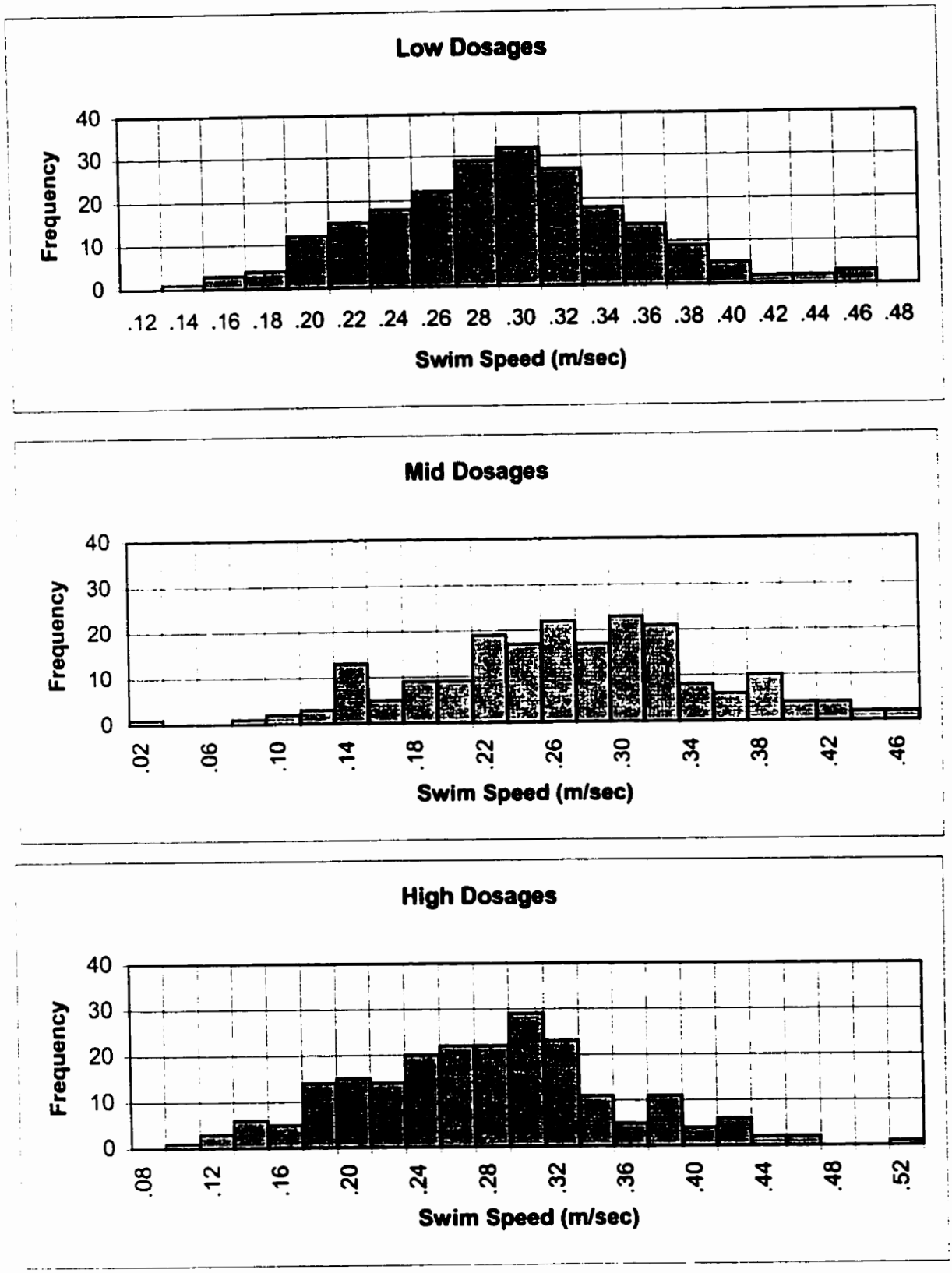
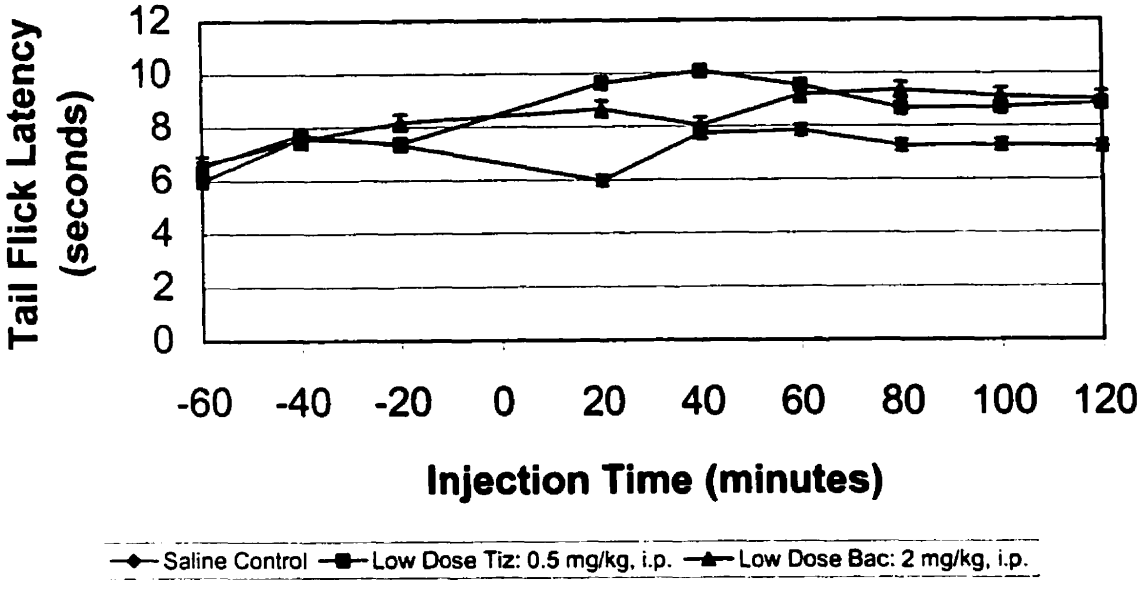


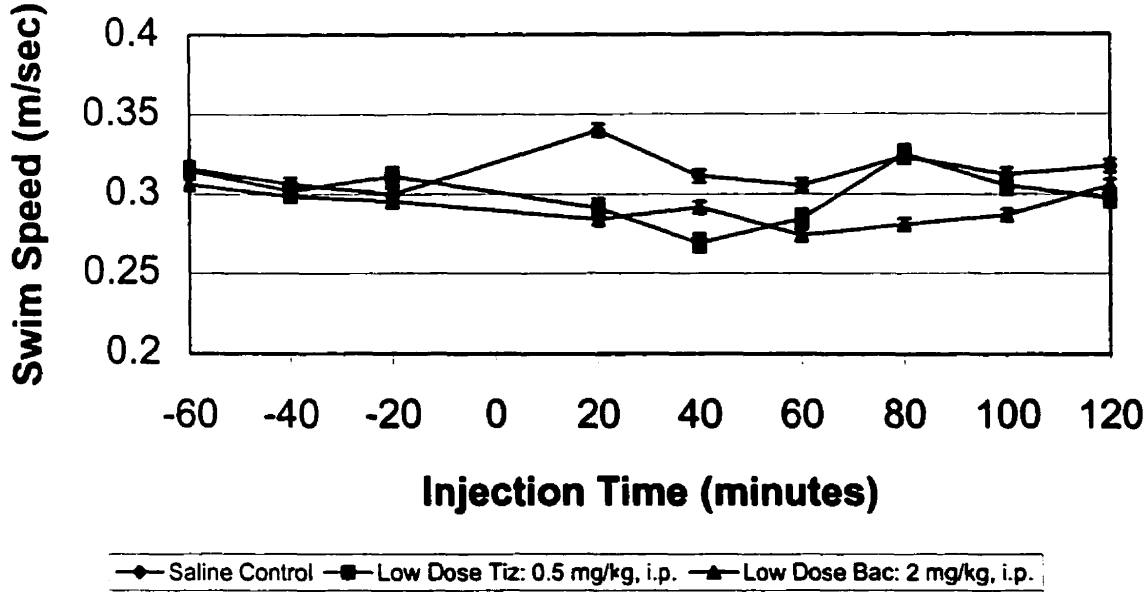

FIGURE 4.4.2

# LOW DOSAGE COMPARISONS

## FIGURE 4.5.1 - Analgesia

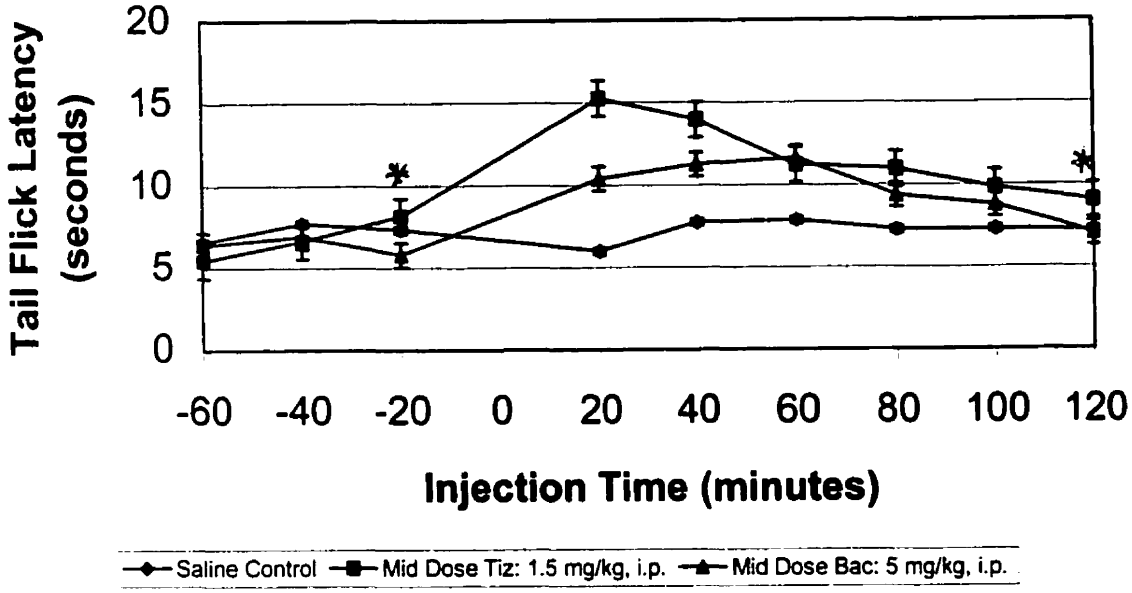



## FIGURE 4.5.2 - Motor Performance

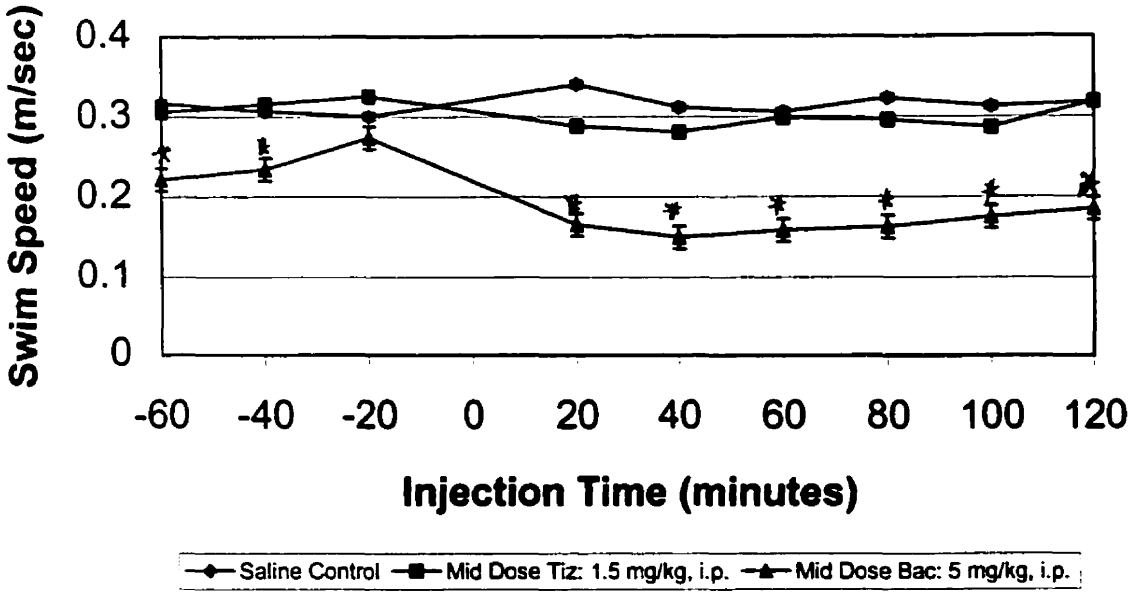


# MID DOSAGE COMPARISONS

## FIGURE 4.6.1 - Analgesia

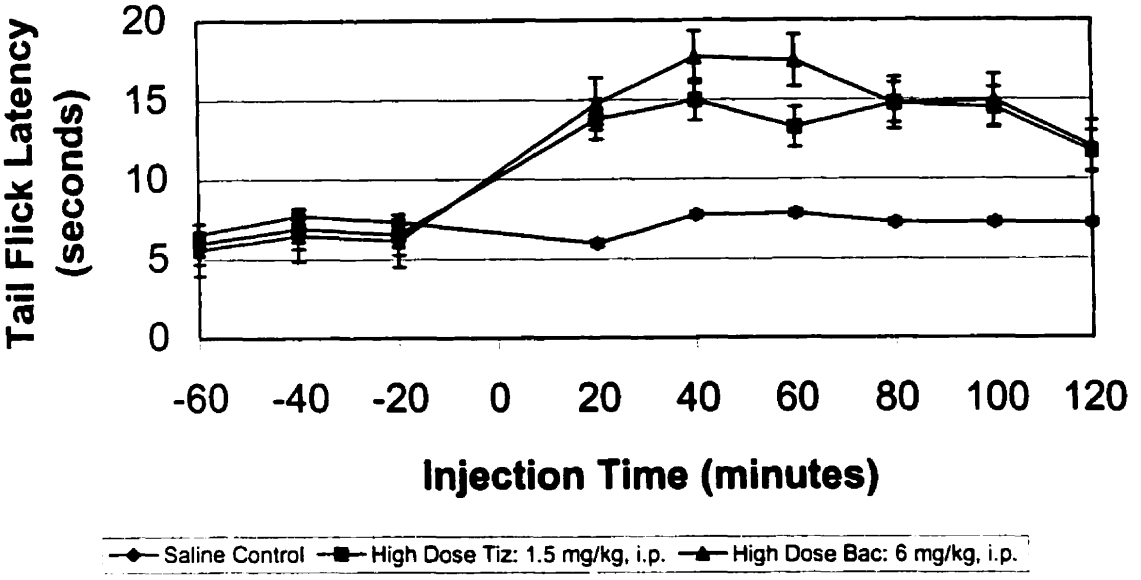



## FIGURE 4.6.2 - Motor Performance

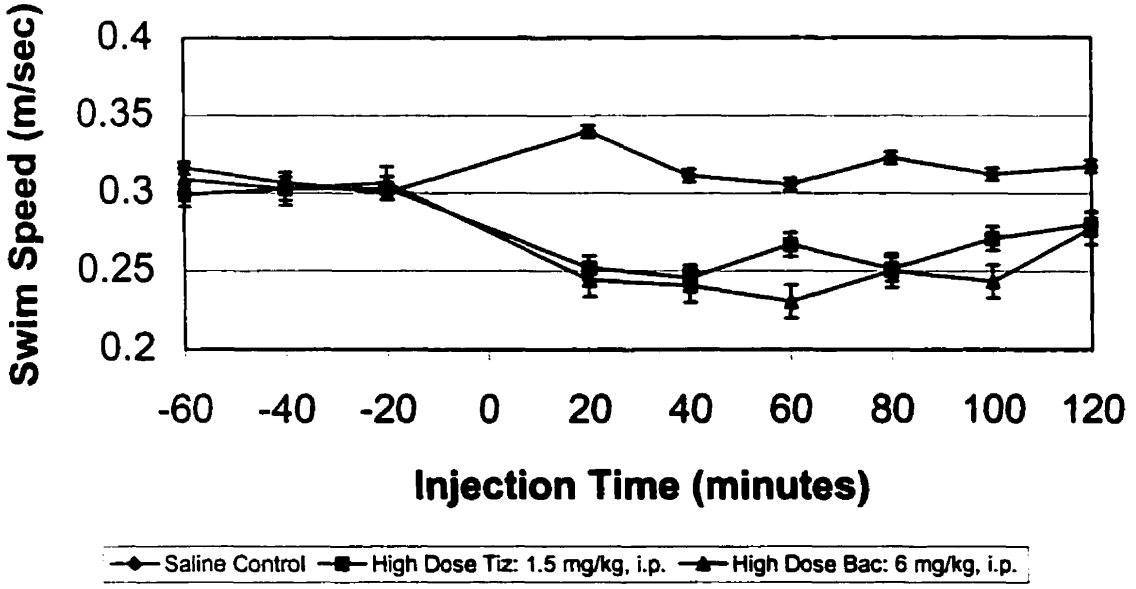


# HIGH DOSAGE COMPARISONS

## FIGURE 4.7.1 - Analgesia




## FIGURE 4.7.2 - Motor Performance



Low Dose Grouped Analgesia Data — **Descriptive Statistics**

| Drug | N | Mean(-60) | Mean(-40) | Mean(-20) | Mean(20) | Mean(40) | Mean(60) | Mean(80) | Mean(100) | Mean(120) |
|---|---|---|---|---|---|---|---|---|---|---|
| BAC | 72 | 6.6 | 7.5 | 8.2 | 8.7 | 8.0 | 9.2 | 9.4 | 9.1 | 9.0 |
| SAL | 72 | 6.5 | 7.7 | 7.3 | 6.0 | 7.8 | 7.8 | 7.3 | 7.3 | 7.2 |
| TIZ | 72 | 6.0 | 7.6 | 7.4 | 9.6 | 10.1 | 9.6 | 8.7 | 8.7 | 8.9 |
| Drug | N | S.E.(-60) | S.E.(-40) | S.E.(-20) | S.E.(20) | S.E.(40) | S.E.(60) | S.E.(80) | S.E.(100) | S.E.(120) |
| BAC | 72 | 1.1 | 0.7 | 0.7 | 1.5 | 1.1 | 0.7 | 1.4 | 1.3 | 1.4 |
| SAL | 72 | 0.5 | 0.4 | 0.7 | 0.7 | 0.8 | 0.8 | 0.7 | 0.8 | 0.5 |
| TIZ | 72 | 0.5 | 0.9 | 0.7 | 2.4 | 1.8 | 0.9 | 0.8 | 0.5 | 0.8 |

Low Dose Grouped Motor Data

| Drug | N | Mean(-60) | Mean(-40) | Mean(-20) | Mean(20) | Mean(40) | Mean(60) | Mean(80) | Mean(100) | Mean(120) |
|---|---|---|---|---|---|---|---|---|---|---|
| BAC | 72 | 0.306 | 0.298 | 0.295 | 0.284 | 0.291 | 0.274 | 0.281 | 0.287 | 0.305 |
| SAL | 72 | 0.316 | 0.306 | 0.300 | 0.340 | 0.311 | 0.305 | 0.323 | 0.312 | 0.317 |
| TIZ | 72 | 0.315 | 0.302 | 0.311 | 0.291 | 0.269 | 0.284 | 0.325 | 0.305 | 0.297 |
| Drug | N | S.E.(-60) | S.E.(-40) | S.E.(-20) | S.E.(20) | S.E.(40) | S.E.(60) | S.E.(80) | S.E.(100) | S.E.(120) |
| BAC | 72 | 0.021 | 0.017 | 0.026 | 0.020 | 0.030 | 0.018 | 0.014 | 0.020 | 0.015 |
| SAL | 72 | 0.026 | 0.026 | 0.019 | 0.026 | 0.029 | 0.022 | 0.023 | 0.020 | 0.030 |
| TIZ | 72 | 0.017 | 0.027 | 0.021 | 0.024 | 0.019 | 0.020 | 0.016 | 0.017 | 0.020 |

Mid Dose Grouped Analgesia Data

| Drug | N | Mean(-60) | Mean(-40) | Mean(-20) | Mean(20) | Mean(40) | Mean(60) | Mean(80) | Mean(100) | Mean(120) |
|---|---|---|---|---|---|---|---|---|---|---|
| BAC | 54 | 6.4 | 6.9 | 5.8 | 10.4 | 11.3 | 11.6 | 9.4 | 8.8 | 7.0 |
| SAL | 72 | 6.5 | 7.7 | 7.3 | 6.0 | 7.8 | 7.8 | 7.3 | 7.3 | 7.2 |
| TIZ | 72 | 5.4 | 6.6 | 8.1 | 14.0 | 14.0 | 11.2 | 11.0 | 9.8 | 9.0 |
| Drug | N | S:E.(-60) | S.E.(-40) | S.E.(-20) | S.E.(20) | S.E.(40) | S.E.(60) | S.E.(80) | S.E.(100) | S.E.(120) |
| BAC | 54 | 0.1 | 0.3 | 0.2 | 2.1 | 1.7 | 1.9 | 0.8 | 0.7 | 0.4 |
| SAL | 72 | 0.5 | 0.4 | 0.7 | 0.7 | 0.8 | 0.8 | 0.7 | 0.8 | 0.5 |
| TIZ | 72 | 0.4 | 0.6 | 0.9 | 2.4 | 1.8 | 0.5 | 0.6 | 0.6 | 0.4 |

Mid Dose Grouped Motor Data

| Drug | N | Mean(-60) | Mean(-40) | Mean(-20) | Mean(20) | Mean(40) | Mean(60) | Mean(80) | Mean(100) | Mean(120) |
|---|---|---|---|---|---|---|---|---|---|---|
| BAC | 54 | 0.221 | 0.234 | 0.274 | 0.165 | 0.149 | 0.158 | 0.162 | 0.175 | 0.185 |
| SAL | 72 | 0.316 | 0.306 | 0.300 | 0.340 | 0.311 | 0.305 | 0.323 | 0.312 | 0.317 |
| TIZ | 72 | 0.305 | 0.315 | 0.325 | 0.288 | 0.280 | 0.298 | 0.295 | 0.287 | 0.318 |
| Drug | N | S.E.(-60) | S.E.(-40) | S.E.(-20) | S.E.(20) | S.E.(40) | S.E.(60) | S.E.(80) | S.E.(100) | S.E.(120) |
| BAC | 54 | 0.020 | 0.014 | 0.015 | 0.035 | 0.017 | 0.016 | 0.016 | 0.017 | 0.013 |
| SAL | 72 | 0.026 | 0.026 | 0.019 | 0.026 | 0.029 | 0.022 | 0.023 | 0.020 | 0.030 |
| TIZ | 72 | 0.017 | 0.020 | 0.018 | 0.018 | 0.022 | 0.023 | 0.019 | 0.028 | 0.023 |

High Dose Grouped Analgesia Data

| Drug | N | Mean(-60) | Mean(-40) | Mean(-20) | Mean(20) | Mean(40) | Mean(60) | Mean(80) | Mean(100) | Mean(120) |
|---|---|---|---|---|---|---|---|---|---|---|
| BAC | 72 | 5.6 | 6.5 | 6.2 | 14.7 | 17.7 | 17.4 | 14.7 | 14.9 | 12.0 |
| SAL | 72 | 6.5 | 7.7 | 7.3 | 6.0 | 7.8 | 7.8 | 7.3 | 7.3 | 7.2 |
| TIZ | 72 | 6.0 | 6.9 | 6.5 | 13.8 | 15.0 | 13.3 | 14.8 | 14.5 | 11.7 |
| Drug | N | S.E.(-60) | S.E.(-40) | S.E.(-20) | S.E.(20) | S.E.(40) | S.E.(60) | S.E.(80) | S.E.(100) | S.E.(120) |
| BAC | 72 | 0.4 | 0.6 | 0.8 | 2.0 | 1.6 | 1.3 | 2.1 | 1.8 | 1.4 |
| SAL | 72 | 0.5 | 0.4 | 0.7 | 0.7 | 0.8 | 0.8 | 0.7 | 0.8 | 0.5 |
| TIZ | 72 | 0.5 | 0.7 | 0.5 | 2.4 | 2.2 | 2.0 | 1.8 | 1.9 | 1.8 |

High Dose Grouped Motor Data

| Drug | N | Mean(-60) | Mean(-40) | Mean(-20) | Mean(20) | Mean(40) | Mean(60) | Mean(80) | Mean(100) | Mean(120) |
|---|---|---|---|---|---|---|---|---|---|---|
| BAC | 72 | 0.308 | 0.302 | 0.306 | 0.244 | 0.240 | 0.230 | 0.250 | 0.243 | 0.278 |
| SAL | 72 | 0.316 | 0.306 | 0.300 | 0.340 | 0.311 | 0.305 | 0.323 | 0.312 | 0.317 |
| TIZ | 72 | 0.299 | 0.302 | 0.303 | 0.252 | 0.246 | 0.267 | 0.251 | 0.271 | 0.280 |
| Drug | N | S.E.(-60) | S.E.(-40) | S.E.(-20) | S.E.(20) | S.E.(40) | S.E.(60) | S.E.(80) | S.E.(100) | S.E.(120) |
| BAC | 72 | 0.027 | 0.030 | 0.025 | 0.030 | 0.023 | 0.023 | 0.033 | 0.020 | 0.024 |
| SAL | 72 | 0.026 | 0.026 | 0.019 | 0.026 | 0.029 | 0.022 | 0.023 | 0.020 | 0.030 |
| TIZ | 72 | 0.020 | 0.015 | 0.028 | 0.020 | 0.026 | 0.033 | 0.020 | 0.025 | 0.038 |

## TABLE 4.5.3

### LOW DOSE ANALGESIA COMPARISONS
Repeated Measures Analysis – General Linear Model Procedure
Univariate Tests - Type III Anova, Within Subject Design
Multivariate Tests – MANOVA

ANOVA: Univariate Tests of Hypothesis for Within Subject Effects

| SOURCE OF VARIATION | DEGREES OF FREEDOM | SUM OF SQUARES | MEAN SQUARE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Drug | 1 | .2934028 | .2934028 | .01 | .9312 |
| Time | 8 | 134.8193055 | 15.8524132 | 2.30 | .0334 |
| Drug*Time | 8 | 27.4709722 | 3.4338715 | .74 | .6566 |

MANOVA: Manova Test Criteria and Exact F statistic for the Hypothesis of no Drug Effect

| STATISTIC | NUM DF | DEN DF | VALUE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Wilk's Lambda | 1 | 7 | .99885813 | .01 | .9312 |
| Pillai's Trace | 1 | 7 | .00114187 | .01 | .9312 |
| Hotelling-Lawley Trace | 1 | 7 | .00114187 | .01 | .9312 |
| Roy's Greatest Root | 1 | 7 | .00114318 | .01 | .9312 |

### LOW DOSE MOTOR COMPARISONS
Repeated Measures Analysis – General Linear Model Procedure
Univariate Tests - Type III Anova, Within Subject Design
Multivariate Tests – MANOVA

ANOVA: Univariate Tests of Hypothesis for Within Subject Effects

| SOURCE OF VARIATION | DEGREES OF FREEDOM | SUM OF SQUARES | MEAN SQUARE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Drug | 1 | .00273006 | .00273006 | .72 | .4257 |
| Time | 8 | .01528243 | .00191030 | 1.88 | .0818 |
| Drug*Time | 8 | .01070563 | .00133820 | 1.22 | .3025 |

MANOVA: Manova Test Criteria and Exact F statistic for the Hypothesis of no Drug Effect

| STATISTIC | NUM DF | DEN DF | VALUE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Wilk's Lambda | 1 | 7 | .90731115 | .72 | .4257 |
| Pillai's Trace | 1 | 7 | .09268885 | .72 | .4257 |
| Hotelling-Lawley Trace | 1 | 7 | .10215773 | .72 | .4257 |
| Roy's Greatest Root | 1 | 7 | .10215773 | .72 | .4257 |

**TABLE 4.6.3**

MID DOSE ANALGESIA COMPARISONS

*(1.5 mg/kg, i.p. Tizanidine vs. 5 mg/kg, i.p. Baclofen)*

Repeated Measures Analysis – General Linear Model Procedure

Univariate Tests - Type III Anova, Between (Drug) or Within (Time) Subject Design

Multivariate Tests – MANOVA

ANOVA: Univariate Tests

| SOURCE OF VARIATION | DEGREES OF FREEDOM | SUM OF SQUARES | MEAN SQUARE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Drug | 1 | 64.0100595 | 64.0100595 | 4.25 | .0616 |
| Time | 8 | 737.6155952 | 92.2019494 | 4.38 | <.0001 |
| Drug*Time | 8 | 92.4270238 | 11.5533780 | 1.43 | .1954 |

MANOVA: Manova Test Criteria and Exact F statistic for the Hypothesis of no Time*Drug Effect

| STATISTIC | NUM DF | DEN DF | VALUE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Wilk's Lambda | 8 | 5 | .28922582 | 1.54 | .3304 |
| Pillai's Trace | 8 | 5 | .71077418 | 1.54 | .3304 |
| Hotelling-Lawley Trace | 8 | 5 | 2.45750602 | 1.54 | .3304 |
| Roy's Greatest Root | 8 | 5 | 2.45750602 | 1.54 | .3304 |

MID DOSE MOTOR COMPARISONS

*(1.5 mg/kg, i.p. Tizanidine vs. 5 mg/kg, i.p. Baclofen)*

Repeated Measures Analysis – General Linear Model Procedure

Univariate Tests - Type III Anova, Between (Drug) or Within (Time) Subject Design

Multivariate Tests – MANOVA

ANOVA: Univariate Tests

| SOURCE OF VARIATION | DEGREES OF FREEDOM | SUM OF SQUARES | MEAN SQUARE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Drug | 1 | .37277452 | .37277452 | 20.62 | .0007 |
| Time | 8 | .08461120 | .01057640 | 9.15 | <.0001 |
| Drug*Time | 8 | .02597812 | .00324727 | 2.81 | .0077 |

MANOVA: Manova Test Criteria and Exact F statistic for the Hypothesis of no Time*Drug Effect

| STATISTIC | NUM DF | DEN DF | VALUE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Wilk's Lambda | 8 | 5 | .25997414 | 1.78 | .2724 |
| Pillai's Trace | 8 | 5 | .74002586 | 1.78 | .2724 |
| Hotelling-Lawley Trace | 8 | 5 | 2.84653643 | 1.78 | .2724 |
| Roy's Greatest Root | 8 | 5 | 2.84653643 | 1.78 | .2724 |

## TABLE 4.7.3

### HIGH DOSE ANALGESIA COMPARISONS
Repeated Measures Analysis – General Linear Model Procedure
Univariate Tests - Type III Anova, Within Subject Design
Multivariate Tests – MANOVA

ANOVA: Univariate Tests of Hypothesis for Within Subject Effects

| SOURCE OF VARIATION | DEGREES OF FREEDOM | SUM OF SQUARES | MEAN SQUARE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Drug | 1 | 24.1736111 | 24.1736111 | .27 | .6199 |
| Time | 8 | 2371.641250 | 296.455156 | 21.93 | <.0001 |
| Drug*Time | 8 | 82.3351389 | 10.2918924 | .96 | .4732 |

MANOVA: Manova Test Criteria and Exact F statistic for the Hypothesis of no Drug Effect

| STATISTIC | NUM DF | DEN DF | VALUE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Wilk's Lambda | 1 | 7 | .96297287 | .27 | .6199 |
| Pillai's Trace | 1 | 7 | .03702713 | .27 | .6199 |
| Hotelling-Lawley Trace | 1 | 7 | .03845085 | .27 | .6199 |
| Roy's Greatest Root | 1 | 7 | .03845085 | .27 | .6199 |

### HIGH DOSE MOTOR COMPARISONS
Repeated Measures Analysis – General Linear Model Procedure
Univariate Tests - Type III Anova, Within Subject Design
Multivariate Tests – MANOVA

ANOVA: Univariate Tests of Hypothesis for Within Subject Effects

| SOURCE OF VARIATION | DEGREES OF FREEDOM | SUM OF SQUARES | MEAN SQUARE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Drug | 1 | .00199511 | .00199511 | .21 | .6575 |
| Time | 8 | .09061222 | .01132653 | 6.83 | <.0001 |
| Drug*Time | 8 | .00708564 | .00088570 | .70 | .6882 |

MANOVA: Manova Test Criteria and Exact F statistic for the Hypothesis of no Drug Effect

| STATISTIC | NUM DF | DEN DF | VALUE | F-VALUE | Pr > F |
|---|---|---|---|---|---|
| Wilk's Lambda | 1 | 7 | .97029577 | .21 | .6575 |
| Pillai's Trace | 1 | 7 | .02970423 | .21 | .6575 |
| Hotelling-Lawley Trace | 1 | 7 | .03061358 | .21 | .6575 |
| Roy's Greatest Root | 1 | 7 | .03061358 | .21 | .6575 |

# Chapter Five: Conclusions

## 5.1 Overview

In this study, a comparison of mean motor performance in normal rats receiving either baclofen or tizanidine at comparable analgesic dosages was performed to test the hypothesis that tizanidine and baclofen produce a dose-dependent difference in motor performance at comparable analgesic dosages. Comparability was based on latency of the tail flick response at multiple dosage levels, to determine dose response curves and analgesic indices. There was sufficient evidence to accept the hypothesis that there is a difference in the motor effects of tizanidine and baclofen, with tizanidine having less effect than baclofen at comparable mid dosage levels, 1.5 mg/kg tizanidine and 5.0 mg/kg baclofen. However, the difference did not apply to low or high dose comparisons. These results suggest that the common, but inconsistently, reported side effect of muscle weakness from patients treated with antispasticity medications could be reduced with tizanidine relative to baclofen at selected moderate dosages.

## 5.2 Time Course of Drug Effects

Drug activity was monitored for two hours post-injection, to allow responses to return to baseline values. A characteristic dose-response pattern was observed in all analgesic and motor data; however, in the low dose comparison of motor data, there was no apparent time effect ($p=0.0818$), while it did appear with analgesia data ($p=0.0334$). This may be explained by an extremely short acting motor effect of these drugs at these doses (for 20 or 40 minutes, as observed from the dose-response curves) but a longer acting analgesic effect (for 20 to 80 minutes, as observed from the

dose-response curves.) This insignificance was not related to the sensitivity of the model, as observed by significant time effects in motor responses at mid and high doses ($p<0.0001$).

5.3 Drug Comparisons

In previous studies, the selective antinociceptive effects of tizanidine relative to baclofen were demonstrated by selective depression in response to noxious (heat) but not innocuous (air) stimulation, and the specificity of tizanidine for group II rather than group I afferents (30, 31, 152). In the present study, both drugs attenuated the characteristic tail flick response to noxious stimulation, as demonstrated by elevated tail flick latencies and their corresponding analgesic indices: 29.9 seconds with mid dose tizanidine and 20.4 seconds with mid dose baclofen. While the extent of spinal and supraspinal drug effects, or pre- and post-synaptic effects, and hence afferent specificity cannot be identified in the present study, the results do not contradict the hypothesis that tizanidine may induce nociception without depressing myorelaxation to the same extent as baclofen.

Previous studies demonstrate selective motor effects of tizanidine relative to baclofen. The non-selective effect of baclofen, which abolished both components of the dorsal root-elicited ventral root reflexes (DR-VRP) of roots L4 and L5, was reversed by a GABAb antagonist, whereas the selective effect of tizanidine, which abolished only part of the DR-VRP, the RS-2-amino-5-phosponopentanoate (AP5) component, was alpha2-adrenergic selective. (150) In the present study, alterations in motor function associated with tizanidine or baclofen are described in terms of impaired motor performance. Since neither compound weakens muscle directly, the decreased performance is an indirect indication of depressed neuromuscular transmission.

Hence, in this study, both drugs impaired motor performance as demonstrated by a reduction in swim speed and their corresponding performance indices: -0.12 m/s with mid dose tizanidine and -0.46 m/s with mid dose baclofen. These results support earlier evidence that tizanidine and baclofen differ with respect to their effects on muscle strength. (169)

## 5.4 Dose Comparisons

The concentration of available drug in the blood varies with dose, where it is expected that at low doses, receptors are undersaturated, at mid doses receptors are saturated and at higher doses, receptors are supersaturated, and thus receptor activation is less selective and specific. Thus, mid doses are expected to have the greatest separation between analgesic action and motor effects.

### 5.4.1 Models of Comparability

a. Antinociceptive Index Score:

A method of quantifying analgesic responses was described by Nance and Sawynok by an antinociceptive index score. It was based on the following equation:

Antinociceptive Index = $\Sigma$ [(Trial Latency - Mean Baseline Latency)]

This represents an approximate area under the curve. (104)

b. Analgetic Index Score:

Another analgesic index was described by Wilson and Yaksh based on the following equation:

Antinociceptive Index = [(Trial Latency - Control Latency) / (Cut off time - Control Latency) ] * 100

Thus, a value is derived from the product of percent analgesia and the duration of analgesia. Consequently, the analgesic index is the area under the time-effect curve of the drug. (176)

In the second formula, antinociceptive drug effects are measured relative to an external control, such as a saline treated group. However, in this study, analgesic indices were measured by the first formula, which uses an internal control. Using the repeated measures design, all animals received all doses and each animal was also its own control, where antinociceptive drug effects were measured relative to baseline or pre-drug injection tail flick latencies. Comparable analgesia was based on these calculated antinociceptive indices as well as statistically insignificant dose response curves, due to the ability of the tail flick test to show dose response with i.p. tizanidine and baclofen.

Comparability was based on analgesia response to the tail-flick test at three dosage levels: low, mid and high. Comparable low dosages (0.5 mg/kg tizanidine and 2 mg/kg baclofen) were defined as the analgesic threshold response, comparable mid doses (1.5 mg/kg tizanidine and 5 mg/kg baclofen) were defined as a producing moderate analgesia and comparable high doses (3 mg/kg tizanidine and 6 mg/kg baclofen) were defined as producing maximum analgesia, without harmful effects to the animal.

It may be noted that while analgesic indices were consistent with dose response results, there was a discrepancy in motor performance indices at mid dose. Specifically, mid dose baclofen produced a more negative performance indice than either high doses of baclofen or tizanidine. This suggests that the analgesic index method to quantify sensory function may not be applicable to a performance index to quantify motor function.

5.4.2 Receptor Theories for Tizanidine

Antagonist experiments have revealed that the antinociceptive effect of tizanidine was antagonized by yohimbine, an α2-adrenergic antagonist, but not by prazosin, an α1-adrenergic antagonist, dopamine, serotonin or GABA receptor antagonists. (103) More recently, tizanidine has demonstrated interaction with both central α2-adrenergic receptors and I-receptors to increase presynaptic inhibition of motor neurons. (158) Animal studies by Coward et al in 1994 demonstrated tizanidine acts to inhibit descending influences from the locus ceruleus on polysynaptic pathways and to decrease excitation of interneurons in spinal cord circuits without directly acting on alpha motor neurons, or without acting on monosynaptic spinal reflexes (21). Furthermore, substance P has demonstrated a role in the spinal modulation of analgesia. (115) Thus, the sites of receptor interaction for tizanidine have been associated with α2-adrenergic and imidazoline receptors, as well an interaction with substance P. (99, 104)

In the present study, tizanidine is administered intraperitoneally, with activation of spinal and supraspinal sites. According to the proposed model of receptor interaction:

Tizanidine → I1-receptors + SP + α2-AR (+ α1-AR)

The spinal mechanism of action for tizanidine is initiated by activation of noradrenergic α2-adrenoceptors and I1-receptors, leading to reduced release of excitatory amino acids from interneurons that results in reduced polysynaptic reflexes producing antinociception and muscle relaxation. I1-receptors and α2-AR are colocalized in many tissues and cell lines and tizanidine has demonstrated selectivity for I1-binding sites over α2-AR in human platelets. (123) It has also been suggested that in spinal rats, a weak facilitatory effect of tizanidine on MSR or PSR

reflexes may be attributed to weak agonistic actions on α1-AR. Further, it has been suggested that α1-antagonists reduce spinal reflexes when the descending noradrenergic pathways are intact, but this effect is blocked by spinalization. (114) Similary, the supraspinal mechanism of tizanidine is initiated by activation of α2-AR, in this case leading to reduced facilitation of cerulospinal pathways to produce antinoceptive and myorelaxant effects. (21) Hence, the suggested mechanism of action of tizanidine, or its spinal and supraspinal effects, has been described to modulate motor function via interneurons by the: i) inhibition of descending circuits (from locus ceruleus) on polysynaptic pathways, and ii) decreased excitation of interneurons in spinal cord circuits. (21)

5.4.3 Receptor Theories for Baclofen

The non-selective depression of both mono- and polysynaptic reflexes with baclofen has been demonstrated in various studies, through either intrathecal, intravenous or intraperitoneal administration. (139, 152) The stereospecific and stereoselective effects, for L - (-) - baclofen have also been shown in various studies, where analgesic potency is in the order of L-baclofen > Racemate (L- and D-baclofen) >>> D-baclofen. (82, 144). Baclofen is antagonized by phaclofen at postsynaptic GABAb receptor sites (154) and it may act on the spinal (segmental) pathway either directly (at GABAb receptors) or indirectly by noradrenergic pathways. Since antispasticity effects of baclofen are evident after SCI, there must be a significant site of spinal action.

In the present study, baclofen is administered intraperitoneally with activation of both spinal and supraspinal sites. According to the proposed model of receptor interaction:

Baclofen → GABAb + SP + α2-AR

The role of descending NA pathways on the effects of baclofen in antinociception was studied by Sawynok and Dickson in 1985. Experiments involved co-administration of neurotoxins (6-OHDA pre-treatment, i.th.) or amine antagonists (phentolamine post-treatment, i.th.) with i.p. baclofen. Results suggested that "descending NA pathways are important mediators of the antinociceptive effect of baclofen following intraperitoneal (i.p.) administration" (144). This conclusion was based on two main observations: 1) i.th. 6-OHDA reduced the antinociceptive effect of i.p. baclofen and 2) i.th. phentolamine reversed i.p. baclofen's antinociceptive effect. Further analysis demonstrated that baclofen "interacts with central NA pathways by a number of mechanisms including inhibition of LC firing and inhibition of NA release from nerve terminals." (145) In addition, high doses of baclofen were reported to activate central α2-adrenergic receptors in *in* vitro studies. (46) These studies suggested baclofen can produce analgesia by mechanisms unrelated to central GABAb receptors, which was attributed to activation of noradrenergic (α2-AR) neurons. In summary, the spinal sites of action of baclofen at interneurons has been described by reduced Ca2+ influx and reduced neurotransmitter release from 1a afferent terminals with decreased 1a afferent firing while the supraspinal site of baclofen has implicated the noradrenergic system.

5.4.4 Multiple mechanisms of dose selectivity

There are several modulatory mechanisms, with suggested 'functionally multiplicative interaction,' which may explain dose effects, including spinal vs. supraspinal sites of action, presynaptic vs. postsynaptic sites of action, stereoselective and stereospecific drug interactions, and

various receptor interactions at various doses. "The question thus arises as to whether the low dose effect (antinociception) is mediated by an action which is pharmacologically distinct from the high dose effect" (muscle relaxation)? That is, does dose selectivity occur by activation of different types of receptors, or by varying degrees of one effect, on the same physiological and pharmacological system? (176) Furthermore, does dose selectivity involve spinal or supraspinal sites, or a synergistic effect, based on dose and the differential sensitivity of the two regions? In addition, do doses involve specific drug actions to mediate behavioural (motor and sensory) effects, where tizanidine and baclofen have previously demonstrated stereoselective responses.

In the present study, a primary consideration in determining dose effects was the actual dose. Based on previous findings, the principal site of action of baclofen is at GABAb receptors distributed widely throughout the CNS, particularly in laminae I-III of the dorsal horn, while the principal site of action of tizanidine is at I1-receptors distributed in a network of neurons throughout the CNS, particularly in motor neurons. At comparable analgesic mid doses, it is suggested that these principal sites would be optimally activated, contributing to the disparity in motor effects between the two drugs. Another consideration for determining the distribution of drug within the body is the route of administration. It is relevant to the onset, intensity and duration of drug action and may be either enteral, involving the GI tract, or parenteral, bypassing the GI tract. Common routes of administration include oral, intraperitoneal and intrathecal routes. (39, 50, 124, 126)

The oral route has the advantages of ease, convenience and non-invasiveness. By enteral administration, many factors determine the availability of drug within the body, including: drug permeability through the gut wall, solubility in GI fluids and other GI parameters, such as gastric emptying or food effects. Potential disadvantages of this route include: gastric irritation, slow or

unpredictable drug absorption and difficulties in quantifying plasma drug concentrations. As a result, oral dosing in the rat may be facilitated by gastric intubation (gavage) or a solution or suspension, or dilution of a drug in a food mixture. An additional consideration in the drug-diet mixture is that it requires uniform distribution of the drug throughout the mixture and establishing a feeding pattern for the drug-diet mixture and may necessitate enhancing palatability to ensure drug delivery.

In contrast, the i.p. route bypasses the GI tract while maintaining the advantages of ease of application and convenience. Disadvantages include: a relatively slow onset of drug action, the potential for drug overdose resulting from a bolus injection in sensitive animals, drug absorption from the peritoneal cavity, hepatic inactivation of the drug before it reaches its site of action or pronounced volume effects in small animals unrelated to any pharmacologic effects of the drug. Another parenteral route of administration is the intrathecal route. Advantages include a rapid onset of drug action, a direct effect on the spinal cord and suitability for drugs that cross the blood-brain barrier slowly. Potential disadvantages of this route include invasiveness of the catheterization procedure or for direct injection to the subarachnoid space.

In this study, drugs were administered intraperitoneally, controlling for overdose and volume effects, and produced activation of both spinal and supraspinal sites.

Most drug-receptor interactions display numerous levels of drug action. "Interaction of a drug with its molecular target then has effects on the cell, subsequently on a tissue, eventually on an organ system and ultimately on the intact organism." (14) Hence, to identify the site of drug activity attributing to an altered behavioural pattern, experimentation at various levels of organization is required. In this study, only one level is considered, the whole animal or behavioural response. A

further investigation to localize the discrete site of drug action would consider isolated organ systems, tissue systems or cellular preparations. Considering the observed behavioural effects in this study in addition to the literature on these additional levels of investigation, a number of suggestions were made to explain the results obtained in the in vivo animal system. Both tizanidine and baclofen demonstrated the characteristic relationship between dose and pharmacologic response in sensory and motor tests. Limitations in analyzing drug-receptor interactions based on dose response curves include the importance of plasma concentration, rather than the dose administered, to the pharmacologic effect. Further, the complex non-linear relationship between a physiological response and receptor occupancy, and the lack of information on the actual concentration of drug at receptors requires careful consideration of dose response curves in extrapolating the affinity of a drug for its receptors. As shown by the graded response curves, in which a low dose produced a small effect with larger doses producing greater effects, it follows that "the response of the drug is directly related to the number of receptors with which the drug effectively interacts." (22) Experimental literature reveals specific binding of tizanidine to I1-R and α2-AR in binding assays. It is suggested that at low doses, tizanidine interacts primarily with the higher affinity I1-R site; however, at increasing doses, tizanidine would produce a greater amount of non-specific binding, including activation of α1-AR similar to the results in spinal rats in which tizanidine had a weak facilitatory effect on reflexes. Antagonist studies with phaclofen show selectivity of baclofen for the GABAb site, which explains results at low and moderate doses, while in vitro studies suggest non-specific binding may be attributed to activation of central α2-AR at high baclofen doses. The observations in this study lead to further questions on multiple functional systems, such as whether the principal sites of action were saturated at the mid analgesic doses tested.

Additional considerations not explored in this study are the development of tolerance with repeated receptor activation associated with chronic application, the synergistic effects of tizanidine and baclofen or analgesic differences produced at comparable motor dosages.

5.5 Model of sensorimotor integration

Sensory and motor responses mutually modulate each other through motor control of sensory input and sensory feedback of the effects of motor activity, allowing refinement of both sensitivity and movement. (34) "Swimming requires the integration of multisensory inputs, a rapid processing of information, and a subtle balance control." (54, 121) It is a dynamic activity that involves both ankle extensors and flexors in the coordinated cycling of legs in this motor task. (48, 121) These characteristics made it a suitable model to test the functional integration of strength, endurance and coordination. (54, 121) In this study, complex reflexes are studied from behavioural responses. The swim task involved a trained motor task, in which forelimbs remained in an outstretched position for support, in contrast to other studies in which lesioned guinea pigs or guinea pigs receiving high doses of baclofen swam using all four limbs. (121) Potential factors limiting muscle performance include: oxidative metabolism, or maximal oxygen uptake, muscle wasting and impaired muscle activation in the central nervous system. (83) However, it is not expected that fatigue or deterioration of the muscle had a significant role in accomplishing the motor task. Another potential confounding factor was the effect of stress-induced analgesia associated with forced swimming as described previously. (163) It has been noted that antinociception induced by an interaction of stress and GABA agonists is greater than the analgesic response of the GABA agonist alone. The evidence is not in complete agreement with respect to the involvement of GABAergic systems in this

response, but it is agreed that swim stress does not affect the GABAb receptor mechanism. (163, 180) Since the present study did not involve the GABAa system, and did involve a trained motor task, stress was not expected to be a relevant factor. A further confounding factor was the ability of certain doses to produce a motor-sensory confound, or the impaired ability of a subject to produce a motor response associated with a nociceptive stimulus at certain doses. It is expected that establishing a cut off time in the sensory evaluation and selecting doses below this level, limited this confounding factor. A third confounding factor may have been the effect of temperature. While precautions were taken to ensure a constant environmental temperature in this study, by using warm water in the swim tank at the normal body temperature, there is evidence that tail flick temperature, as measured by increasing and decreasing tail temperature, and core body temperature, as indicated by colonic temperature, had a negligible effect on latencies in the tail flick test. (86).

## 5.6 Scientific contribution

### 5.6.1 Significance of results

Based on findings of this study, we can accept the hypothesis that tizanidine and baclofen produce a dose-dependent difference in motor performance but only at moderate doses.

### 5.6.2 Final Comment

In summary, the physiology and pharmacology of tizanidine and baclofen were considered for two integrated behaviours: analgesia and motor performance. The integrated sensorimotor model used in this study was an effective way to compare these two drugs with distinct pharmacological profiles and mechanisms. The findings from this study confirm previous evidence of the analgesic

and myorelaxant properties of baclofen and tizanidine. A comparative difference in the effects of tizanidine and baclofen on motor performance at a moderate, selected, comparable analgesic dosage was demonstrated, while not at extreme low or high doses. This suggests the interaction of multiple mechanisms or different receptor involvement, in the motor response compared to the analgesic response.

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