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Interaction of Ethanol and Insulin on Motor Activity and Hypoglycemia in Rats

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INTERACTION OF ETHANOL AND INSULIN ON MOTOR ACTIVITY AND HYPOGLYCEMIA IN RATS

by

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ABSTRACT

INTERACTION OF ETHANOL AND INSULIN ON MOTOR ACTIVITY AND HYPOGLYCEMIA IN RATS.

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The present study investigated the effects of ethanol on insulin-induced hypoglycemia in rats. Measurements were made on the interaction of insulin at a dose of 2 units/kilogram in combination with four ethanol doses on motor activity and on blood glucose levels. Nine rats were administered eight treatment conditions (insulin or no insulin; combined with ethanol dosage of either: 0, 300, 600, or 1000 mg/kg). In a counterbalanced sequence of treatments, the rats were injected with insulin and placed into activity detectors for 20 minutes. At this time their blood glucose was measured, they were injected with ethanol, and placed back into the activity detectors for 60 minutes. Blood glucose was again measured after the 60 minutes had elapsed. Insulin caused a decrease in motor activity both prior to and after ethanol injections. Without insulin a dose of 1000 mg/kg of ethanol was required to significantly decrease activity. However, with insulin pre-treatment, significant decreases were seen at 300 mg/kg and higher doses of ethanol. Insulin decreased blood glucose before and after ethanol injections, but ethanol did not influence blood glucose levels, nor did it interact with the insulin-produced hypoglycemia.

This thesis is dedicated to my mother, for all her love and support throughout the years.

ACKNOWLEDGEMENTS

There are several people I would like to thank for the completion of this thesis. Without these individuals, I would not be where I am today.

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I would also like to thank my mother for her constant demonstrations of love. Throughout my life, she has given me strength and courage to reach my goals. I am very appreciative of all the things that she has done for me and I will always be thankful for her.

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CHAPTER 1

INTRODUCTION

Over the past 15 or 20 years, it has been recognized that ethanol administration may be associated with the development of hypoglycemia (Begleiter, 1978). Although there are many causes of hypoglycemia (see Appendix), it is estimated that 70 percent of all reactive hypoglycemias are induced by ethanol (Cohen, 1976). Therefore, the focus in this study will be on the relationship between hypoglycemia and ethanol.

Hypoglycemia is a condition that results when the insulin level is too high and there is an insufficient amount of glycogen available for the conversion into glucose. Symptoms include sweating, faintness, hunger, tachycardia, muscle weakness, headache, and confusion (Cohen, 1976). Some of the symptoms of hypoglycemia resemble that of an acute intoxicated condition (e.g., stupor and coma) or withdrawal from alcohol (e.g., delirium tremens). Even a sober alcoholic may have a low blood sugar level producing hypoglycemic symptoms (e.g., weakness, headache, a rapid pulse, sweating, and hunger) (Cohen, 1976).

Since hypoglycemia, like ethanol intoxication, can influence behavior and have adverse effects on glucose regulation, it is likely that the two occurring together may have even more pronounced effects. These two abnormal

states may interact by means of ethanol's complex and poorly understood actions on glucose metabolism. Therefore, ethanol's effects on glucose metabolism will be discussed.

Ethanol Effects

A primary reason for long-term ethanol abuse is that the neural effects of ethanol are reinforcing or rewarding. There is evidence that the monoamine neurotransmitters (i.e., dopamine and serotonin), GABA, and glutamate contribute to the reinforcing properties of ethanol. After ethanol consumption, dopamine levels increase in the nucleus accumbens and other reward centers. The D_2 type of dopamine receptor is thought to be involved in the reinforcing effects of ethanol (Crabbe, 1989).

Acute exposure to ethanol produces several physiological effects. Although it is unclear whether ethanol acts on lipids or proteins in the neuronal cell membrane, it is apparent that ethanol does alter the function of neuron-specific proteins. For example, there is evidence suggesting that the activity of the chloride ion channel linked to the A-type receptor of the GABA neurotransmitter (the major inhibitory neurotransmitter) enlarges during exposure to large amounts of ethanol. GABA's inhibitory effect makes the neuron less likely to fire causing the depression of the central nervous system (Nakahiro, Arakawa, & Narahashi, 1991). This action may be

the reason for the anxiolytic, sedative, and motor impairment effects of ethanol (Givens & Breese, 1990).

Glutamate, the major excitatory transmitter in the central nervous system, is also affected by acute exposure to ethanol. Glutamate activates the N-methal-D-aspartate acid (NMDA) receptors and the amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Mayer & Westbrook, 1987). Evidence suggests that by blocking the NMDA receptors ethanol has an depressant effect. This impairment of neural activity contributes to intoxication and is also involved in the cognitive impairment and amnesia that is evident at moderate to high levels of ethanol consumption. It was reported that acute ethanol exposure decreases the learning of new information and interferes with long-term potentiation (Crabbe, 1989).

It remains unclear as to the mechanism of ethanol's action on dopaminergic transmission. Studies suggest that the most likely mechanism is increased activity of dopaminergic neurons in the ventral tegmental area (VTA) of the brain (Gessa, Muntoni, Collu, & Vargiu, 1985). When the activity of neurons in the VTA are increased, dopamine is released from the presynaptic terminals of these neurons in the nucleus accumbens. It seems that ethanol's effects occur in the VTA because altered firing rates have been observed primarily in this region of the brain (Crabbe, 1989). There is evidence to suggest that acute ethanol

exposure results in long-term alterations in neuronal functioning and structure at the synapses. Animals who are exposed for days to ethanol have decreased $GABA_a$ receptor function in neuronal tissue (Morrow, Suzdak, Karanian, & Paul, 1988). It has been proposed that the altered function could result from a change in the expression (e.g., the DNA- and RNA-driven production) of the sub-unit components of the receptor. Studies have also demonstrated that changes in the $GABA_a$ receptor subunit expression and increases in NMDA receptors in certain brain regions (e.g., hippocampus) may contribute to withdrawal symptoms (Cermak, 1990).

Continual exposure to ethanol can result in tolerance for and physical dependence on the drug. Tolerance for ethanol results from changes in ethanol's effects on the brain as well as the body's increased ability to reduce ethanol levels via metabolism (Cermak, 1990).

The adverse effects of ethanol are many and include negative effects on the absorption of many vitamins and minerals. Absorption of vitamin B_1 (thiamine), vitamins B_2 (riboflavin), B_3 (nicotinamide), B_6 (pyridoxine), folic acid, magnesium, zinc, and calcium are most affected by excessive quantities of ethanol. Ethanol also interferes with the metabolism of the essential cis-linoleic acid and essential fatty acids (Lieber, 1992).

Long-term consumption of ethanol can damage the liver, nervous system, brain, heart, esophagus, stomach, and the

pancreas. It can also increase the risk of certain types of cancer (e.g., cancer of the liver, esophagus, larynx, and mouth). Some of the metabolic disturbances that have been correlated with a high ethanol intake include an increased risk of gout, a worsening of diabetes, and raised blood levels of fat. Thus, there are many medical and nutritional complications of ethanol. One of the problems that has gained attention is the effects of ethanol on glucose metabolism (Lieber, 1992). Therefore, an understanding of how glucose is metabolized and its effects on the body will be discussed.

Glucose Metabolism, Regulation, and Availability

The most important substrate for energy metabolism in the brain is glucose. It is responsible for over 90% of oxidative metabolism. Research has demonstrated that the entry of glucose to some tissues (e.g., muscle, heart, fat cells, and lactating mammary glands) is facilitated catalytically by the presence of insulin. Further, there is a specific insulin-dependent carrier that forms a sugar-carrier complex involved in the transport across the cell barrier. This explains the competition for entry between glucose and other sugars when they are both present in the perfusate or extracellular medium (Razada, Phillips, & LeRoith, 1987).

The supply of glucose to the cells is considered to have a central role in eating behavior. When food is

consumed, the carbohydrates and proteins are broken down into glucose and amino acids that can be used as fuel. However, only glucose can be used as fuel in the brain because the central nervous system normally restricts amino acids from passing the blood-brain barrier (Galluscio, 1990).

Two nutrient reservoirs control the absorption and fasting phases of metabolism. The short-term reservoir is in the cells of the liver and the muscles and provides glycogen, which is a complex, insoluble carbohydrate. Cells located in the liver convert glucose into glycogen and store the glycogen. When glucose and insulin are in the blood, a portion of the glucose will be used for energy, and the rest is stored as glycogen for future use. After the digestive tract absorbs all the food, the blood glucose level begins to drop (Carlson, 1995). When cells in the brain detect that the blood glucose levels are declining, the sympathetic axons that innervate the pancreas increase their activity. This activity stops the secretion of insulin and signals the pancreas to begin secreting glucagon, which converts glycogen into glucose (Carlson, 1995).

The long-term reservoir consists of adipose tissue. When too much food is consumed, glycogen is converted into fat and stored as adipose tissue. When there is not enough food, the fat cells start converting triglycerides into fuels that the cells in the body use (free fatty acids),

with the exception of those in the central nervous system. The CNS energy supply is provided only by the glucose supplied by the liver (Carlson, 1995).

According to glucostatic theories of eating, it is the level of available glucose in the blood that determines hunger and satiety. Immediately after a meal, the available glucose is metabolized and the organism is satiated. When the blood sugar becomes too low, the organism becomes hungry again. However, even with a high level of glucose in their blood, diabetics continue to be hungry due to the fact that they also have low insulin level that is necessary for effective utilization of glucose in the tissues (Galluscio, 1990).

Under normal conditions, the level of glucose in the blood varies little. Even if the individual is fasting, the liver converts glycogen, fats, and proteins into glucose to keep blood glucose at an optimal level. However, changes in blood levels of insulin and glucagon can affect the availability of glucose to the cells (Galluscio, 1990).

Insulin is important in promoting glucose uptake in muscles, adipose tissue, and the liver. It also accelerates glycogen synthesis in muscle and adipose tissue. Insulin is the glucose regulatory hormone; it facilitates the entry of glucose into cells and suppresses glucose production, thus lowering the plasma glucose level (Cryer, 1981).

The effect of insulin is that glucose will be used for current energy needs or stored as fat or glycogen. Glucagon has the opposite effect. By stimulating the liver to transform stored glycogen to glucose, blood glucose levels are raised. Thus, insulin and glucagon provide a feedback system to control the intake and use of nutrients. After an individual eats, insulin level rises, glucose enters into the cells, and the person no longer feels hunger. As time goes on, blood glucose levels fall, the pancreas releases more glucagon and less insulin, and the individual starts to feel hunger again (Galluscio, 1990).

Under normal conditions as plasma glucose rises, insulin secretion increases and plasma glucose then declines; as plasma glucose falls, insulin secretion decreases and then plasma glucose rises. Although insulin is considered the dominant glucoregulatory factor, there is evidence to suggest that glucoregulation is accomplished also by the glucose-raising actions of various glucose counter-regulatory factors (Cryer, 1985). Glucagon and epinephrine are considered the best candidates as fast acting counterregulatory factors, while growth hormone and cortisol are considered to be more slow acting factors (Cryer & Gerich, 1990).

The dominant controls of fuel metabolism during both the fed and fasted states are hormonal. During a fast, the increased rate of gluconeogenesis is a result of a decrease

in circulating insulin and an increase in glucagon (Lieber, 1992).

Hypoglycemia

Hypoglycemia is a condition that can result when there is too much insulin in the bloodstream, and especially if glycogen is not available to be converted into glucose, causing blood glucose levels to drop drastically. This homeostatic imbalance can lead to a disruption of cognition and memory. Other symptoms of hypoglycemia include sweating, muscular tremors, blurred vision, confused thinking, headache, fatigue, and numbness (Cryer & Gerich, 1990).

Certain physiological changes that can occur during hypoglycemia include a systolic blood pressure response, increases in glomerular filtration rate and cerebral blood flow, and a decrease in intraocular pressure. As the plasma volume decreases, viscosity is increased, platelet aggregation is activated, and erythrocyte 2.3-diphosphoglycerate levels decline. Reports of changes in EEG have been found in many patients to persist for at least one month after a hypoglycemic episode. There have also been reports of lower intelligence quotients and nonspecific impairments on tests of cognitive and neuropsychologic function in children with insulin-dependent diabetes mellitus (IDDM), who have suffered repeated incidents of hypoglycemia (Cryer & Gerich, 1990).

Finally, hypoglycemia can be fatal. During an attack of severe hypoglycemia, permanent damage can occur to the heart. Under normal circumstances, provided that there is adequate coronary circulation, the cardiac muscles contain glycogen that is available should the blood sugar fall too low. However, if the coronary vessels are narrowed, then the myocardium may be damaged (Razada, et. al., 1987).

The brain is also vulnerable to even very short durations of hypoglycemia. Hemiplegia or an extensor plantar response is likely to continue for some hours after the blood sugar returns to normal. In some cases, despite the restoration of the blood sugar, the individual may fall into a coma (Razada, et. al., 1987).

One of the most common contributing factors to hypoglycemia is the consumption of refined carbohydrates. These foods contain high levels of sugar and glucose which cause the blood sugar to increase rapidly resulting in an excessive production of insulin and a subsequent overcompensating reduction in blood glucose level. Chronic stress, food intolerance, too much or too little thyroid hormone, deficiencies of nutrients, drug effects, missed meals, excessive tea and coffee consumption, cigarette smoking, and alcohol use have all been correlated with hypoglycemia (Cryer & Gerich, 1990).

To promote glucose recovery from hypoglycemia, a rapid injection of glucagon must be administered. The resultant

burst of glucose production that restores euglycemia is for the most part due to glycogenolysis (liver glycogen breakdown to glucose). However, recovery from prolonged hypoglycemia at first involves glycogenolysis which subsequently becomes gluconeogenesis (the synthesis of glucose from amino acids in protein and glycerol in fat) (Cryer & Gerich, 1990).

As depicted here, the consequences of either ethanol intake or hypoglycemia can be very serious. Additionally, the effect that ethanol has on a hypoglycemic individual can result in even more serious consequences. Due to the high incidence of ethanol abuse, and the possibility of frequent cases of hypoglycemia, it is important to understand the effects that occur when combining the two.

Ethanol and Glucose Regulation

As noted previously, ethanol has effects on the liver, glycogen, pancreas, and adrenal functioning. There are many complex ways ethanol affects glucose regulation. One result of ethanol's adverse effects on glucose regulation is hypoglycemia.

Although ethanol-induced hypoglycemia was first observed around 1941, ethanol was not considered to be an important contributing factor in hypoglycemia until 1963. The multifactorial inhibition of gluconeogenesis, that provides the major source of blood glucose during starvation, appears to be the main mechanism in ethanol-

induced hypoglycemia. This occurs because in the metabolism of ethanol and acetaldehyde, hydrogen ions are produced. The hydrogen ions attach themselves to NAD (nicotinamide adenine dinucleotide). This forms $NADH_2$, which forces the chemical reactions toward lactate and away form the precursors of carbohydrate. Changes that occur in plasma insulin and glucagon concentrations do not play a major role in the pathogenesis of ethanol related hypoglycemia (Lieber, 1992).

Glucose intolerance is often observed in alcoholic cirrhotic individuals and in individuals who manifest chronic liver disease of other etiologies. This probably results from the damaged liver's inability to store glycogen. Glycogen is poorly stored in necrotic hepatocytes. (Lieber, 1992).

Ethanol-induced hypoglycemia does not appear to be mediated by insulin. Under fasting conditions, there is a reduction in the concentration of circulating insulin during ethanol administration. It was postulated that ethanol-induced hypoglycemia reduces insulin secretion through a feedback mechanism occurring between glucose concentration, the rate of insulin secretion, and plasma insulin levels (Lieber, 1992).

It was postulated that ethanol induces insulin resistance (Yki-Jarvinen, Koivisto, & Taskinen, 1988). The main product of ethanol oxidation in the liver is acetate.

In humans, 70-80% of the ethanol oxidized appears as free acetate in the hepatic vein. The oxidation of acetate in extrahepatic tissues has been shown to suppress the combustion of other substrates, including glucose, and this could, in turn, slow the glucose disappearance rate. This is known as glucose intolerance (Lieber, 1992).

Diminished insulin responses can also explain glucose intolerance; that is, the glucose-induced insulin secretion could be stopped by epinephrine released in response to ethanol. It has also been postulated that ethanol interferes with the integrity of the microtubular system of pancreatic B-cells (Lieber, 1992). The hyperinsulinemia caused by ethanol has been ascribed to a priming effect of ethanol on the B-cells (Friedenberg, Metz, & Mako, 1971). Ethanol action of the B-cells might be regulated by its metabolites, acetaldehyde and acetate, which exhibited a marked blocking effect on glucose and arginine induced insulin secretion (Lieber, 1992). It has also been demonstrated that ethanol causes the release of secretin which enhances glucose-induced insulin secretion (Straus, Urbach, & Yalow, 1975).

In summary, a high ethanol intake can produce the following carbohydrate shifts and produce hypoglycemia: decreased intake or loss of nutrients, an impaired absorption of carbohydrates and vitamins needed for carbohydrate metabolism, diversion of pyruvate to lactate

preventing gluconeogenesis, reduction of liver glycogen stores, and indirect stimulation of insulin secretion (Cohen, 1976).

Ethanol's Effects on Locomotion

Ethanol and hypoglycemia appear to have similar effects on motor activity. It has been reported that hypoglycemia decreases locomotor activity (Koontz & Duncan, 1994), but little information is available as to the effects of both ethanol and insulin on locomotor activity. Therefore, some of ethanol's effects on activity will be discussed.

Although moderate doses of ethanol (1.0 to 2.4g/kg) cause increases in locomotor activity in mice, it remains unclear as to the direction of locomotor activity in rats especially using small or moderate doses of ethanol. It has been demonstrated that low to moderate doses of ethanol produced increases in activity level in monkeys, rats, mice, and gerbils (Waller, Murphy, McBride, Lumeng, & Li, 1986). However, it has also been found that moderate doses produce a decrease in motor activity in rats (Duncan & Cook, 1981). Duncan and Baez (1981) argued that the effects of ethanol on motor activity appear to be situation-specific, bi-phasic, and occur differently in various species.

It remains inconclusive as to whether motor activity will decrease or increase when ethanol is administered. It will be of interest to determine the effects on motor activity when low, moderate, and high doses of ethanol are

administered to rats. It will also be of interest to find out the effects on activity when ethanol is combined with insulin.

Research Purpose

The present experiment involved the combined effects of ethanol and insulin-produced hypoglycemia on rats' spontaneous motor activity and on glucose regulation. Since hypoglycemia, like ethanol intoxication, can influence behavior and produce altered states of consciousness, it is likely that the two occurring together may have even more pronounced effects (Lieber, 1992). As noted previously, these two abnormal states may interact by means of ethanol's complex and poorly understood actions on glucose metabolism (Lieber, 1992). Therefore, in the present study, blood glucose levels were measured in an attempt to determine whether ethanol treatment causes hypoglycemia, and also whether it alters the hypoglycemia produced by insulin treatment.

It is evident that there are many theoretical and practical reasons to investigate the interaction of ethanol and insulin. Hypoglycemia occurs often in persons suffering from insulin-dependent diabetes mellitus (IDDM), and less often and to a lesser extent in some other persons (Cryer & Gerich, 1990). Since hypoglycemia and ethanol can both produce altered states of consciousness, it is likely that the combination of the two may have adverse effects.

Therefore, the present study investigated the effects of combining insulin and ethanol on rats' motor activity, and also the effect of ethanol on insulin-induced hypoglycemia.

Subjects were injected with insulin and or ethanol and placed into an activity box where their movement was recorded. The rats' blood glucose was measured before and after the ethanol injections.

Formal Hypotheses

1). The first hypothesis was that the results of Koontz and Duncan (1994), that hypoglycemia causes reduced motor activity in rats, would be replicated. As hypoglycemia develops, brain function becomes progressively more impaired. One manifestation is the reduction in normal levels of ongoing motor activity. As the animal becomes affected, little locomotion or grooming occurs. Gradually the animal becomes unresponsive to handling or other stimulation, eventually becoming comatose (Koontz & Duncan, 1994).

2). The second hypothesis was that insulin and a low dose of ethanol would increase spontaneous motor activity. Lieber (1992) found that ethanol causes catecholamine release and disinhibits behavior at low doses. Hypoglycemia also causes the release of catecholamines, therefore a combination of these treatments might cause an increase in motor activity.

3). It was predicted that since ethanol decreases activity at higher doses, hypoglycemia would potentiate this effect. Thus, at higher doses of ethanol a greater decrease in locomotor activity will be evident in hypoglycemic, compared to euglycemic rats.

4). The final prediction was that ethanol would produce hypoglycemia, and also that ethanol would increase the degree of hypoglycemia caused by the insulin treatment.

CHAPTER 2

METHOD

Subjects

Nine male rats of the Long-Evans strain, approximately 90 days old, served as subjects in this study. The rats were obtained from Charles River Laboratories (Wilmington, MA) and had a mean body weight of 350g. They were housed in individual cages with water freely available. The use of these animals in this experiment was approved by the Old Dominion University Institutional Review Board for utilization of animals in research.

Apparatus

Four Opto-Varimax Mini "A" animal activity meters (manufactured by Columbus Instruments) were used to measure ambulatory activity, total activity, and rearing. The testing enclosures were four translucent plastic storage containers, each measuring 58(l) x 42(w) x 23(h) cm. Each test-box was equipped with two linear arrays of 16 infra-red light sources paired with sensing photocells. Successive interruptions of adjacent infra-red light beams in the lower array by the rats registered as ambulatory activity counts. Accumulated activity counts were recorded at successive 15-min intervals by means of an IBM 386 PC, Columbus Instruments interface equipment, and appropriate software. Ambulatory counts were a measure of locomotion made by the rats, and total counts were a measure of any type of

movement made by the rats (e.g., walking, scratching, biting). Rearings were detected by activation of the upper array of photocells in the activity detector box. Rearings are any type of movements when the rats' head or body was elevated at least 2 cm above the floor.

Blood glucose was measured by means of a Lifescan "One-Touch" glucometer by Johnson and Johnson. This device is primarily intended for the monitoring of blood glucose levels in humans suffering from diabetes. This instrument is of sufficient accuracy and dependability to be used for research purposes (Messier & Kent, 1994). "First-Choice" Reagent strips for blood glucose testing, manufactured by Polymer Technology International, Inc. were also used to measure the blood glucose levels.

Drugs

Two units/kg of Lilly Regular Iletin insulin, diluted with water, were used to produce hypoglycemia. This was demonstrated to be an effective dose in producing hypoglycemia (Koontz & Duncan, 1994), reducing blood glucose to about 60-70% of pre-injection levels at 30-min post-injection. Koontz and Duncan (1994) reported that this dose produced a slight decrease in spontaneous motor activity in rats during the 30-min post-injection period.

Ethanol doses were 0, 300, 600, and 1000 mg/kg. It has been demonstrated that lower doses of this range cause little or no decrease in spontaneous motor activity, and the

larger doses produce marked decreases in activity in rats (Duncan & Baez, 1981; Duncan & Cook, 1981). The rationale for using each ethanol dosage was that this dose-range produces dose-related decrements in motor activity. The rationale for the insulin dosage was that this dose produces moderate hypoglycemia and slight to moderate activity decrease.

Experimental Design

A 2 (0, 2 units/kg insulin) x 4 (0, 300, 600, 1000 mg/kg ethanol) within-subject factorial design was employed. The independent variables for this study were the dose of insulin and the dose of ethanol. One of the dependent variables was the degree of hypoglycemia, measured by the rats' blood glucose level. The second dependent variable, motor activity, was measured by the number of activity counts in the activity detector box.

Each rat was subjected to all eight experimental conditions (combinations of insulin and ethanol doses). The sequence of treatments were counterbalanced in order to control for tolerance to the ethanol and adaptation to the injection and other experimental procedures.

Procedure

For three consecutive days, the rats were adapted to being handled and placed into the activity detectors for 30 minutes. Novelty has been demonstrated to cause stress, which in turn causes sympathetic nervous system activation.

Adrenalin causes glucose to be released from the muscles and the liver which could counteract hypoglycemia. (Antelman, Eichler, Black, & Kocan, 1980). Therefore, it was important that the rats be in a familiar environment. After this adaptation phase was completed, the sequence of experimental treatments commenced.

The rats were subjected to 8 treatment days with a day off after each treatment. Twenty-four hours prior to each experimental day, food was removed from each rat's home cage. At the beginning of each session, the rats were weighed to determine the amount of insulin and ethanol for injection. Depending upon the treatment condition, the rats were either injected with water, or with two units/kg of insulin via an intraperitoneal (IP) injection. The rats were immediately placed into the activity detector box for 20 minutes. Each rat's tail was then pricked with a stylette, to obtain a drop of blood for glucose measurement. Next, each rat was injected (IP) with the appropriate amount of ethanol or water and placed back into the activity detector box for another 60 minutes. Immediately after being taken out of the box, another blood drop was drawn from the tail for a second blood glucose measurement. The rats were given food immediately after this final procedure.

CHAPTER 3

ANALYSIS OF THE DATA

A 2 (0, 2 units/kg of insulin) x 3 (300, 600, 1000 mg/kg of ethanol) analysis of the variance (ANOVA) was performed to determine the effects of insulin on total ambulatory activity during the first 20 minutes, prior to ethanol injections. There was a significant main effect for insulin, $F(1, 71) = 37.86$, $p < .0003$, indicating that a decrease in activity level was produced by the insulin treatments.

The second independent variable (ethanol dose) was actually a "dummy variable" for this analysis, since no ethanol manipulation had been yet made. Thus, there was no main effect of this variable, nor was there an interaction effect. Mean ambulatory activity counts pooled across the four treatment days within each insulin condition, was 310 for the insulin conditions, and 535 for the no-insulin condition.

A 2 (0, 2 units/kg of insulin) x 4 (0, 300, 600, 1000 mg/kg of ethanol) within-subjects ANOVA was performed to determine how insulin and ethanol doses affected total and ambulatory motor activity. The results indicate that there was a significant main effect for insulin, $F(1, 71) = 20.67$, $p < .002$ and for ethanol, $F(3, 71) = 4.77$, $p < .01$. There was not a significant interaction between insulin and

ethanol, $F(3, 71) = 1.89$, $p < .16$. Figures 1 and 2 illustrate the entire 60-min period for the ambulatory andtotal activity measures. A similar pattern of results were seen in the two activity measurements. A decrease in activity was evident when insulin was administered, and when a high dose of ethanol without insulin was administered. At lower ethanol doses, the combination of insulin and ethanol also caused a decrease in activity. Figure 3 depicts the activity level across the four 15-min time periods at each of the three of the ethanol doses with insulin. Figure 4 illustrates the three ethanol doses at each time period with out insulin. There were no apparent interactions of either independent variable with time. Activity levels generally decreased as time progressed in all treatment conditions.

Although the ANOVA indicated no significant interaction effects, the hypotheses stated above identified additional comparisons of means of the various treatment conditions. Results from the Tukey's test, which was used to compare individual pairs of experimental conditions (critical difference=634.6), give support to the interpretation that without insulin, a dose of 1000 mg/kg of ethanol was required to significantly decrease activity. However, with insulin significant decreases were seen at 300 mg/kg of ethanol, as well as with 600 and 1000mg/kg ethanol.

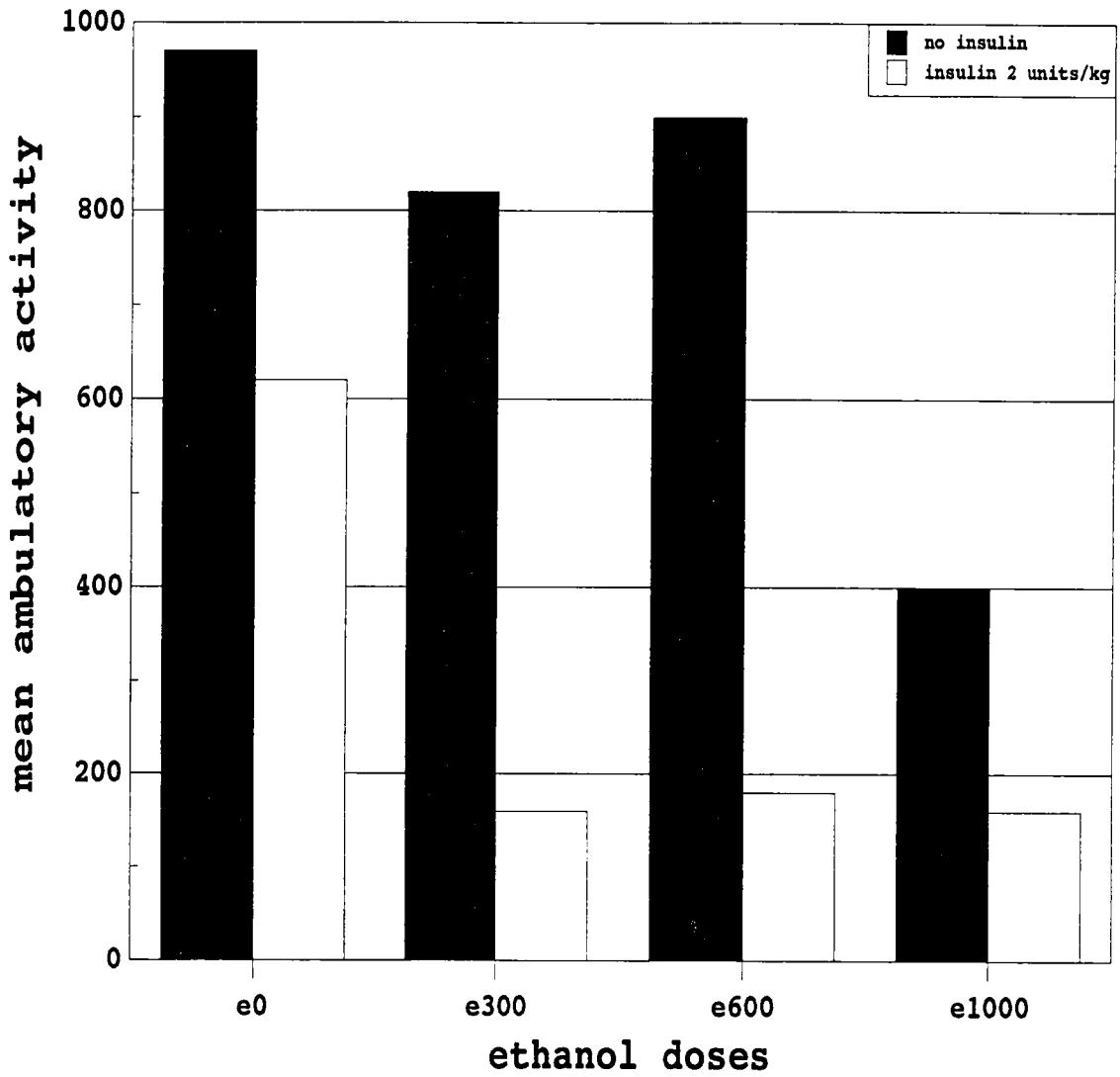

Figure 1. Ambulatory activity means for entire session.

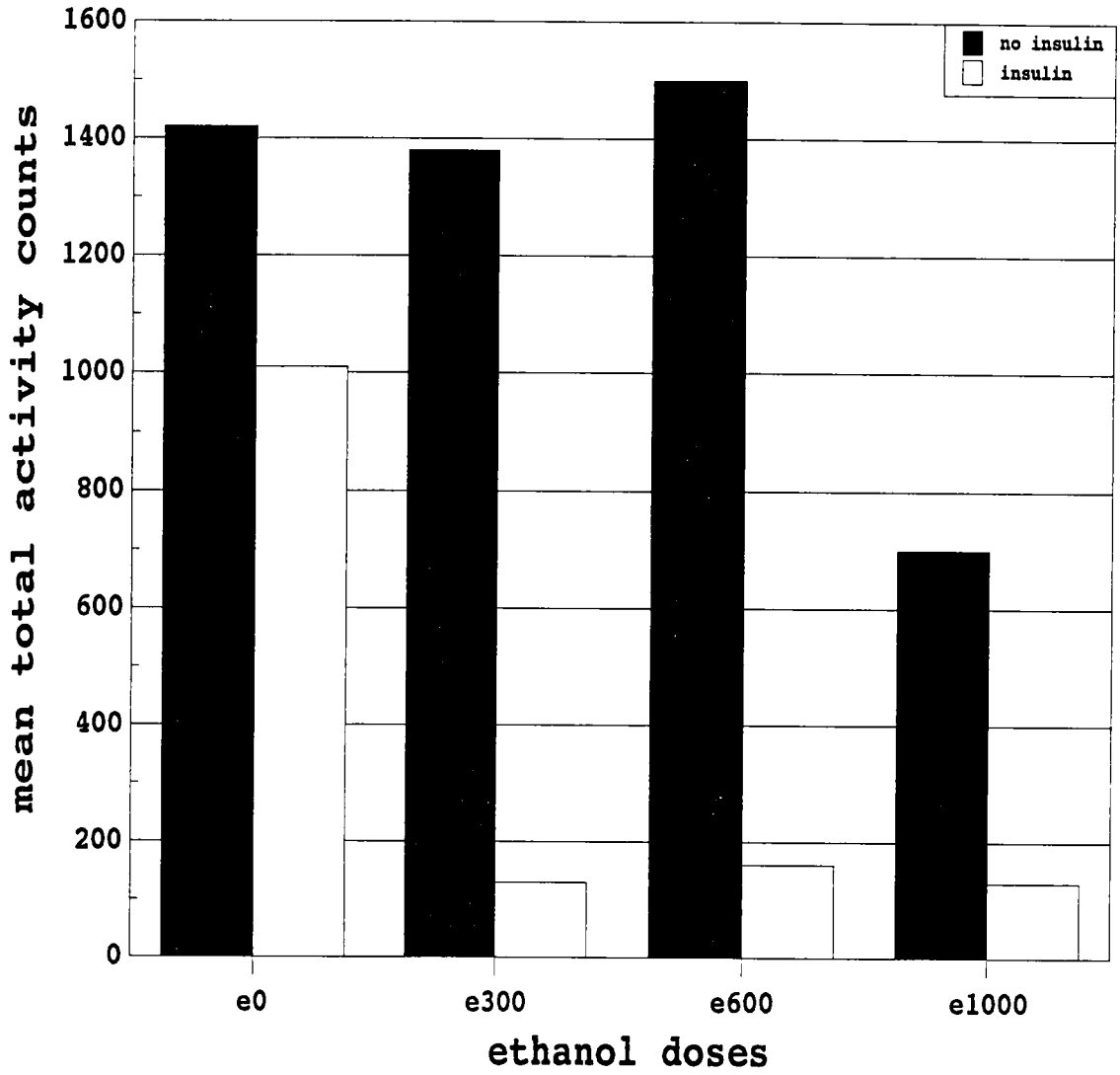

Figure 2. Total activity means for entire session.

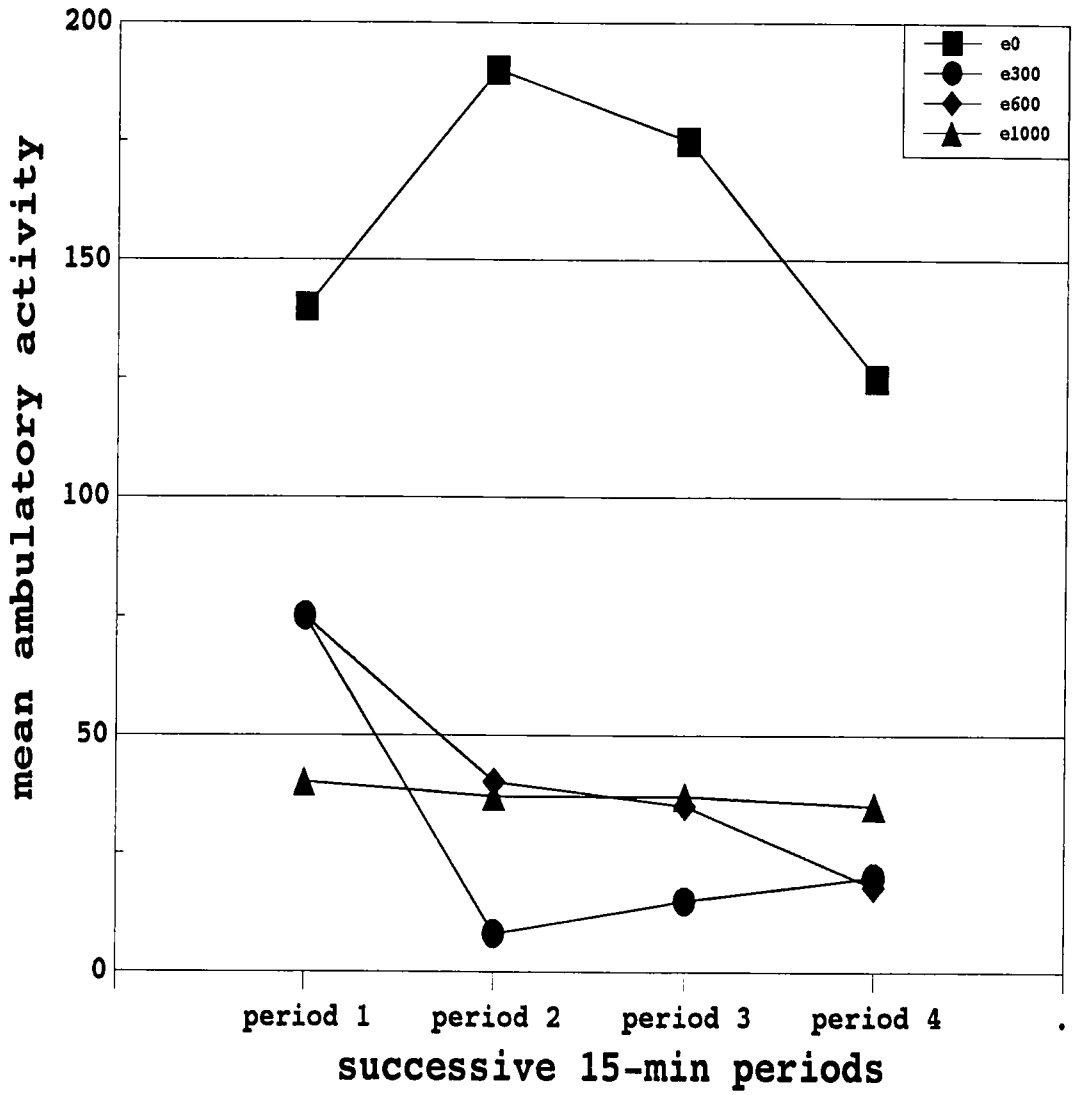

Figure 3. Mean activity level for each 15 minute time period, insulin combined with three ethanol doses.

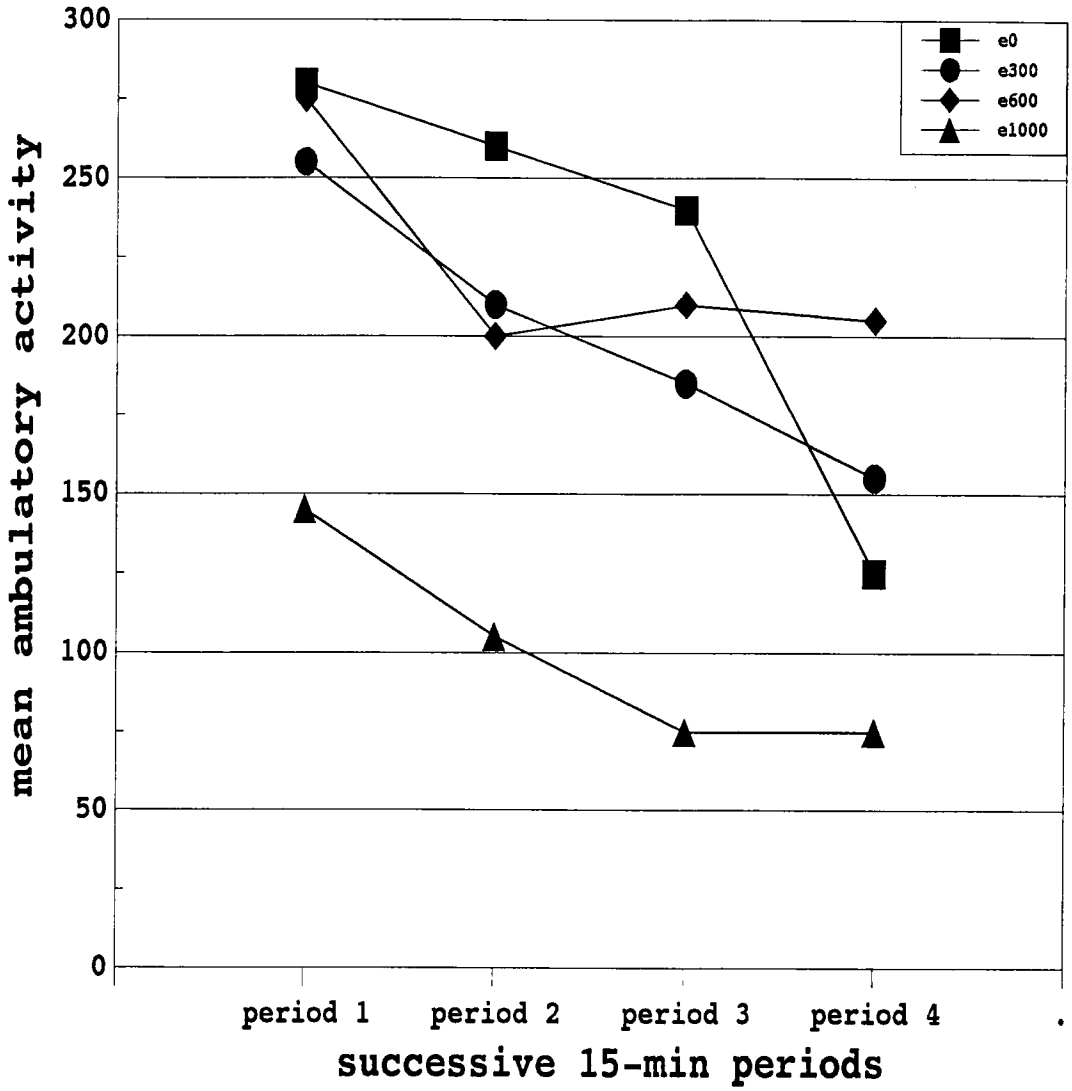

Figure 4. Mean activity level for each 15 minute time period, three ethanol doses, no-insulin.

These post-hoc tests, conducted on the total counts for the entire one-hour sessions, indicated an identical pattern of results for both the ambulatory, and the total-activity counts. The "rearing" dependent variable shows results which are very similar to those of the ambulatory, and the total activity measures. Therefore, these data are not reported here. Activity measures were examined for each treatment day, pooled across the various experimental conditions for that day, and no apparent sequence-related effects were found.

An ANOVA was performed to find the effects of insulin on blood glucose at 20 minutes after injection. The results indicate that there was a significant main effect of insulin at this time prior to any ethanol injections $F(1, 71) = 49.95$, $p < .0001$. The means were 70.5 mg/deciliter for the no insulin condition and 47.25 mg/deciliter for the insulin condition. The standard error of measurements were ± 17 and ± 11.2 respectively.

A 2 x 4 ANOVA was performed on blood glucose measurements taken 60-min after ethanol injections (80 min after insulin injection). There was a significant main effect for insulin, $F(1, 71) = 250.88$, $p < .0001$, but not for ethanol, $F(3, 71) = .62$, $p < .61$. An interaction was not found between insulin and ethanol, $F(3,71) = 1.03$, $p < .40$. Figure 5 describes the means for the blood glucose prior to any ethanol manipulations, 20 minutes after insulin

injection. Figure 6 illustrates the effects of insulin, ethanol, and their combination on blood glucose level, 80 minutes after insulin injection and 60 minutes after ethanol injection.

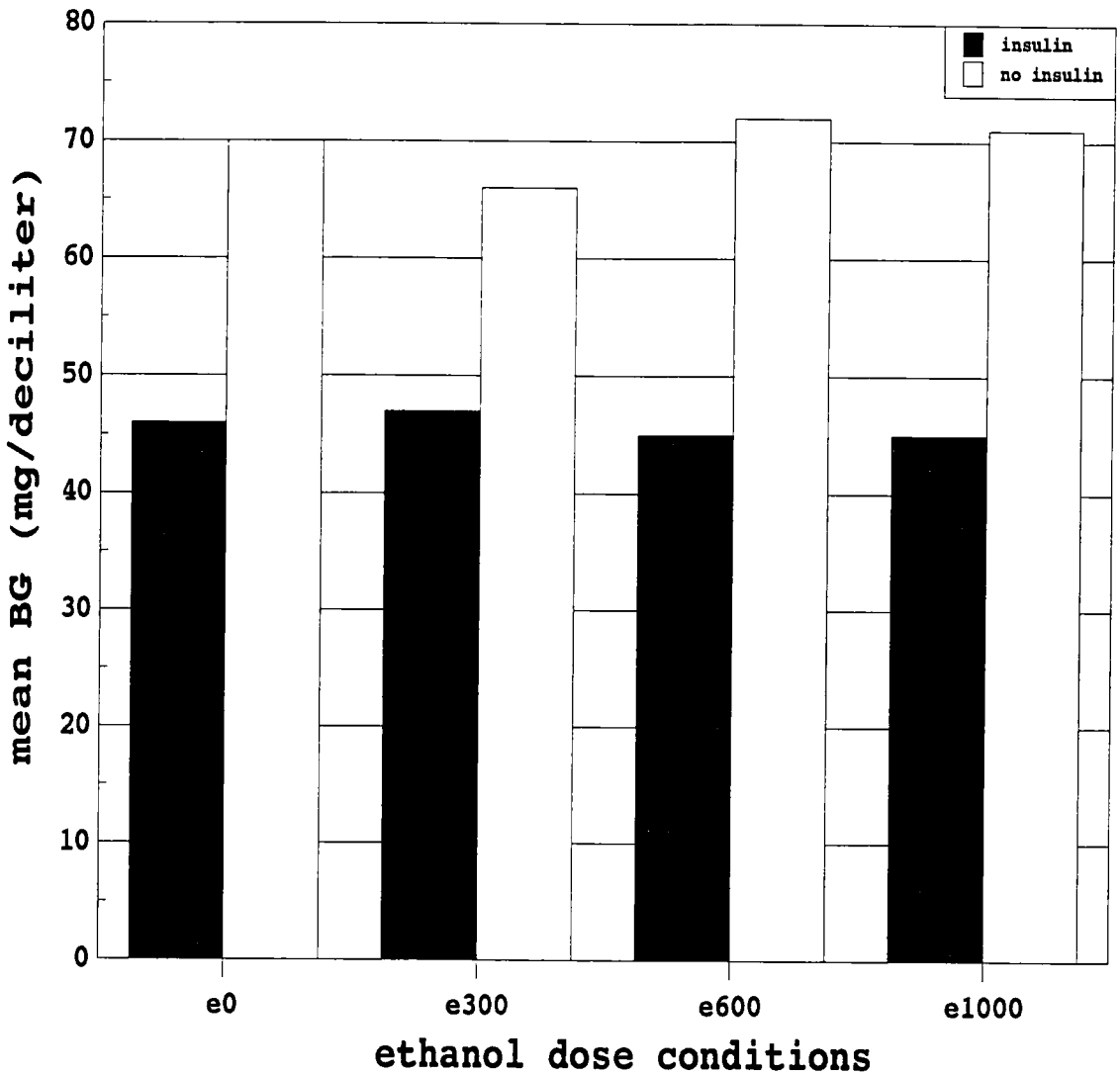

Figure 5. Means for the blood glucose prior to any ethanol manipulations, 20 minute post insulin injection.

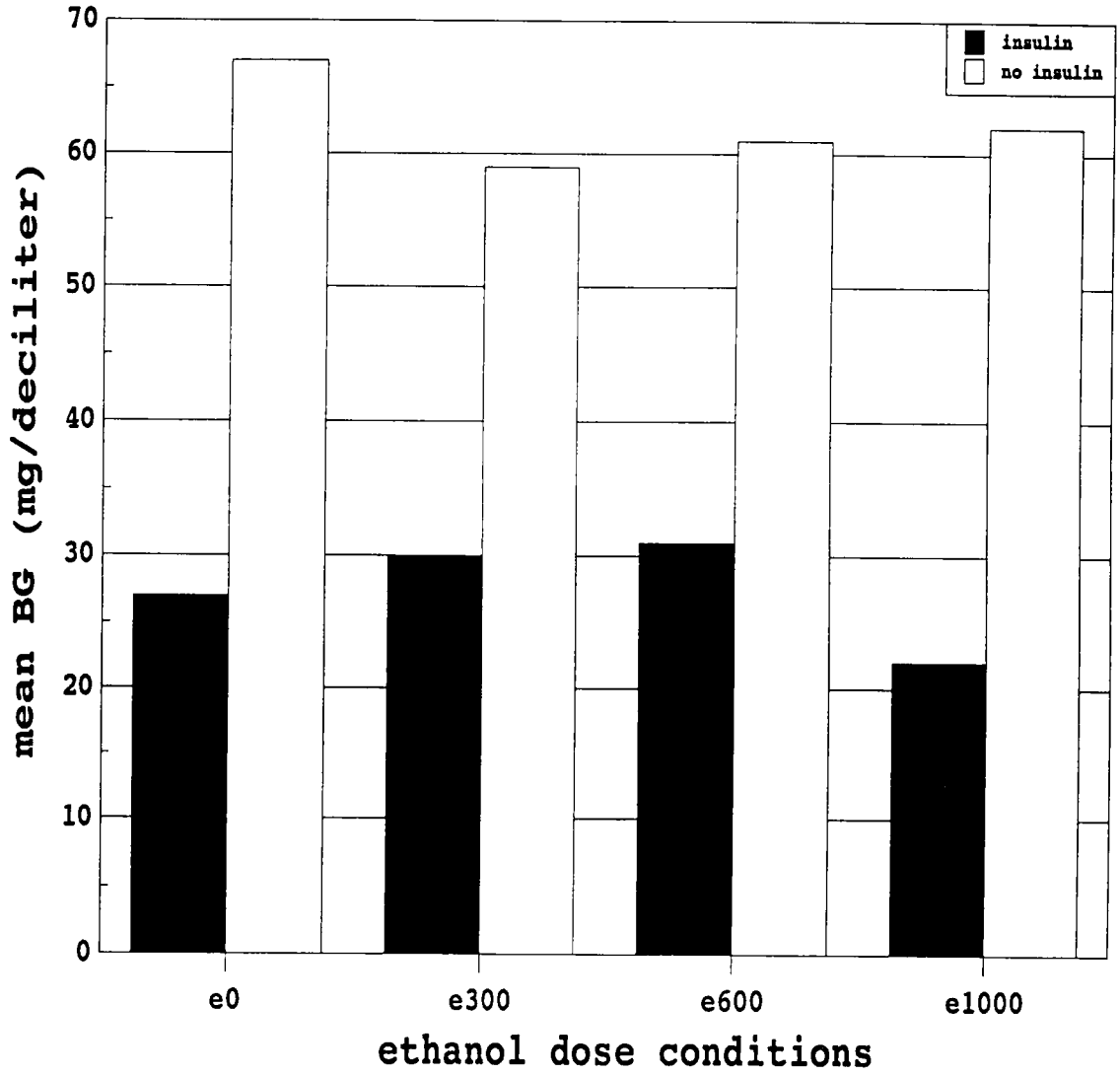

Figure 6. Effects of insulin, ethanol, and their combination on blood glucose level, 80 minute post insulin injection and 60 minute post ethanol injection.

CHAPTER 4

DISCUSSION

For many years, it has been recognized that the ingestion of ethanol may be associated with hypoglycemia. In fact, 70 percent of all reactive hypoglycemias result from the consumption of ethanol (Cohen, 1976).

The similar physiological symptoms (e.g., rapid pulse, numbness, weakness, lethargy, coma) that occur in persons suffering from hypoglycemia or chronic ethanol intoxication has led to the belief that these two conditions have similar effects on glucose regulation and metabolism (Begleiter, 1978). Although it was demonstrated that ethanol inhibits gluconeogenesis both in vivo and in vitro, other mechanisms also seem to be involved (e.g., enhancement by ethanol of the insulin response to carbohydrate and the impaired secretion of several counter-regulatory hormones; cortisol, growth hormone, catecholamines) (Begleiter, 1978).

Although earlier research suggests that ethanol and insulin have some similar physiological effects, more research is needed on the behavioral effects when these two drugs are combined. It has been reported that when insulin is administered by itself, motor activity decreases (Koontz & Duncan, 1994). However, the direction of activity change when ethanol is administered depends on several other factors (Duncan & Baez, 1981). There is no information in the research literature pertaining to the activity level

when these two drugs are combined, using different ethanol doses. Therefore, the goals in this study were to obtain information on the locomotor activity level when these two drugs are combined and to ascertain the physiological effects (e.g., blood glucose levels) from the combination.

The initial findings of this study replicated results from earlier research (Duncan & Koontz, 1994). Therefore, the first hypothesis was confirmed. Insulin, in the absence of ethanol significantly decreased activity and blood glucose levels within the first 20 minutes. Furthermore, blood glucose and motor activity decreased further during the subsequent 60 minutes, even when ethanol was not combined with insulin. These results suggest that as hypoglycemia progresses, brain function becomes more impaired. As a direct result of this impairment, ongoing motor activity decreased below normal levels. As time progressed, the animals gradually became less responsive to handling or other stimulation.

A dose-related activity decrease was evident when ethanol was administered without insulin. This response difference was not apparent at 300 or 600, but did occur at 1000mg/kg of ethanol. These findings are consistent with previous research (Duncan & Cook, 1984).

The study was also motivated by the expectation that the combination of ethanol and insulin would have differential effects on motor activity level as a function

of ethanol dose. Specifically, it was expected that at a low dose of ethanol, spontaneous motor activity would increase in the hypoglycemic animals. The reasoning behind this was that ethanol causes catecholamine release and disinhibits behavior at low doses and hypoglycemia also causes the release of catecholamines (Lieber, 1992). It was postulated that because ethanol has some disinhibitory effects on activity, some of the behavioral depressant hypoglycemic effects might be relieved. However, this hypothesis was not supported by the results. The difference was found in the opposite direction.

One possible explanation for failure to find the predicted interaction (the second hypothesis) is that there was no significant activity increase caused by a low ethanol dose (without insulin). There was a trend for some disinhibitory activity increase at the 600mg/kg ethanol dose. Duncan and Baez (1981) stated that the disinhibitory effect is very situation-specific. Therefore, the best explanation is that there was no disinhibitory effect without insulin. Hence, there was no enhancement of this effect from hypoglycemia.

The third hypothesis proposed that ethanol at higher doses should decrease activity, and when combined with insulin the hypoglycemia should potentiate this effect. This is because ethanol has mainly a depressant effect which is similar to the eventual hypoglycemic effect so they might

be expected to potentiate each other (Lieber, 1992). This hypothesis was confirmed. Even without insulin the level of activity with a 1000mg/kg dose of ethanol was significantly lower when compared to the other groups. With the insulin, however, the activity was dramatically reduced and this effect was also seen at ethanol doses of 300 and 600mg/kg. Thus, insulin greatly enhanced the depressant effects of ethanol, causing significant differences at very low doses. The low doses of ethanol in the hypoglycemic state had effects similar to a higher dose in the normal state.

The final hypothesis was not supported. The combination of ethanol and insulin did not increase hypoglycemia. Results from this study revealed that ethanol did not produce hypoglycemia, and ethanol did not further decrease the insulin-produced hypoglycemia. The short-term nature of the experiment probably resulted in the ethanol not producing nor potentiating hypoglycemia. The effects might have been seen at higher doses with chronic treatment (Duncan & Baez, 1981). Another probable reason why the ethanol did not further decrease the insulin-produced hypoglycemia was that the compensatory processes prevented further decreases in blood glucose (Messier & Kent, 1994). In the combination treatment conditions, the rats exhibited signs of being in a severe hypoglycemic state. They were motionless and did not respond to handling, and three rats died from the combination of a high dose of ethanol and

insulin. These fatalities were probably due to the extremely low blood glucose in combination with central nervous system depressant effects of ethanol.

Conclusion

In conclusion, the findings in this study suggest that there was a strong potentiation of ethanol's behavioral depressant effects by hypoglycemia, however there was no potentiation of low dose ethanol disinhibitory effect. There was also no enhancement of insulin-produced hypoglycemia by ethanol. Furthermore, hypoglycemia was not evident without insulin treatment.

The interaction found between ethanol and insulin could have resulted from several factors. First, insulin causes behavioral depression by lowering the availability of the central nervous system's only energy source (glucose). This glucose deprivation results in severe impairment of all neural activity, and at extreme levels convulsions and death may occur (Lieber, 1992). Another factor contributing to this interaction is that ethanol disrupts central nervous system functioning by altering the function of neurotransimitters, such as GABA, Glutamate Acid, etc. (Crabbe, 1989). Apparently, these two different types of impairment combined to cause much greater depression than either type alone.

Regarding the lack of interaction of a disinhibitory nature with low-dose ethanol it cannot be concluded that it

does not occur because it might be seen at very low ethanol doses. Weigand (1990) found that ethanol doses of 60 and 120 mg/kg could interact with other drug states when conditioned place preference was the dependent variable. Lower doses of ethanol and perhaps lower degrees of hypoglycemia might reveal an interaction. In the present study a strong interaction was found at the lowest ethanol dose (300mg/kg). Thus, future research should explore lesser degrees of both types of central nervous system impairment.

A possible reason for the failure to find ethanol-related hypoglycemia in this study was that the rats were only deprived of food for 24 hours. As previously discussed in the introduction, most ethanol effects on glucose metabolism are associated with long-term ethanol exposure, especially in fasting humans or animals (Lieber, 1992). Only acute ethanol treatments were administered in the current study, and although these animals were 24 hours food deprived, they did not experience an extreme level of "fasting."

Although the present study did not demonstrate that ethanol can cause hypoglycemia or an increase in insulin produced hypoglycemia, the behavioral depressant effects (including, extreme lethargy and coma) were dramatically increased by ethanol. Individuals need to be aware that insulin and ethanol have some similar physiological effects

on the body and in particular, on the brain. When taken together, brain function is lowered to a point that coma or death may result. Even if ethanol is consumed alone, the carbohydrate shift in the body can cause a hypoglycemic state (Cohen, 1976). Thus, it is very important for drinkers to consume food during or after consuming ethanol. This information should be of important interest to IDDM patients, heavy drinkers, and the medical community. Further research should involve a wider range of ethanol doses, chronic ethanol treatment, and more subtle levels of hypoglycemia.

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APPENDIX

CAUSES OF HYPOGLYCEMIA

I. Endogenous (Spontaneous) Causes

- A. Excessive glucose utilization
  1. Insulinoma
  2. Insufficient amount of anti-insulin hormones: growth and thyroid hormones, glucagon, cortisol, and epinephrine
  3. Hypoglycemia in neonatal infants that results from having diabetic mothers
- B. Insufficient glucose production
  1. Poor glucose release
  2. Glycogen storage disease
  3. Liver disease
  4. Fructose-1-6-diphosphotase deficiency
- C. High glucose production or excretion
  1. Renal glycosuria
  2. Pregnancy
  3. Exercise
  4. Fever
  5. An excessive amount of glucose can be utilized from certain tumors

II. Exogenous (Reactive) Causes

A. Extreme amount or quick absorption of carbohydrate

1. Insulin overshoot hypoglycemia

2. Dumping syndrome

B. Specific nutrients can prevent glycogenolysis

1. Leucine sensitivity (maple syrup urine disease)

2. Fructose intolerance

3. Galactosemia

C. Drugs

1. Too much glucose utilization

a. Exorbitant amount of glucose injection

b. Propanolol, oxytetracyline, EDTA, etc. can increase the insulin effect

c. High amount of sulfonurea injection

d. Alcohol, sulfa drugs, dicumarol, phenylbutazone, can intensify the sulfonurea effect

e. An excessive amount of phenformin injection

2. Insufficient amount of glucose production

a. Alcohol

b. Haloperidol

c. Salicylates

d. Chlorpromazine

e. Propozyphene

f. Aminobenzoic acid

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