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THE ROLE OF GLUCOREGULATION
IN ALCOHOL INTAKE

by

Ye Him Loo

A thesis submitted in conformity with the requirements
for the degree of Master of Science,
Graduate Department of Pharmacology,
in the University of Toronto

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ABSTRACT

THE ROLE OF GLUCOREGULATION IN ALCOHOL INTAKE  Loo, Ye Him M.Sc. (1998). Department of Pharmacology, University of Toronto.

The glucostatic theory has been implicated in controlling feeding behavior in experimental animals. Since alcohol is a food substance, alcohol intake may also be regulated by a glucostatic mechanism. Naive Wistar rats drinking alcohol and water under the limited access procedure provide a model of human alcohol consumption used in this thesis to explore the role of glucoregulatory processes in alcohol intake.

Peripheral glucose injections and ATP did not reduce alcohol consumption. Although glucose utilization was increased after glucose injections in the chronically food-deprived rats, glucose injections again did not attenuate alcohol intake. Endogenously elevated blood glucose had no effect on alcohol consumption in the streptozotocin-induced diabetes. The glycogenolytic inhibitor, trifluoperazine and the ATP synthesis inhibitor, 2,4-dinitrophenol were found to have no effect on the naltrexone-induced reduction of alcohol intake. Although an inverse relationship was found between the blood glucose and alcohol intake using two $\alpha_2$-adrenoceptor antagonists, the results from idazoxan were not robust. Taken together, these findings suggest that glucoregulatory processes do not play a role in the control of alcohol intake.
This thesis is dedicated to my parents. Without their love and support, I would not have the opportunity to complete this work.

I wish to thank my supervisor, Dr. Larry Grupp and Steve Harding for their guidance, assistance and most importantly our friendship. The work presented in this thesis would not have been successful without their invaluable contributions.

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LIST OF ABBREVIATIONS

5-HT-5-hydroxytryptamine
cAMP-Adenosine 3',5'-cyclic monophosphate
i.p.-intraperitoneal
s.c.-subcutaneous
AA-Alcoholic Anonymous
ADH-Alcohol Dehydrogenase
ALDH-Acetaldehyde Dehydrogenase
ANG-Angiotensin
ATP-Adenosine triphosphate
BZD-Benzodiazepine
DA-Dopamine
GABA-Gamma-aminobutyric acid
NAD+-Nicotinamide adenine dinucleotide (oxidized form)
NADH-Nicotinamide adenine dinucleotide (reduced form)
NIAAA-National Institute on Alcohol Abuse and Alcoholism
NMDA-N-methyl-D-aspartate
1. GENERAL INTRODUCTION

1.1 ALCOHOL ABUSE AND ALCOHOLISM

Since the dawn of history, alcoholic beverages have been consumed all over the world. Second only to caffeine, alcohol is one of the most popular psychoactive substances used by humans today. From the spontaneous-fermented beverages of relatively low alcohol concentration to the introduction of distillation techniques in the Middle Ages by the Arabs, the alchemists believed that alcohol was the long sought after elixir of life and was regarded as a “panacea” for all kinds of diseases. Although moderate drinking has been accepted as a part of our modern normal social lives, excessive alcohol consumption and alcohol abuse have caused serious medical, social and economic problems in our society.

Due to the widespread nature of this problem across different cultures, solid criteria for alcoholism are needed in order to make a diagnosis and prescribe appropriate treatments. The World Health Organization (WHO) suggests that the cardinal manifestation of addiction is “loss of control” of the use of a particular substance (Edwards et al., 1981). The WHO-proposed definition of a drug-dependence syndrome is “a cluster of cognitive, behavioural and physiological phenomena that include a compulsion for a stereotypical drug-taking habit, evidence of tolerance and withdrawal, use of drug to alleviate withdrawal symptoms, high priority of drug-seeking behaviour, and rapid reinstatement of the syndrome after a period of abstinence”. The following elements have been suggested by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) to better characterize alcoholism, which is also known as the disease called “alcohol dependence syndrome” (NIAAA, 1996).
- **Craving:** A strong need, or compulsion, to drink.
- **Loss of control:** The frequent inability to stop drinking once a person has begun.
- **Physical dependence:** The occurrence of withdrawal symptoms, such as nausea, sweating, shakiness, and anxiety, when alcohol use is stopped after a period of heavy drinking. These symptoms are usually relieved by drinking alcohol or by taking another sedative drug.
- **Tolerance:** The need for increasing amounts of alcohol in order to get “high”.

Scientifically, two different forms of alcoholism have been proposed and characterized by Cloninger and colleagues (Cloninger et al., 1981) and it still remains one of the most popular classification models for alcoholism. Type 1 alcoholism, or milieu-limited alcoholism, is associated with mild alcohol abuse in the parents, frequency and severity of abuse determined by environment, and is usually a mild form of abuse with better prognosis. Type 2 alcoholism, also known as male-limited alcoholism, is conversely highly heritable and is characterized by severe abuse by the father and no abuse by the mother, with no effect of environment on the frequency of abuse. Recently, another typology method has been proposed, Type A or B alcoholism. Babor and colleagues (1992) based their typology on the assumption that the heterogeneity among alcoholics is attributable to a complex interaction among genetic, biological, psychological and sociocultural factors. For example, certain genetically influenced vulnerability factors (e.g. family history, childhood temperament, and behaviour problems) may predispose subjects to a more severe form of alcohol abuse with worse outcome (i.e. type B). This suggests that one could identify higher risk type B subjects before their problems become severe. Subjects lacking these risk factors (i.e. type A subjects) may develop a less severe and more treatable form of alcoholism that is more...
environmentally influenced. The merits of the latest system are still under investigation.

Alcohol abuse, however, is distinct from alcoholism in that it does not usually include a strong craving for alcohol, loss of control or physical dependence. In addition, alcohol abuse is less likely to produce tolerance than alcoholism. A working definition for alcohol abuse is that the pattern of drinking is accompanied by one or more of the following situations within a 12 months period: (NIAAA, 1996)

- Failing to fulfill major work, school or home responsibilities.
- Drinking in situations that are physically dangerous, such as driving a car or operating machinery.
- Recurring alcohol-related legal problems, such as being arrested for driving under the influence of alcohol or for physically harming someone while intoxicated.
- Continued drinking in spite of ongoing relationship problems that are caused or worsened by the effects of alcohol.

While alcohol abuse is basically different from alcoholism, it is important to note that many effects of alcohol abuse are also experienced by alcoholics.

In 1996, according to the statistics released by the NIAAA, the economic cost of all alcohol-related problems in the United States is estimated to be about $100 billion a year (NIAAA, 1996). Nearly 14 million Americans, i.e. 1 in every 13 adults, abuse alcohol or are alcoholic. The spectrum of problems ranges from medical conditions such as liver cirrhosis and cancers, traffic accidents, domestic violence to loss of productivity. In human terms we can never measure in dollars the value of lost human lives, wrecked families, deteriorated personalities and human misery.

One classical pharmacological intervention to treat alcoholism has been by disulfiram
(Antabuse). Disulfiram inhibits the hepatic enzyme acetaldehyde dehydrogenase which oxidizes acetaldehyde to acetate, thereby increasing acetaldehyde accumulation in the body. A severe reaction occurs within minutes with signs of increased pulse rate, respiration and cardiac output which can be life-threatening at times (Kalant & Khanna, 1989). In theory, alcoholics are given disulfiram so that fear of a reaction will deter them from drinking. Hence, instead of being a cure for alcoholism, disulfiram merely acts as a deterrent. Besides, the unpleasant nature of this therapy has led to very poor compliance and therefore very little value in the treatment of alcoholism (Kristenson, 1992).

Currently, anti-craving substances such as acamprosate and naltrexone have been approved for use in the prophylaxis of relapse in alcoholics (Spanagel & Zieglsberger, 1997). Naltrexone, an opioid receptor antagonist, interferes with the positive reinforcing effects of alcohol presumably through its actions on the endogenous opioid systems (Sinclair, 1990; Spanagel et al., 1992). Acamprosate, on the other hand, exerts its actions on the NMDA receptor and voltage-gated ion channels, thereby reducing the hyperexcitability that occurs during alcohol withdrawal (Littleton, 1995). Although naltrexone and acamprosate can prevent relapse after long abstinence periods, there is no evidence that these compounds also reverse lasting changes in sensitivity to alcohol that is induced by their long-term exposure. It is possible that drugs or manipulations which would modulate other systems such as dopamine, GABA, serotonin or renin-angiotensin, etc. can one day be used in the treatment of alcoholism (Myers, 1994).
1.2 PHARMACOLOGY OF ALCOHOL

1.2.1 Pharmacokinetics

Ethanol, CH₃CH₂OH, is rapidly absorbed from the stomach, intestine and colon after oral ingestion. The main site of the absorption takes place in the small intestine via passive diffusion. The rate-limiting-step in the absorption process, on the other hand, is the rate at which the stomach empties its contents into the small intestine (gastric emptying). Slower gastric emptying, e.g. presence of food in the stomach, can reduce the rate of absorption of alcohol and decrease the maximum blood alcohol levels attained. Since alcohol has a low oil/water partition coefficient and is completely miscible in water, alcohol is distributed throughout the total body water after complete absorption. In general, after consumption of one standard drink (A standard drink is defined as 12 ounces of beer, 5 ounces of wine, or 1.5 ounces of 80-proof distilled spirits.), the blood alcohol concentration of a normal healthy individual peaks within 30 to 45 minutes. Because of its low partition coefficient, alcohol does not dissolve readily in fat tissues. Hence, women achieve a higher blood alcohol concentration than men (Saunders & Paton, 1981) when they consume an equivalent amount of alcohol is due to the higher subcutaneous fat storage in females.

Elimination of alcohol from the body occurs mainly in the liver through enzyme-mediated metabolism (95%) although a minute amount will be excreted unchanged in the breath, sweat, faeces and urine (5%). In the liver, alcohol can be metabolized through one of four pathways within the hepatocytes outlined below.

1. An enzyme called alcohol dehydrogenase mediates the conversion of alcohol to acetaldehyde. Acetaldehyde is rapidly converted to acetate by acetaldehyde dehydrogenase (ADH) and is eventually metabolized to carbon dioxide and water. In
normal circumstances, ADH is by far the most important hepatic enzyme responsible for the *in vivo* oxidation of alcohol and it constitutes most of the elimination of alcohol from the body. ADH, a cytosolic enzyme, functions in the presence of its co-factor NAD\(^+\) and the reaction follows first-order kinetics at lower concentration of alcohol. At higher doses the metabolism is strictly independent of the blood alcohol concentrations and follows a zero-order kinetics reaction.

2. The second pathway of alcohol metabolism involves the microsomal ethanol-oxidizing system (MEOS), which is essentially a part of the cytochrome P450 system. The cytochrome P450-2E1 and P450-3A have shown a high substrate specificity for alcohol. This system is NADPH-dependent and requires the presence of molecular oxygen for the conversion of alcohol to acetaldehyde which is again further metabolized by ALDH to acetate. Chronic exposure to alcohol can lead to an induction of these cytochrome P450 enzymes and results in the increased rate of alcohol oxidation observed in heavy drinkers. This induction may be a factor in the development of tolerance and is also the major cause of the drug-interaction problems in the alcoholics particularly in the aging population.

3. Thirdly, being very minor in significance, is the process associated with the catalyse enzyme system located in the peroxisomes. Catalyse functions in the presence of hydrogen peroxide, H\(_2\)O\(_2\), which is derived from an independent oxidizing system.

4. Lastly, minute amounts of alcohol are conjugated with glucuronic acid or sulfuric acid.

In conclusion, the absorption and metabolism of alcohol can be influenced by several factors such as food, body weight, genetics, gender and interaction with other concurrent medications.
Nevertheless, the typical rate of alcohol metabolism is about 10 grams per hour for a healthy 70 kg male.

1.2.2 Metabolic Effects of Alcohol

The development of fatty liver, very often seen in alcoholics, is produced by an excess of alcohol oxidation in the liver. Alcohol metabolism appears to increase oxygen utilization by liver cell, thereby reducing the availability of oxygen for other important cellular functions. This hypoxia state in the liver may lead to chronic liver inflammation and cirrhosis of the tissues. The change of NAD$^+$ to NADH, resulting from the oxidation of alcohol and acetaldehyde affects other metabolic processes which require NAD$^+$ as a co-factor. The following are some examples caused by the disruption of other NAD$^+$/NADH metabolic processes: elevation of serum lactate by the reduction of pyruvate to lactate accompanied by the oxidation of NADH to NAD$^+$; the excess of glycerol-3-phosphate and fatty acids leads to increased esterification and accumulation of neutral glycerides in the liver (fatty liver); the increase in NADH and decrease in pyruvate result in a reduced rate of gluconeogenesis; and in the brain, monoamineoxidase (MAO) is inhibited, so that metabolism of dopamine, serotonin and noradrenaline reduced.

1.2.3 Effects of Alcohol on CNS

There is a public misconception that alcoholic drinks are stimulating. Actually, alcohol is primarily a CNS depressant. The stimulation that occurs briefly during the rising phase of the blood alcohol concentration results largely from depression of inhibitory control mechanisms in the brain. Upon intoxication, a general impairment of nervous systems occurs, and a condition similar to
general anesthesia follows. In fact, the effect of alcohol on cell membrane is comparable to that observed in general anesthesia: expansion and fluidization of the excitable membrane.

Chronic heavy drinking and alcoholism can have serious repercussions for the functioning of the entire nervous system, particularly the brain. These effects include changes in emotions and personality as well as impaired perception, learning and memory. Neuropathological and imaging techniques have provided evidence of physical brain abnormalities in alcoholics, such as atrophy of nerve cells and brain shrinkage.

Alcohol can have a negative effect on certain neurological processes, such as temperature regulation, sleep and coordination. For example, vasodilation of the cutaneous blood vessels due to impairment of the thermoregulatory centre may produce a life-threatening decline in body temperature (i.e. hypothermia). Alcohol also interferes with the normal sleep patterns. Rapid-eye-movement (REM) sleep is suppressed by relatively small doses of alcohol. Another prominent effect of chronic alcohol consumption is damage to the cerebellum, resulting mainly in the loss of muscular coordination. This damage appears most often as imbalance and staggering or impairment of hand-eye coordination.

One of the most severe consequences of long-term alcoholism on mental functioning is Korsakoff’s syndrome (KS), a memory disorder in which a person appears to forget the incidents of his or her daily life as soon as they occur. Because of this dramatic loss of memory, patients with KS virtually live in the past. Some alcoholics may have a genetic component or predisposition to develop this amnesic condition; these patients may have an enzyme deficiency that prevents their bodies from using thiamin (a B vitamin) efficiently. This deficiency, coupled with a diet high in alcohol and low in thiamin, may lead to brain damage and amnesia.
Another disorder, Wernicke's encephalopathy, frequently occurs with KS, leading to the diagnosis in the patient of Wernick-Korsakoff syndrome. Patients with Wernicke's encephalopathy exhibit confusion, uncoordinated gait, and abnormal eye movements. Like KS, Wernicke's encephalopathy is thought to be caused by thiamin deficiency. The syndrome's acute manifestations can often be reduced by thiamin administration.

The effects of alcohol are both specific and non-specific. The non-specific actions of alcohol resemble to those of general anaesthetics, as mentioned above. Prior to the early 70s, the specific actions of alcohol were thought to be produced by GABA mediated chloride flux resulting in synaptic inhibition in a fashion similar to those observed with the barbiturates. This effect can be reversed by the administration of a GABA antagonist and this occurs at concentrations which do not relate with the membrane fluidizing effects (Seeman, 1972). Hence, this evidence suggests that the GABA receptor complex may be a specific target for alcohol actions.

On the other hand, as the role of neurotransmitters and their receptors in the CNS began to be better understood, it became apparent that alcohol was in fact able to alter central neurotransmission. It is now clear that there is no single neurotransmitter system which is responsible for mediating all of the central effects of alcohol.

1.2.4 Effects of Alcohol on Nutrition

Alcoholism has profound deleterious effects on nutritional status. (Denney & Johnson, 1984) Several alcohol-induced nutrient deficiencies in turn affect the metabolism of alcohol, aggravating its hepatotoxicity and nutritional status. Alcohol depresses appetite, displaces other foods and nutrients from the diet, and decreases the value of food by interfering with digestion and absorption. Even
when nutrients are absorbed, alcohol prevents them from being fully utilized by altering their transportation, storage and excretion. Patients hospitalized for medical complications associated with alcoholism are often severely malnourished. Such medical complications are known to be due to the direct and indirect effects of alcoholism on nutrient availability and utilization (Lieber, 1988).

When alcohol is consumed, its calories may spare nitrogen utilization, thus preventing the muscle and other organs from breaking down their proteins for use as energy sources. When alcohol replaces carbohydrate calories, i.e. glycogen storage is depleted in liver, an increase occurs in nitrogen losses as urea (Brenout et al., 1987). Also, the ingestion of alcohol depresses protein synthesis, inhibits hepatic and muscular mitochondrial protein synthesis (Schreiber et al., 1974), depresses serum protein fraction and total protein (Stamm et al., 1984), impairs absorption and secretion of amino acids by liver, increases serum concentration of branch-chain amino acids, and inhibits the secretion of albumin and plasma glycoprotein by the liver (Tuma et al, 1988).

The calories in alcohol are inefficiently utilized by the body, especially when the dose is high or when the subject is an alcoholic. Even when alcohol is ingested as extra calories, it causes less weight gain than calorically equivalent amounts of carbohydrate or fat (Guthrie et al., 1990), and no weight gain occurs in lean individuals (Crouse & Grundy, 1984). The lack of energy gained from alcohol is a direct toxic effect of alcohol metabolism in the liver. Chronic alcohol consumption results in a generalized depression in hepatic mitochondria energy metabolism. It decreases both the rate and efficiency of adenosine triphosphate (ATP) synthesis via the oxidative phosphorylation system (Cunningham et al., 1990). Also, hormonal imbalances leading to reduced hepatic ATP content (Spait et al., 1982), increased ATPase activity (Arai et al., 1984), and the inhibition of glucose metabolism under oxygen-poor conditions may explain the lack of weight gain commonly
observed in alcohol abusers.

Alcohol also affects carbohydrate metabolism via its regulation of pancreatic enzymes. SanKaran et al. (1989) showed that acinar content of amylase and the acinar response to cholecystokinin (CCK)-octapeptide are significantly lower in rats fed diets containing 26% of calories from alcohol. Human alcohol abusers with liver cirrhosis have decreased insulin sensitivity and glucose-6-phosphatase. The resultant changes in these pancreatic enzymes lead to the glucose intolerance and insulin resistance observed in chronic alcohol abusers.

Many biochemical abnormalities in lipid metabolism result from intoxication or chronic alcohol usage. When alcohol is present in the system, it displaces fat as the primary fuel in the liver, resulting in hepatic accumulation of fat. These lipids originate from dietary lipids that reach the bloodstream and the liver as chylomicrons, adipose tissue lipids that are transported to the liver as fatty acids. Metabolic disturbances in the equilibrium resulting from alcohol ingestion affect hepatic lipid synthesis, increasing hepatic lipogenesis via the stimulation of lipogenic enzymes, decreasing lipid breakdown, decreasing hepatic secretion of lipids, and enhancing hepatic uptake of circulating lipids (Arakawa et al., 1975).

Alcohol has detrimental effects on calcium, magnesium, iron, zinc, selenium, thiamin, riboflavin, folic acid, niacin, biotin, ascorbic acid, and vitamins A, D, E and K. For example, research has shown that alcohol may directly affect calcium (Ca$^{2+}$) balance by altering water balance through its diuretic actions (McIntyre, 1984). Negative Ca$^{2+}$ balance may also occur secondarily to fat malabsorption in human alcoholic patients (Morgan, 1982). In trace quantities, selenium (Se) is an essential nutrient and induces antioxidant activity. Low blood Se levels are associated with abnormal liver structure and functions. This may contribute to hepatic injuries via increased lipid
peroxidation and the associated damage of liver cell membranes (Korpela et al., 1985). Low vitamin A status has been observed in Norwegians who consumed large amounts of alcohol (Kvale et al., 1983). Deficiency of vitamin A has very serious biochemical consequences, it increases the susceptibility to neoplasia and carcinogenesis (Nettesheim & Williams, 1976).

In summary, abuse of alcohol depletes tissue levels and/or activities of the inherent antioxidant enzymes and dietary antioxidants that protect the biological systems against reactive electrophiles generated from the oxidative degradation or metabolism of alcohol. Thus, the multifactorial effect of alcoholism on nutritional status is an undisputed factor in the pathogenesis of liver and/or other organs damages.

1.2.5 Interaction between Alcohol and Glucose Homeostasis

The maintenance of plasma glucose concentrations within relatively stable limits is a primary homeostatic mechanism in man. This is presumably because hypo- and hyperglycaemia are associated with severe metabolic and functional abnormalities. Episodes of hyperglycaemia may cumulatively lead to the onset of chronic complications in susceptible tissues, at least in diabetic patients, whereas hypoglycaemia is associated with irreversible brain damage and mental retardation.

The steady-state plasma glucose concentration is determined by the balance between the rate of glucose production by the liver, to a lesser extent, the kidney, and the rate of glucose utilization. Both gluconeogenesis and glycogenolysis contribute to hepatic glucose production, whereas many tissues (e.g. skeletal muscles and brain) make important contributions to the rate of glucose utilization and oxidation.

The balance between the rates of glucose production and utilization is determined to a certain
extent by the balance between insulin and its counterregulatory hormones, including glucagon, cortisol, growth hormone, and the catecholamines. In the liver, insulin reduces glucose production via the inhibition of gluconeogenesis and stimulates net glucose disposal. The maintenance of normal glucose homeostasis is dependent upon the coordinate regulation of three interdependent processes: (1) insulin secretion of the pancreatic β cells, (2) the suppression by insulin of hepatic glucose output, and (3) the stimulation by insulin, in association with hyperglycaemia, of peripheral and splanchnic glucose uptake.

In fasted human volunteers, alcohol administration reduces rates of glucose synthesis from lactate and rates of glucose appearance in the postabsorptive state. In individuals with adequate hepatic glycogen reserves, the inhibition of gluconeogenesis is presumably compensated for by increased rates of glycogenolysis. Besides its effects on gluconeogenesis, alcohol also impairs the hepatic storage of glycogen as a result of the diminished glucose synthesis. It is now recognized that a substantial proportion (up to 70%) of the liver glycogen deposited in response to carbohydrate refeeding is synthesized from glucose following its conversion to lactate and related 3-carbon metabolites. Sustained gluconeogenesis is, therefore, a prerequisite to active glycogen deposition following refeeding. In the rats, the inhibition of hepatic gluconeogenesis secondary to alcohol oxidation may, therefore, not only lead to fasting hypoglycaemia, but also severely compromise the capacity of the liver to store glycogen.

Whereas glucose tolerance may be impaired by the simultaneous administration of alcohol, the consensus view is that alcohol pretreatment leads to potentiation of the insulin secretory response on subsequent oral or intravenous glucose challenge. Plasma insulin levels are elevated if alcohol and glucose are given concurrently, implying that pretreatment is not an absolute prerequisite to
alcohol stimulation of glucose-mediated insulin secretion.

Insulin release from the pancreatic $\beta$ cells is biphasic, the initial burst of release within the first few minutes being followed by a sustained phase of gradual release of the hormone over several hours. Alcohol pretreatment increases both first- and second-phase insulin secretory responses to glucose loading in normal subjects, but the stimulation of the first-phase response may be attenuated in obese and diabetic subjects.

The potentiation by alcohol of glucose-induced insulin secretion may provoke a profound, often symptomatic, reactive hypoglycaemia following the ingestion of carbohydrate-rich foods. The potentiation of glucose-induced insulin secretion by alcohol may also cause severe hypoglycaemia in sulfonylurea-treated diabetic patients, in athletes following severe exercise in cold weather, and may also lead to nocturnal hyperinsulinaemia in healthy individuals who drink alcoholic beverages with their evening meals.

It is well established that alcohol, in addition to decreasing hepatic glucose production, impairs peripheral glucose utilization and causes acute insulin resistance. The mechanism of acute alcohol-induced insulin resistance remains elusive. Indirect calorimetric studies indicate that in normal individual alcohol decreases total body fat oxidation and protein oxidation, and attenuates the rise in carbohydrate oxidation after glucose infusion. The implication is that alcohol acts as a preferred oxidative fuel, largely replacing fat and protein as the substrate for oxidation, suppressing glucose mobilization and oxidation induced by insulin.
1.3 ANIMAL MODELS OF HUMAN ALCOHOL CONSUMPTION

An animal model is an essential part of basic scientific research. Many aspects of alcoholism and alcohol abuse have been revealed using studies carried out in rodents and primates. Animals are used in three principal ways in research on alcoholism: to model the drinking behaviour of human alcoholics; to learn from the brain and other organs of drinking animals how brain chemistry leads to a drinking behaviour; and to study how alcohol damages organs per se.

An animal model is preferred to human subjects in basic research because of practical and ethical issues. Secondly, due to the complexity of factors controlling alcohol intake, many factors and systems are at play contributing to the influence of alcohol intake. Thus, an animal model will be most valuable in mechanistic studies where particular parameters can be looked at individually and most of the extraneous variables eliminated. This kind of study, in such a highly controlled manner, simply may not be possible with humans either in a natural or laboratory setting. Therefore, an appropriate choice of animal model is in effect a crucial element in determining the usefulness of the data collected.

Alcohol is taken orally by humans and hence an animal model with the same route of intake must be met. Intravenous self-administration in primates or intragastric self-administration, though useful in the study of alcohol tolerance and withdrawal, appears to be inappropriate in studying self-administration in a manner similar to the human condition. One of the apparent reasons is that the olfactory and gustatory cues are both absent in the above two cases and these events may be important in evoking the brain's response to alcohol intake.

One of the major obstacles in the development of a suitable animal model of alcohol addiction is related to the motivational aspect of alcohol intake. Procedures have been designed with
food or water deprivation in which the animals are “forced” to drink alcohol simply for its caloric value or to quench its thirst. This approach leads to complications in the interpretation of the results because the rat may not be drinking alcohol for its reinforcing properties but rather to survive. These procedures are not favourable in the study of voluntary alcohol intake (choice vs. forced) since in the human situation, people choose to drink alcohol because of its drug effects.

In the limited access procedure of alcohol self-administration used in the present thesis which is modified and developed by MacDonall & Marcucella (1979), rats are given access to a choice of alcohol and water in separate graduated drinking tubes. Access to alcohol is restricted to the drinking session on each day to the time they spent in the drinking cages, usually one hour. The rats spend the rest of the day in their separate home cages in which they have access to food and water ad lib.

The rats, being nocturnal animals, are tested in a reverse light dark cycle environment in which they will have the access of alcohol during the dark and active phase of the day. Another advantage of this procedure is that it can train alcohol-naive rats to drink in a “bout” fashion in a relatively short period of time, typically from 3 to 4 weeks. It has also been well established that rats drinking in this paradigm consume enough alcohol to achieve pharmacological relevant concentrations of blood alcohol up to 50 mg%. Also, since the drinking occurs as a bout, the experimenters know that animals will drink when they go into the drinking cages and therefore if they give drugs before drinking sessions, they know that the drug will be working at the time when the animals would be drinking. In conclusion, this procedure has provided a scientifically sound model of human alcohol consumption and will be employed in all experiments presented in this thesis.
1.4 BIOLOGICAL MECHANISMS IN ALCOHOLISM

1.4.1 The Dopamine Reward System

The dopamine reward system plays an important role in the neurochemical basis of compulsive use of alcohol and other drugs. The endogenous reward system has a discrete neuroanatomical location in the hypothalamus and electrical stimulation of these “pleasure centres” in animals lead to reward-like behaviour (Dackis & Gold, 1985). The circuit of the reward system includes descending fibers from the lateral hypothalamus that project to the ventral tegmental area (VTA) via the medial forebrain bundle. The ventral tegmental area is the location of major dopamine neurons. Interruption of dopamine neurotransmission decreases the reward behaviour produced by electrical stimulation of the lateral hypothalamus.

Central stimulants that are readily self-administered appear to activate dopamine neurons in the reward centre. The ventral tegmentum projects to other dopamine areas in the striatum, limbic system, and cerebral cortex. Lesions in the nucleus accumbens located in the limbic system results in a decrease in the self-administration of cocaine in animals (Roberts et al., 1980).

A great deal of attention has been devoted to behavioural studies examining the effects of various manipulations of dopaminergic neurotransmission on alcohol intake. An early study by Myers and Melchoir (1975) suggested a potential role for dopamine (DA) in the mediation of alcohol drinking behaviour by showing that intracerebroventricular injection of 6-hydroxy-dpamine (6-OHDA) resulted in a decreased preference for alcohol in the rat.

In a double-blind clinical trial with 50 chronic alcoholics, the rated craving for alcohol was reduced from strong to very mild by administration of the DA agonist bromocriptine (Borg, 1983). In addition, microinjections of the non-specific DA agonist d-amphetamine and the D$_2$/D$_3$ agonist...
quinpirole into the nucleus accumbens increase alcohol-reinforced lever pressing (Hodge et al., 1992), while responding is decreased when the D\textsubscript{2} antagonist raclopride is injected into this area (Samson et al., 1993). Consistent with the idea that reductions in responding following antagonist injection into the nucleus accumbens may be due to decreased DA activity, the injection of quinpirole into the VTA, which decreases cell firing rates, presumably by activating somatodendritic DA receptors (Chiodo, 1988), also resulted in a decrease in alcohol-reinforced responding (Hodge et al., 1993).

1.4.2 Effects of Alcohol on other Neurotransmitters and Reinforcement

In addition to the dopamine-mediated effects on the CNS, a number of neurotransmitters in the brain have been shown to affect the CNS either by its own circuitry or in combination with the mesolimbic DA reward system while under the influence of alcohol.

A. Gamma-aminobutyric acid (GABA)

Behavioural studies have demonstrated the ability of GABA mimetic drugs to potentiate the sedative and incoordinating effects of alcohol in rodents, whereas GABA antagonists and inverse agonists have been shown to attenuate these effects (Allan & Harris, 1987).

Most data linking the GABAergic system to alcohol actions are concerned with the mediation of the direct CNS effects of alcohol. There are also several lines of evidence, however, which support a role for this neurotransmitter system in alcohol-induced positive reinforcement, by way of an enhancement of GABAergic function. Notably, an early study demonstrated the maintenance of alcohol withdrawal with the BZD agonist diazepam (Deutsch & Walton, 1977). In more recent studies, the partial inverse agonist at the BZD receptor, Ro 15-4513, was found to reduce alcohol
intake in several lines of rats, including an alcohol-preferring strain, without modifying other consummatory behaviours (e.g. McBride et al., 1988).

B. Serotonin

Today, there is considerable evidence from both animal and human studies that 5-HT plays a role in the modulation of alcohol intake and dependence, and in the development of tolerance to alcohol. The general consensus is that there exists an inverse relationship between the functioning of the cerebral serotoninergic system and alcohol drinking preference. Pharmacological manipulations that reduce cerebral 5-HT concentrations increase alcohol intake in animals; conversely, procedures which increase 5-HT release or turnover produce a reduction in alcohol intake. Moreover, studies using genetically bred lines of alcohol-preferring, alcohol-naive rats (e.g. P/NP rats) have demonstrated lower basal concentrations of 5-HT and its main metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Murphy et al., 1987), as well as a decreased turnover rate (Morinan, 1987) and a reduction in the number of 5-HT fibres (Zhou et al., 1991), in various brain areas of these rats as compared to their genetically bred, non alcohol-preferring counterparts.

Within the context of the central reinforcing effect of alcohol, several observations support the existence of functional interactions between 5-HT and DA neurotransmission. In vivo microdialysis studies in the nucleus accumbens have shown that alcohol administration will preferentially stimulate DA (Imperato et al., 1986; Yoshimoto et al., 1991) but also 5-HT (Yoshimoto et al., 1991) release in the nucleus accumbens and in the frontal cortex of rats (Portas et al., 1994). Furthermore, microinjections of 5-HT into the VTA (Guan & McBride, 1989) are capable of enhancing the release of DA in the nucleus accumbens. One line of evidence indicates that the mechanism of this excitatory action of 5-HT may be via the 5-HT₃ receptor, whose
stimulation by agonists increases DA release in the nucleus accumbens (Jiang et al., 1990). Indeed, systemic administration (Carboni et al., 1989) or local application through a microdialysis probe (Yoshimoto et al., 1991) of 5-HT, antagonists has been shown to attenuate the stimulatory effects of alcohol on DA release in the nucleus accumbens.

C. Norepinephrine

A number of early studies implicated noradrenergic systems in the mediation of alcohol self-administration, as neurochemical lesions of noradrenergic neurons (Brown et al., 1977) or inhibition of norepinephrine synthesis (Davis et al., 1978) were found to result in a suppression of voluntary alcohol consumption. However, the noradrenergic and dopaminergic systems should not necessarily be completely dissociated from one another with respect to their involvement in the mediation of alcohol reinforcement. dopaminergic mechanisms in this phenomenon has repeatedly been reported in the literature. In this context, Wise and Bozarth (1985) proposed that while dopamine is likely to be the principal neurotransmitter responsible for the central mediation of alcohol reward, alcohol reinforcement may occur via inhibition of noradrenergic activity in the locus coeruleus resulting in a release of dopaminergic neurons from tonic noradrenergic inhibition.

D. Endogenous Opioids

It is known, for example, that a number of the behavioural and pharmacological effects of alcohol, such as hypothermia, euphoria, analgesia and motor activation, as well as the development of tolerance and dependence, are similar to those produced by opiates (Kalant, 1977). The traditional view of the involvement of endogenous opioids in the voluntary consumption of alcohol is referred to as the opioid deficiency hypothesis (Reid et al., 1991), which states that, analogous to what is believed to be the case for serotonergic neurotransmission, alcoholics suffer from a deficiency in
central opioidergic neuron activity, which is compensated for by the consumption of alcohol.

Two opioid receptors have been more particularly implicated in alcohol effects, the $\mu$ and $\delta$ subtypes. An early study by Altshuler et al. (1980) revealed that the blockade of these receptors by naltrexone reduced rhesus monkeys' intravenous self-administration of alcohol, suggesting the opiate receptor blockade had diminished the reinforcing properties of alcohol.

E. Other Neuropeptides

In addition to the endogenous opioids, a number of other neuropeptides have been studied for their involvement in the mechanisms underlying alcoholism. Notably, the neuropeptide cholecystokinin (CCK) has been shown to inhibit intake of alcohol solutions and feeding behaviours in a variety of species (Kulkosky et al., 1988), including rats selectively bred for alcohol sensitivity (Kulkosky et al., 1993). The renin-angiotensin (R-A) system has also been shown to be a robust modulator of alcohol consumption (review by Grupp, 1991). The relationship between this hormone system and alcohol drinking behaviour is an inverse one as a rise in R-A activity depresses alcohol intake while a fall in R-A activity enhances alcohol intake. In addition, there is evidence for the involvement of neurotensin in the effects of alcohol (Grupp & Harding, 1996). In particular, a down-regulation of the receptors of this peptide was observed upon chronic administration of alcohol to mice (Erwin et al., 1993).

1.4.3 Reinforcement and Alcoholism

It may seem evident that a person will repeat an action that brings pleasure and/or reward. The process by which such an action becomes repetitive is called positive reinforcement. Normally, this process functions to sustain behaviours essential to the individual or species, such as eating,
drinking, or reproductive behaviour. Evidence suggests that alcohol and other drugs of abuse are chemical surrogates of such natural reinforcers. Alcohol and other drugs of abuse that cause a rewarding mental state (e.g. euphoria) function as positive reinforcers upon initial exposure. These drugs may be more powerfully and persistently rewarding than the natural reinforcers to which the human brain is accustomed. Thus, continued exposure to such drugs can initiate increased drug-seeking behaviour and build the foundation for addiction.

Animal research has proved that alcohol is reinforcing (Yoshimoto et al., 1992). Studies have shown that some of alcohol’s actions in the brain and bloodstream can cause an animal to seek alcohol and even to work for it (e.g. pressing a lever) to repeat the effects it elicits (Koob, 1992). After alcohol-seeking behaviour has been established, the brain undergoes certain adaptive changes to continue functioning despite the presence of alcohol. As a consequence of this adaptation, however, certain abnormalities occur in the brain when alcohol is removed. Thus, periods of abstinence are marked by feelings of discomfort and craving, motivating continued alcohol consumption. This kind of motivation, based not on reward but on avoidance of unpleasant stimuli; is called negative reinforcement. Both positive and negative reinforcement are involved in the maintenance of alcoholism.

In conclusion, drinking alcohol can provide pleasant subjective feelings and these effects are produced by its basic actions on the CNS. These effects are the properties that reinforce alcohol-seeking and alcohol-drinking behaviour. Nevertheless, it is apparent that the drinking process cannot occur indefinitely without control. Therefore, it is reasonable to hypothesize that there are biological mechanisms in our bodies which can suppress the drinking process. One of the possible mechanisms which can control the termination of the drinking behaviour may be the feeding-related satiety signal
as will be discussed in more detail in the following section.

1.5 FEEDING-RELATED SATIETY

1.5.1 Alcohol as a Food

In contrast to other drugs of abuse, alcohol is unique in that it is the only drug that has any caloric value. The caloric value of alcohol is about 7 cal/g, almost twice as much as sugar per weight. While the role of glucoregulatory processes in the consumption of alcohol awaits more direct support, there is a number of promising findings suggesting that alcohol intake may be controlled by mechanisms similar to those that mediate food intake and maintain nutritional balance.

Over the past five decades, many studies have been conducted on the influence of dietary factors on alcohol intake (Lester & Greenberg, 1952; Pekkanen et al., 1978; Forsander & Sinclair, 1988). The overall results of these studies indicate that through some unknown mechanism, alteration in the diet can cause a change in alcohol consumption. For example, an early study by Rogers and Pelton (Rogers, 1957) reported that glutamine supplements diminished the desire to drink, decreased withdrawal, and improved the ability to sleep in subjects with extensive histories of heavy drinking. More recently, Yung et al. (1983) evaluated the possible relationship between drinking and diet in recently detoxified alcoholics. Those who maintained sobriety longer consumed significantly more carbohydrates, including twice as much sugar. It is unknown whether sobriety caused the increase in sugar intake, or if the increased sugar intake facilitated sobriety, or in fact, if some unidentified underlying mechanisms were responsible for both.

Mechanistically, the neurochemical mediation of food intake parallels that of alcohol
reinforcement. Although there appears to be multiple neurochemical systems involved in both food intake (Halmi, 1995) and alcohol reinforcement, the role of endogenous opioid system is important. For example, the opioid agonists (enkephalins, β-endorphin, and morphine) increase food intake and alcohol consumption (Reid et al., 1984) whereas the opioid antagonist naloxone reduces food intake (Margules et al., 1978) and also reduces alcohol consumption and reinforcement (Reid et al., 1984; Froehlich et al., 1990). Recent research indicates that δ and κ opioid receptors may play key roles in alcohol reinforcement and food intake (Morley et al., 1986; Froehlich et al., 1991). Related to these findings, is a recent experiment carried out in our laboratory (Grupp et al., 1997), in which we found that morphine-induced increase in alcohol consumption can be attenuated by peripheral (i.p.) glucose injections. In addition, the angiotensin (ANG) II-inhibited alcohol consumption can be attenuated by tolbutamide injections. Tolbutamide, a sulfonylurea, has been shown to inhibit glycogenolysis induced by Ca²⁺-mediated second messenger hormones such as ANG II, through a direct action on the liver and through its ability to stimulate insulin secretion (Mine et al., 1986). These findings suggest that the mechanisms which maintain glucose homeostasis may be involved in the regulation of alcohol intake. More research on these systems and their interaction with other important neurochemical systems (e.g. dopamine, serotonin and norepinephrine) is necessary to find out their roles in the complex mediation of food intake and alcohol reinforcement.

In a recent paper, Myers (Myers, 1994) catalogued over 60 drugs which could alter alcohol consumption in animals and/or humans. Interestingly, many of these modulators of neurotransmitter systems which can reduce alcohol intake also have effects on the regulation of blood glucose. For instance, the opiate antagonist naltrexone (McCubbin et al., 1989), the dopaminergic agonist quinpirole (Roane & Paul, 1991), the dopaminergic antagonist haloperidol (Wannarka, 1983), the
noradrenergic agonist isoproterenol (Potter & Ellis, 1975), the serotonin agonist 8-OH DPAT (Chaouloff & Jeanrenaud, 1987), the peptides CCK (Levine & Morley, 1981) and angiotensin II (Koide et al., 1985) all elevate circulating blood glucose levels and reduce alcohol intake, yet the role of glucose in their effects on alcohol consumption has never been studied.

1.5.2 Glucostatic Theory “Revisited”

As already mentioned, the mechanism mediating feeding behaviour is very complex and may involve many systems. In particular, glucose has long been recognised as a modulator of food intake by its actions as a satiety signal (Mayer, 1953). More recently, fatty acids as well as glucose are viewed as major sources of metabolic fuels whose utilization may produce satiety signals. Within the context of alcohol acting as a food-like substance, the possible role of these satiety signals in modulating alcohol intake has never been examined. However, the recent study from our laboratory (Grupp et al., 1997) demonstrating that peripheral glucose injections attenuated morphine-stimulated alcohol consumption is in concordance with this putative function.

According to Mayer’s (1953) glucostatic theory, a change in glucose utilization can be sensed by specific cells in the liver with a special affinity for glucose and serves as a stimulus for satiety or hunger. It proposed that the glucostatic control of food intake is integrated into the homeostatic mechanisms of the brain which depend mostly on glucose oxidation sensed by the glucosensitive cells in the liver. To demonstrate that glucose utilization is involved in the control of feeding, Mayer observed an inhibition of feeding after parenteral administration of glucose in the original experiment. However, attempts to induce satiety by parenteral administration of glucose have led to equivocal results. While Mayer and many others (Russek, 1970; Novin et al., 1974; Yin et al.,
1979) observed an inhibition of feeding after parenteral administration of glucose, some investigators did not (Adair et al., 1968; Scharrer et al., 1974). Since the reason for the conflicting evidence is unclear, the role of glucose utilization in the regulation of food intake is questioned and interest in the glucostatic theory has gradually decreased.

It was not until later that the studies of Tordoff and Friedman (1985; 1986) initiated renewed interest in the study of glucose utilization as a possible mechanism for the control of food intake. They found that hepatic portal glucose infusions decreased food intake. Rats with either hepatic portal or jugular catheters were infused with glucose and equitonic saline. During the infusions, their food contained nonnutritive chocolate or chicken flavour, depending on the infusate received. Hepatic portal glucose infusions decreased food intake relative to saline and no infusion conditions, but jugular glucose did not. When allowed to choose between the flavours associated with each infusate, rats with hepatic portal catheters preferred the flavour eaten during glucose infusion, and rats with jugular catheters showed no preference. These results suggest that a mechanism located in the liver can reduce food intake without producing malaise and that portal glucose can act as an unconditioned stimulus for the acquisition of a learned food preference.

In a follow-up experiment (Tordoff and Friedman, 1988), they found that hepatic glucose, fructose and mannitol infusions reliably and equally decreased food intake when compared with placebo and saline control infusions. However, an interesting finding from these studies was, unlike glucose, fructose infusions given by either the hepatic portal or jugular route decreased food intake when the infusions were given before the rats ate. It was suggested that this difference in response to glucose and fructose is due to the difference in tissue utilization of these fuels. While glucose can be metabolized extensively throughout the body, fructose is only poorly metabolized by brain and
limited peripheral tissues (Park et al., 1957). Hence, it is likely that larger quantities of infused fructose would reach the liver and exert its effects on hepatic metabolism. Based on this significant finding, the authors concluded that the utilization of metabolic fuels in the liver is essential to generate a satiety signal from the metabolic cascade.

All in all, in this thesis, attempts will be made to explore the interaction between gluoregulatory processes and alcohol consumption. In order to further justify the role of glucose acting as a satiety signal, in the following section, some latest developments in the investigations of glucose as a satiety signal will be presented.

In the classical study, Mayer (1953) found excellent correlations between glucose utilization, measured by arterio-venous (A-V) differences in glucose concentration, and the subjective feelings of satiety. A large A-V glucose difference (10 mg%), an indirect indicator of glucose utilization, was found to coincide with satiety. Both centrally-located and hepatic sensors are involved in monitoring glucose concentration and are highly influenced by insulin action, and liver glycogen content is monitored indirectly and gives rise to afferent signals influencing food intake. This pioneering work has provided one of the first piece of evidence suggesting the involvement of glucose as a hunger/satiety signal.

A comprehensive review of the literature has provided a more recent and complete picture of the mechanism of glucose acting as a feeding-related satiety signal. In summary, there are several lines of evidence supporting the notion:

1. Hepatic portal vein infusion of glucose into animals have demonstrated that glucose can inhibit feeding behaviour significantly. Russek's (1963) early hypothesis about the hepatic metabolic regulation on feeding behaviour focused on hepatic glucose metabolism. His studies showing
that hepatic portal vein infusion of glucose was more effective than systemic infusions in suppressing food intake in dogs provided strong evidence for a glucosensitive mechanism in liver for control of feeding behaviour. Tordoff and Friedman (1986; 1988) and other investigators (Novin et al., 1974; Yin et al., 1979) attempted to replicate this effect in the rats and had successful results.

Reduction in food intake is not due to discomfort (implantation of catheter) because the rats prefer a flavour associated with infusions that depress intake. In fact, if glucose infusions are noxious, rats would avoid flavours associated with infusion. Thus, direct infusions of glucose into hepatic portal vein, like ingestion of food, are able to trigger a stimulus to terminate feeding.

2. The nonmetabolizable glucose antagonist (anti-metabolite) 2-deoxy-glucose (2-DG), on the other hand, has been shown to increase feeding behaviour (Novin et al., 1973; Thompson & Campbell, 1977). In order to control feeding behaviour, a signal from the liver is required to initiate the appropriate response in the brain. Russek (1963) assumed that the hepatic glucosensors were hepatocytes which were hyperpolarized by glucose or some metabolites of the glycolytic pathway. In fact, Meyer and Scharrer (1991) proved that glucose had a short-term hyperpolarizing effect on hepatocyte membranes. Equally in importance is that 2-DG has a similar effect in that it can also hyperpolarize hepatocytes. In previous liver perfusion studies, Niijima (1969) showed that the discharge rate of hepatic vagal afferents to central sites decreased when glucose was added into the medium. In contrast, 2-DG increased the firing rate (Niijima, 1983), and taken together, these findings imply that the afferent nerve activity is specifically related to glucose utilization.
3. Evidence indicates that a vagal afferent pathway transmits a signal from liver to brain to control food intake in rats. The hepatic vagal innervation is implicated in the control of feeding by experiments showing that hepatic branch vagotomy affects feeding behaviour by abolishing the diurnal pattern of feeding behaviour (Delprete & Scharrer, 1994), altering behavioural adaptation to changes in diet, and attenuating the effects of various treatments that stimulate or suppress food intake (Delprete & Scharrer, 1990). Secondly, it also prevents the eating response to 2-DG or 2.5-anhydromannitol which presumably elicits feeding by its actions in liver (Tordoff et al., 1991). In summary, based on the evidence mentioned, the utilization of glucose seems to play an important role in the control of food intake. Therefore, the role of glucose utilization in alcohol consumption will be examined in this thesis.

1.5.3 The Latest Development: ATP as a Satiety Signal

Reports of an increase in food intake after administration of the fructose analogue 2.5-anhydro-mannitol (2.5-AM; Tordoff & Friedman, 1988) are consistent with the hypothesis that ATP availability plays a role in the sensory process of the hepatic sensors for the oxidation of fatty acids and perhaps other substrates that are being implicated in feeding control. In addition to inhibiting gluconeogenesis and glycogenolysis (Hanson, 1984), 2,5-AM has been shown to decrease the cytosolic ATP:ADP ratio in isolated hepatocytes (Stevens et al., 1985). The hyperphagic effect of 2,5-AM originates in the liver because hepatic branch vagotomy blocked the feeding response to 2,5-AM and after administration of radioactive 2,5-AM, significant quantities of radioactivity were found in the liver but not in the brain (Tordoff et al., 1991).

The results described above point to a mechanism for the control of feeding that integrates
information from changes in peripheral glucose and fatty acid metabolism. The liver is apparently involved in this integrative control, but the nature of its role is unclear because the mechanism of integration remains unresolved. It is possible that separate signals associated with glucose metabolism arise in the liver or other peripheral tissues and are then integrated in the brain. Accordingly, the findings implicating decreased hepatic ATP as a signal for feeding supports this interpretation because ATP production is a final common pathway in the metabolism of both glucose and fatty acids.

In an effort to elucidate the metabolic control of alcohol intake behaviour we are faced with the fundamental problems of identifying the signal responsible for inducing satiety. Given the latest evidence of ATP being a candidate stimulus for the control of feeding, it is reasonable to assume that the intake of alcohol may be regulated via identical mechanisms. Hence, the possible role of ATP in the control of alcohol intake will also be addressed in this thesis.

1.6 HYPOTHESES AND OBJECTIVES OF THESE STUDIES

The goal of alcoholism research, as in all medical research, is to understand the causes of disease and to develop new treatments. As mentioned earlier, Yung et al. (1983) showed that detoxified alcoholics who stayed in sobriety longer consumed more carbohydrates. In addition, in a double-blind, placebo-controlled study, Blum et al. (1989) investigated the effects of an amino acid and vitamin mixture (called "SAAVE") in alcohol and multiple drug abusers in an inpatient treatment program. Patients receiving SAAVE for 3 weeks showed improvement in several physical responses (e.g. muscular coordination, seizure activity and tremors). In light of the positive findings
of nutritional interventions on alcohol drinking and the increasing body of evidence of a glucostatic control in food intake, a causal study of the role of glucoregulatory mechanisms on alcohol consumption is justified. In order to better understand the possible role of glucoregulatory mechanisms in alcohol intake, five experiments were conducted in this thesis using different strategies. The following is an outline of the experiments and the details of the rationale for each experiment will be discussed separately in later sections:

1. Since alcohol is a food substance, the assumption that glucose injections will reduce alcohol intake through a glucostatic mechanism is tested in experiment one. Glucose injections were given intraperitoneally just prior to alcohol drinking to test directly its effects on alcohol intake. The hypothesis of this experiment was as follows: **if glucose injections produce a significant increase in blood glucose, alcohol consumption will be inhibited because of the higher blood glucose levels.** Moreover, since ATP satiety signal may also be involved in the regulation of food intake, the effects of L-malate, which can increase intracellular ATP levels, on alcohol consumption were also examined. In this case, our hypothesis of this experiment was: **the increased ATP satiety signal generated by L-malate injections will inhibit alcohol intake via a feeding satiety mechanism.**

2. In the second and third experiments, two different manipulations were used to study the effects of glucose utilization and diabetes on alcohol consumption. In experiment two, the effects of glucose injections combined with food deprivation on alcohol intake were investigated. In a nutshell, our hypothesis of this experiment was: **since food deprivation has been shown to increase glucose utilization when glucose is administered to food-deprived rats** (Issad et al.,
1987), such increase in glucose utilization may give rise to a satiety signal and decrease alcohol intake. Both acute and chronic food deprivational states were examined in this experiment. In experiment three, aside from the exogenous glucose injections examined in experiment one, the effects of an endogenously elevated and a more lasting increase of blood glucose (produced by diabetes) on alcohol intake were examined. We hypothesized that the diabetic rats might consume less alcohol because of their endogenously elevated blood glucose levels. In this experiment, the rats were made diabetic by injections of streptozotocin which selectively destroys the pancreatic β cells that secrete insulin.

3. In experiments four and five, two different drug manipulations were used to study the role of glucoregulatory mechanisms in alcohol consumption. In experiment four, we attempted to test the hypothesis that the opiate antagonist naltrexone reduced alcohol intake by increasing blood glucose or producing a satiety signal. A glycogenolytic inhibitor, trifluoperazine and an inhibitor of ATP synthesis, 2,4-dinitrophenol; were used to block naltrexone-induced reduction of alcohol intake by decreasing blood glucose and inhibiting ATP satiety signal respectively. In a previous study another opiate antagonist nalmefene has been shown to decrease food intake with an increase in blood glucose (McLaughlin et al., 1986). In addition, naltrexone has also been shown to potentiate hyperglycaemia induced by stress or adrenaline (McCubbin et al., 1989). Given this, we hypothesized that naltrexone's ability to reduce alcohol intake might be antagonized by drugs which could alter glucose or energy balance. In the last experiment, two α2-adrenoceptor antagonists, methoxy-idazoxan and idazoxan, were used to assess their opposite effects on alcohol intake and blood glucose levels.
Methoxy-idazoxan has been shown to be able to decrease alcohol intake (Reid et al., 1994) and produce hyperglycemia due to the release of norepinephrine and increased glucose output (Rosen et al., 1983). On the other hand, idazoxan has been shown to be able to increase alcohol consumption with a concomitant decrease in blood glucose (Grupp et al., 1997). Given this, we attempted to demonstrate in one experiment that there was an inverse relationship between blood glucose levels and alcohol intake.
2. GENERAL METHODS

2.1 Experimental Animals and Housing

The subjects used in all experiments were naive male Wistar rats weighing between 300-350 g at the beginning of the experiment (Charles River, Montreal). In each experiment, the rats were individually housed in hanging wire cages and were maintained on a 12-hour reversed light cycle, with lights off at 7 am and on at 7 pm. During each experiment the rats had a continuous and unlimited access to Purina rodent chow (except during the food deprivation study) and tap water inside their home cages. Food consisted of Purina rodent chow was placed in hoppers attached to the front of each cage. Cages were equipped with automatic watering system to provide continuous and unlimited access of water to the rats.

2.2 The Limited Access Procedure of Alcohol Self-administration

The reversed light cycle allowed for experiments to be carried out during the dark phase of the rats’ circadian cycle, the time during which rats are normally active. All rats were weighed every day. During the daily 40-minute drinking sessions, the animals were transferred from their home cages to the separate drinking cages with alcohol and water solutions available in 20 ml graduated glass tubes in front of each cage. The volume in each tube was read before rats were transferred. No food was available in the drinking cages during the drinking sessions. Sessions were run for 40 minutes and rats were left undisturbed during this time. The final volume of the drinking tubes was read after the session finished and the rats were returned to their home cages. The tubes were
removed from the drinking cages and emptied. The positions of the alcohol and water tubes were alternated each day to prevent the development of a position-preference.

The concentration of alcohol solution offered to the rats was initially 3% weight per volume diluted from 95% alcohol using tap water. The levels of intake of each fluid (i.e. alcohol and water) were plotted on graphs each day. When the rats had shown a clear preference for alcohol, the concentration of alcohol was raised to 6% weight per volume where it remained for the duration of the experiment. When the level of alcohol intake had stabilized after switching to the 6% alcohol solution, experimental manipulations such as drug injections commenced.

The amounts of both alcohol and water drinking during the final six days of the 6% phase was averaged for each rat and serves as that rat’s baseline. Also, this average value of alcohol intake would be used to select the rats from the original group which had acquired consistent drinking of significant amounts of alcohol to produce a pharmacologically relevant blood alcohol concentration. Rats typically metabolize alcohol at 300 mg per kilogram per hour, and this amount is equivalent to 5 mL/kg/session of 6% w/v alcohol (Wallgren & Barry, 1970). This level of intake was considered to be the minimum each rat must drink during baseline in order to be included in the study. Usually, 70-80% of the group trained drink enough to be included in the experiment. The rest of the subjects were discarded from the study. In case of studies utilizing a group design, the selected rats were randomly assigned to treatment groups in such a way that the baseline alcohol intake was approximately equal among groups.

2.3 Treatment

In order to determine the appropriate routes and times of drug administration, the primary
concern is that the drugs act while the animals are in the drinking cages. Hence, the routes and times of drug administrations were based on prior successful experiments from the literature with similar protocols or pilot studies carried out in our laboratory.

2.4 Measurements of Blood Glucose

Blood glucose measurements were taken using the Glucometer Elite Blood Glucose Meter (Bayer Healthcare Division, Etobicoke, Ontario, Canada). To collect the blood sample, a droplet of blood was drawn from the nicked end of the tail and placed in contact with a Glucometer Elite test strip where capillary action drew a small amount (~5 μL) of blood into the reaction chamber. After 60 seconds, a blood glucose reading was displayed and recorded. The limited access procedure was not run and alcohol was not available during these measurements.

2.5 Statistical Analysis

In most of the experiments, a one-way repeated measures analysis of variance (ANOVA) was used to determine if there was a significant change in alcohol intake during the course of experiment. When the test result from the ANOVA was significant (F statistic value less than 0.05), post hoc Duncan’s test were employed to examine the relevant pairwise comparisons. A drug treatment was deemed to be effective if the alcohol intake on the drug treatment day was significantly different from the immediate preceding and following intervening days. In addition, alcohol intake on the drug treatment day had to be significantly different from the alcohol intake on the saline vehicle day. The critical $p$ value of the Duncan tests was set to 0.05.

Where indicated, a two-way repeated measures ANOVA was performed with phase (dose)
as the within group factor and treatment (group) as the between group factor. When significant effects of group, phase or group*phase interaction were found, post hoc multiple Duncan's comparisons were performed. In such studies, a one-way randomized block analysis of variance were used to determine the effect of phase within each group. This analysis compared the effects on drinking in each group with its own baseline. On the other hand, a one-way unweighted means analysis of variance was used to determine the effect of treatment within each phase. This analysis was used to compare the drug effects on drinking during the same phase in the control group or other relevant groups. The use of this procedure was limited to cases in which the experimental design called for a comparison between experimental groups. Blood glucose data were analyzed in the same manner as the fluid consumption. Finally, results of the statistical analyses (ANOVA Tables) for each experiment were listed in the Appendix section.
3. EXPERIMENT ONE

THE EFFECTS OF GLUCOSE AND L-MALATE ON ALCOHOL INTAKE

3.1 Rationale

Glucose is known to inhibit food intake in rats and some other species when infused into the hepatic portal vein (Novin et al., 1985; Tordoff & Friedman, 1986). Recent research into the mechanism of the inhibition of food intake suggests the involvement of a glucostatic mechanism originating in the liver (Russek, 1981; Tordoff et al., 1991). For example, it has been observed that the transient drop or rise in blood glucose levels and glucose utilization in the liver are responsible for meal initiation and termination respectively (Louis-Sylvestre & LeMagnen, 1980; Campfield et al., 1985). If the effect of glucose on meal termination is mediated through the generation of a satiety signal, increasing the blood glucose level should lead to a decrease of food intake. Because of its caloric value, alcohol can be regarded as a food substance. It is therefore possible that the intake of alcohol may also be regulated by the same mechanisms which are involved in the regulation of food intake.

In a recent study carried out in our laboratory, the effects of angiotensin II (ANG II) combined with tolbutamide and the effects of glucose combined with morphine on alcohol consumption were investigated (Grupp et al., 1997). ANG II reduces alcohol intake and produces glycogenolysis (Ma et al., 1979; Koide et al., 1985). Competitive bindings studies have identified angiotensin receptors in the liver and shown that the majority of these are of the AT$_1$ subtype which is the same subtype that mediates the inhibition of alcohol drinking. If ANG II reduces alcohol
intake, in part, through its glycogenolytic action, then injections of a drug which inhibits glycogenolysis should attenuate the ability of ANG II to reduce alcohol intake. Tolbutamide, a sulfonylurea, has been shown to inhibit glycogenolysis induced by Ca$^{2+}$ dependent hormones such as ANG II, through a direct action on the liver and through its ability to stimulate insulin secretion. In summary, the results of that experiment showed that a dose of 100 mg/kg of tolbutamide produced a robust hypoglycaemia together with a reduction in alcohol intake. However, when the 100 mg/kg dose of tolbutamide was combined with ANG II, it did not enhance the inhibitory effect of ANG II on alcohol intake but rather attenuated it and even caused a net increase in alcohol intake. This indicated that the ability of ANG II to reduce alcohol intake was antagonized by a physiological mechanism. In addition, we also found that the morphine-induced alcohol consumption could be attenuated by glucose injections. Taken together, these findings suggest the mechanisms which maintain glucose homeostasis may be directly involved in regulating alcohol consumption.

The encouraging results seen with the glucose-mediated attenuation in morphine-stimulated alcohol intake raised the question of whether glucose on its own could produce a reduction in alcohol intake in rats given the same 40 minutes limited access to alcohol.

In the second part of the present experiment, another possibility was investigated. Besides the glucose-mediated satiety signal, "phosphate-trapping" has also been found to inhibit the ATP synthesis pathway and lead to an increase of food intake (Rawson et al., 1994). On the other hand, substances like L-malate, which can act as a substrate precursor for the synthesis of ATP, have been found to decrease food intake. Therefore, it is possible that the effect of glucose on food intake may, at least in part, be mediated through the ATP generation pathway. Thus, with the use of a substrate provider such as L-malate, alcohol consumption may be blocked via a feeding-related mechanism.
In fact, in a study by Singh et al. (1983), L-malate had the same effects as glucose in attenuating the analgesic action of morphine. As a result, the effects of glucose and L-malate will be tested in the first experiment to explore the role of glucoregulatory processes in alcohol intake.

3.2 Methods

3.2.1 Experimental Animals

The subjects were naive male Wistar rats (Charles River, Montreal) weighing between 280 and 300 g at the start of the experiment. A total of 15 rats (n=15) completed this experiment. After 1 week of acclimation in the vivarium, the animals were introduced to the limited access drinking procedure which was conducted 7 days a week. The animals were maintained on a reversed light cycle and the experiments were conducted during the dark portion of the cycle when the animals were normally active.

3.2.2 Drug Preparations

Alcohol solution was prepared by diluting 95% ethanol w/v with tap water to a required concentration: 3% w/v during the initial training and 6% w/v for the remainder of the experiment.

Glucose (Baxter sterile USP, 5%) injections were given i.p. according to the weights of the rats. L-malate (Sigma Chemical Co.) injections were administered i.p. at the lower doses (18 and 36 mg/kg) and subcutaneously at the higher doses (250, 500, 750 mg/kg).

3.2.3 Procedure

40 Minutes Limited Access

Training and selection: Using the 40 minutes limited access procedure, rats were offered 3% ethanol w/v to drink for the first 2 weeks, followed by 6% ethanol w/v for the next 2 weeks. Based
on the average alcohol intake during the last six days of training with 6% ethanol, the rats which consistently consumed alcohol in amounts exceeding their metabolic limits were selected.

Treatment:

At the beginning of the experiment, glucose in doses of 200, 400, 800 and 1200 mg/kg were administered i.p. to all rats 10 minutes prior to the drinking session on a drug testing day. Each drug testing day was separated by an intervening washout day to allow the animals to recover to their normal drinking levels. The saline vehicle was administered in the same route and schedule as the glucose injections.

Following the testing of parenteral glucose injections, low doses of L-malate, 18, 36 mg/kg, were given i.p. 30 minutes (Singh et al., 1983) before the drinking session. Higher doses of L-malate, 250, 500 to 750 mg/kg, were administered subcutaneously 2 hours (Langhans et al., 1985) before the drinking session.

Finally, a dose of naltrexone at 3 mg/kg, was administered to all the rats 30 min prior to limited access to compare its inhibitory effect on alcohol consumption with glucose and L-malate.

3.3 Results

The effects of glucose and L-malate injections on alcohol intake

Figure 1 illustrates the mean alcohol consumption over the entire experiment. The values of the bars represented the average alcohol intake of the rats on the drug testing days and the error bars indicated the values of the standard error. The filled circles linked by solid lines represented the average alcohol consumption of the rats on the intervening days during which the limited access procedure was run but no injections were given. A one-way repeated measures analysis of variance
revealed a significant change in alcohol consumption over the course of the experiment \([F_{36,318}=4.11, P<0.00001]\). Although the 200, 800 and 1200 mg/kg doses of glucose injections appeared to decrease alcohol intake, post hoc Duncan’s test \([P<0.05]\) revealed that the decreases were not significant. Injections with the saline vehicle did not alter the alcohol consumption in the rats when compared with the immediately preceding or following intervening days. Furthermore, alcohol intake recovered to the baseline level on the intervening days indicating that there was little or no carry-over effect from the injections on the previous glucose testing day.

Alcohol consumption fluctuated during the several trials of L-malate injections, however no clear pattern of the effects of the L-malate injections emerged. Post hoc Duncan’s tests \([P<0.05]\) confirmed that the fluctuations in alcohol intake were not significant relative to the saline injection and the intervening days. On the final drug testing day, naltrexone (3 mg/kg) was given to all rats to compare its effects with the previous glucose and L-malate injections. Alcohol intake was decreased by approximately 10 ml/kg (or 0.6 g/kg) on that day. Post hoc Duncan’s tests \([P<0.05]\) confirmed that this inhibition in alcohol intake produced by naltrexone was significant when compared with the saline injection and the intervening days.
Figure 1. Effect of glucose and L-malate on alcohol intake.

Mean daily intake of alcohol during the 40-minute limited access procedure. _T bars_ represent the standard error of the mean. _Filled circles connected by solid lines_ represent the alcohol intake on intervening days.

GLUC-Glucose

LM-L-malate

SAL-Saline

NALT-Naltrexone

Numbers underneath represent doses in mg/kg.
ALCOHOL INTAKE ml/kg

GLUC-Glucose
LM-L-malate
NALT-Naltrexone
SAL-Saline

• Intervening day

ALCOHOL INTAKE g/kg
The effects of glucose and L-malate injections on water intake

Figure 2 shows the mean water consumption for all the rats during the same period. A one-way ANOVA revealed a significant change in water intake in the course of the experiment \( F_{36,518} = 2.30, P < 0.0001 \). Post hoc analysis \( P < 0.05 \) revealed that the water intake on the first trial of 200 mg/kg glucose injection was significantly higher than those in the rest of the experiment. Although water consumption fluctuated during the course of the experiment, the water consumption remained relatively stable over the experiment and the fluctuations were not statistically significant. Hence, glucose had no effects on water intake except on the first trial and L-malate did not alter water intake at all.

The effect of glucose injections on blood glucose

Figure 3 illustrates the blood glucose time course following an 800 mg/kg i.p. dose of glucose or a saline vehicle injection. A two-way repeated measures ANOVA showed significant effects of Treatment \( F_{1,140} = 63.59, P < 0.0001 \), Time Interval \( F_{5,140} = 16.34, P < 0.0001 \), and Treatment*Time Interval interaction \( F_{5,140} = 6.29, P < 0.0001 \). Duncan's tests \( P < 0.05 \) revealed that blood glucose levels were significantly higher in the glucose-treated group when compared to the saline group for all time intervals. These findings indicated that glucose injections at the 800 mg/kg dose significantly raised blood glucose levels in the rats.
Figure 2. Effect of glucose and L-malate on water intake.

Mean daily intake of water during the 40-minute limited access procedure. *T bars* represent the standard error of the mean. *Filled circles connected by solid lines* represent the water intake on intervening days.

GLUC-Glucose

LM-L-malate

SAL-Saline

NALT-Naltrexone

Numbers underneath represent doses in mg/kg.
GLUC-Glucose
LM-L-malate
NALT-Naltrexone
SAL-Saline

- Intervening day

WATER INTAKE ml/kg

- GLUC 200
- GLUC 200
- GLUC 400
- GLUC 400
- GLUC 800
- GLUC 800
- GLUC 800
- GLUC 800
- SAL
- GLUC 1200
- GLUC 1200
- LM 18
- LM 18
- LM 36
- LM 250
- LM 500
- LM 750
- NALT 3
Figure 3. Effect of 800 mg/kg glucose injections on blood glucose level.

Mean blood glucose levels in the vehicle and glucose treated rats. *Bars* represent the standard error of the mean. Asterisk (*) denotes significance relative to the saline vehicle. T5, T10, T15...T30 min are the blood glucose sampling times and correspond to 5, 10, 15...30 min into the limited access procedure.
3.4 Conclusion

Glucose given peripherally was shown to have no effect on alcohol intake in a limited access paradigm. Although there appeared to be a reduction in alcohol consumption on several trials, the decrease was insignificant and there was no consistent dose-response pattern. The effect of 1200 mg/kg i.p. glucose injection was equivocal because at this dose of glucose, the glucose solutions were prepared by mixing glucose with saline solution to a final concentration of a 10% glucose solution (concentration two times higher than the stock glucose solution). It was noted that when this solution was administered to the rats, the animals became agitated and stretched their bodies dramatically particularly in the abdominal area. Injections of solutions of such a high osmolarity may have caused severe discomfort in these animals by drawing water rapidly away from the intestines because of the osmotic pressure gradient. By the same token, this phenomenon may help explain why the first trial dose of 200 mg/kg glucose injection increased water consumption significantly on the first day of the experiment. Since the physiological effects of the glucose solution injections were novel to the rats on the first trial, the rats might consume more water to replenish the loss of water by osmosis from their intestines. However, these effects of glucose on water consumption were attenuated or abolished afterwards which might be in part due to the rats had been habituated to the physiological effects produced by the injections after numerous trials.

In the second phase of the experiment, L-malate was found to have no effect on both alcohol and water consumption. Since there were no changes in alcohol intake observed throughout the experiment, the opiate antagonist, naltrexone, was used at the end of the experiment as a positive control to demonstrate that this group of animals could respond to a recognized treatment.
To confirm that there was an effect on blood glucose levels produced by the peripheral administration of glucose, a blood glucose time course in these animals was recorded after an i.p. injection of 800 mg/kg dose of glucose. Although there was a significant increase in blood glucose levels produced by the 800 mg/kg glucose injections when compared with the saline vehicle injections, the rise in blood glucose levels however had no effect on alcohol intake. This was the first piece of direct evidence demonstrating that high blood glucose levels \textit{per se} are not involved in modulating alcohol intake. Congruent with the glucostatic theory, glucose utilization rather than levels \textit{per se} may be another aspect which has to be achieved in order to inhibit alcohol intake.
4. EXPERIMENT TWO

THE EFFECTS OF FOOD DEPRIVATION AND GLUCOSE INJECTIONS ON ALCOHOL INTAKE

4.1 Rationale

The previous experiment demonstrated that the peripheral administrations of glucose or L-malate had no effect on alcohol intake. When glucose was given to the rats, however, it raised blood glucose levels significantly. These results suggested that the circulating blood glucose levels per se might not modulate alcohol intake. As already noted in the glucostatic theory, it has been demonstrated that glucose utilization is an equally important aspect which modulates food intake. More recent studies view fatty acids as well as glucose as major sources of metabolic fuels whose utilization produces satiety signals (Rawson & Friedman, 1994; Langhans, 1996). In the previous experiment involving the administration of an exogenous dose of glucose in freely-fed rats, it was reasonable to assume that glucose would be converted into glycogen and stored. Therefore, if the glucose administered was not being utilized as a fuel source, no satiety signal would be generated and alcohol intake would remain unchanged.

In retrospect, glucose utilization seems to be able to provide an explanation for the absence of effects on alcohol intake observed with the glucose injections in the freely fed rats. In an experiment conducted by Tordoff & Friedman (1986), they found that glucose infusions into the hepatic portal vein decreased food intake but did not cause an increase in peripheral blood glucose levels suggesting that most of the glucose administered was utilized by liver and thus may have provided a satiety signal to inhibit food intake. It has been demonstrated in a number of studies that
food deprivation increases glucose utilization robustly in rats (Tsujii et al., 1988; Issad et al., 1987; Garcia-Salguero & Lupianez, 1989). As a result, the present experiment is designed to provide an altered glucose utilization state in the rats by means of food restriction such that injected glucose will be utilized and not stored.

Several studies have shown that food deprivation has an important influence on drug self-administration in laboratory animals (Carroll & Meisch 1979, 1984; Files et al., 1993). In general, the self-administration of drugs that readily serve as reinforcers (e.g. cocaine, opiates and alcohol) increases under food deprivation conditions while self-administration is not altered by food deprivation when the maintenance drug (e.g. diazepam) is not readily reinforcing (Carroll et al, 1981; de la Garza & Johnson, 1987). To date, however, the mechanisms by which food deprivation alters these behaviours is still unclear. It may be that by depriving rats of a reinforcer (e.g. food), drug-seeking behaviour for other alternatives increases. For example, a recent study demonstrated that acquisition of cocaine self-administration was either reduced or completely abolished in rats with concurrent access to glucose or saccharin (Carroll & Lac, 1993). Yet, the role of glucose utilization changes in food deprivation conditions has never been investigated as a possible explanation for the alterations in the drug-seeking behaviour. Since glucose utilization has been cited as an important variable in the control of food intake, the purpose of the present study is to assess the involvement of glucose utilization in the control of alcohol intake.

This experiment is divided into three phases. In the first phase of the experiment we attempt to replicate our findings of the previous experiment by administrating glucose to sated animals. In the second phase we attempt to induce a different glucose utilization state in the rats by 24 hour food-deprivation. In the final phase, instead of inducing an acute food deprivation in the animals,
chronic food-deprivation will be used to study the effects of glucose injections and the changes in glucose utilization status on alcohol drinking.

Since alcohol consumption is known to be increased by manipulations which affect food intake (e.g. food deprivation, Carroll & Meisch, 1984), it is possible that the food-deprived animals may become more sensitive to glucose injections because there is now a greater demand for glucose physiologically. Hence, the exogenous source of glucose administered may be expected to be utilized rather than stored in these animals who are now in a deprived state and alcohol intake may thus be reduced.

4.2 Methods

4.2.1 Subjects

The subjects were male Wistar rats (Charles River, Montreal) weighing between 280 and 300 g at the start of the experiment. After 1 week of acclimation to the vivarium, the animals were introduced to the limited access drinking procedure which was conducted 7 days a week.

4.2.2 Drug Preparation

Alcohol solution was prepared by diluting 95% ethanol w/v with tap water to a required concentration: 3% w/v during the initial training and 6% w/v for the remainder of the experiment.

Glucose (Baxter sterile USP, 5%) injections were prepared fresh daily and were given i.p. according to the weights of the rats.

4.2.3 Procedure

This experiment was divided into three phases, phase 1-food satiation, phase 2-acute food deprivation and phase 3-chronic food deprivation. The feeding conditions will be discussed in the
following section. The 40 minutes limited access procedure was run in all three phases to investigate the effects of glucose injections and food deprivation on alcohol intake.

40 Minutes Limited Access

Please refer to the GENERAL METHODS section for details.

Treatment: A glucose dose of 800 mg/kg i.p. was given to all animals in all of the three phases of the experiment. The saline vehicle was given by the same route and schedule as the glucose injections. Between each drug testing day there was an intervening washout day to allow the animals to recover to their baseline drinking levels. Doses of naltrexone at 3 mg/kg and 6 mg/kg were given to all the rats to compare its inhibitory effects on alcohol consumption with glucose at the end of the experiment.

4.2.4 Feeding Conditions

Rats were maintained under different food levels in each phase of the experiment: in phase one, rats were fed with unlimited access of Purina rodent chow throughout the whole phase. During phase 2 of the experiment, prior to each drug testing day, the rats were food deprived for 24 hrs without any access of food in their home cages or drinking cages. Rats were refed with unlimited access of food immediately after the completion of drug testing and the limited access procedure. In the final phase of the experiment, the rats were maintained at 80-85% of their free feeding weights. Specifically, after completion of phase two of the experiment, four pellets of Purina rat chow were given to all animals initially. The rats were weighed every day and if their weights decreased by less than 5 g compared to the previous day, one pellet of rat chow would be removed from feeding until the target weight was reached. When all animals achieved the desired weights, their weights were kept constant throughout the experiment by adjusting the number of pellets given.
4.3 Results

**Effects of food deprivation and glucose injections on alcohol intake**

Figure 4 shows the mean alcohol drinking of all animals over the course of experiment. A one-way repeated measures analysis of variance revealed a significant change in alcohol consumption \(F_{37,570}=11.42, P<0.00001\). One-way unweighted means analyses of variance were performed for each phase in order to test whether the effects of the glucose injections and the food deprivation treatments were significantly different compared to the saline treatments or the intervening day drinking levels.

In phase one, alcohol intake was not modified by glucose or saline injections confirming the findings of experiment one \(F_{8,135}=0.64, P=n.s.\). During phase two, alcohol intake was altered dramatically on the intervening days after the first trial of the acute food deprivation and saline treatment \(F_{13,210}=15.86, P<0.00001\). Post hoc Duncan's tests \(P<0.05\) revealed that the alcohol intake on the intervening days was significantly lower than the alcohol drinking at the beginning of the experiment and during the acute food deprivation treatment days. Nevertheless, alcohol consumption on the acute food deprivation treatment days with glucose or saline injections was not significantly different from the baseline drinking levels at the start of the experiment. Thus acute food-deprivation did not result in an inhibitory effect of glucose on alcohol intake. In the final phase of the experiment, there was a significant change in alcohol consumption \(F_{14,225}=2.13, P=0.0114\). Alcohol consumption increased in the final phase of the experiment compared to the preceding two
Figure 4. Effect of food-deprivation and glucose injections on alcohol intake.

Mean daily intake of alcohol during the 40-minute limited access procedure. *T bars* represent the standard error of the mean. *Filled circles connected by solid lines* represent the alcohol intake on intervening days.

GLUC-Glucose

AD-Acute food deprivation (24 hr.)

SAL-Saline

NALT-Naltrexone

Numbers underneath represent doses in mg/kg.
Phase 1 - Satiation
GLUC - Glucose
SAL - Saline
AD - 24hr food deprivation
NALT - Naltrexone

Phase 2 - Acute Food Deprivation

Phase 3 - Chronic Food Deprivation
GLUC - Glucose
SAL - Saline
NALT - Naltrexone
phases. Post hoc Duncan’s tests \([P<0.05]\) revealed that the increase in intake of alcohol during chronic food-deprivation was significantly higher than the levels during food-satiation or acute food-deprivation phase. Naltrexone at the 6 mg/kg dose reduced alcohol intake by about 50% and post hoc Duncan’s test revealed that this reduction was statistically significant \([P<0.05]\). On the other hand, the glucose, saline injections and the 3 mg/kg of naltrexone failed to modify alcohol intake over the entire chronic food deprivation phase. Thus chronic food-deprivation also did not enable an effect of glucose on alcohol intake. The significant effect of naltrexone on alcohol intake revealed that the alcohol consumption of these animals could indeed be modified by a drug manipulation.

**Effects of food deprivation and glucose injections on water intake**

Figure 5 shows the mean water intake of the same group of animals over the experiment. One-way repeated measures analysis of variance of the water data uncovered significant effects of treatment over the course of the experiment \([F_{37,570}=2.38, P<0.00001]\). Water consumption was reduced in the final phase of the experiment when compared to phase 1 or phase 2. Post hoc Duncan’s tests \([P<0.05]\) confirmed that water intake in phase 1 or phase 2 was significantly higher than that of phase 3 but water intake of phase 1 and phase 2 were not significantly different from one another.

**Effects of food deprivation and glucose injections on blood glucose levels**

Figure 6 illustrates the effects of food deprivation and glucose injections on blood glucose levels in phase 1 and phase 3 of the experiment. Measurements were taken at 10, 20 and 30 minutes following the beginning of the limited access procedure and were done on a separate day following completion of the alcohol drinking experiment. The limited access procedure was not run on these days.
Figure 5. Effect of food-deprivation and glucose injections on water intake.

Mean daily intake of water during the 40-minute limited access procedure. $T$ bars represent the standard error of the mean. Filled circles connected by solid lines represent the water intake on intervening days.

GLUC-Glucose

AD-Acute food deprivation (24 hr.)

SAL-Saline

NALT-Naltrexone

Numbers underneath represent doses in mg/kg.
Figure 6. Effect of chronic food-deprivation and glucose injections on blood glucose level.

Mean blood glucose levels in sated or chronically food-deprived rats when given glucose (800 mg/kg) or vehicle injections. T10, T20, T30 min are the blood glucose sampling times and correspond to 10, 20, 30 min into the limited access procedure.
A two-way repeated measures analysis of variance showed significant effects of Treatment \(F_{3,120}=125.31, P<0.00001\), Time \(F_{2,120}=13.43, P<0.00001\) and the interaction of Treatment*Time \(F_{6,120}=4.92, P=0.0002\). Post hoc analysis revealed significant treatment differences in blood glucose levels at all time intervals for all four treatment schedules. Glucose injections (800 mg/kg) produced a significant \(P<0.05\) increase of blood glucose both in the food satiation phase and the chronic food deprivation phase. Furthermore, post hoc Duncan's tests \(P<0.05\) revealed that blood glucose levels during the chronic food deprivation phase were significantly lower than those during the food satiation phase both with and without glucose injections. This indicates that chronic food deprivation was a successful manipulation in producing glucose utilization and reducing the glucose storage in the animals in the final phase of the experiment.

4.4 Conclusion

In the first phase of the experiment, under food-satiation conditions glucose injections increased blood glucose levels but again failed to reduce alcohol intake replicating our previous work. Under chronic food deprivation blood glucose is low compared to that of the satiation phase and alcohol drinking is increased. The fact that the 800 mg/kg dose of glucose produces lower blood glucose levels during deprivation than during satiation indicates that the glucose administered has been utilized yet even under these conditions it does not reduce alcohol intake. Therefore, even under conditions when a satiety signal is presumably being produced, alcohol intake is still not affected suggesting that the satiety signal in food intake does not influence in the regulation of alcohol intake. On the other hand, naltrexone at a dose of 3 mg/kg which robustly reduced alcohol intake in previous experiment did not decrease alcohol intake significantly in the chronic food-
deprivation phase. When the dose of naltrexone was increased to 6 mg/kg, it produced a significant reduction in alcohol intake. The attenuation of the naltrexone-induced reduction of alcohol intake could be due to the increased motivation to consume alcohol during chronic food-deprivation.

Interestingly, alcohol intake on the intervening days in the acute food-deprivation phase was dramatically reduced after the first day of trial of 24 hour food-deprivation and the reduction in alcohol intake did not recover in the subsequent intervening days. A possible explanation for this phenomenon might be since the rat consumed a significant amount of alcohol with an empty stomach, this resulted in rapid absorption into the central nervous systems and possibly a very high level of intoxication in a relatively short period of time. It is possible that the unpleasant pharmacological effects of alcohol produced by this rapid absorption might have led to subsequent aversion to alcohol when the rats were sated.
5. EXPERIMENT THREE

THE EFFECTS OF EXPERIMENTALLY-INDUCED DIABETES AND INSULIN ON ALCOHOL INTAKE

5.1 Rationale

Glucose intolerance is a metabolic disorder that has been implicated in alcohol preference in animals (Goas et al., 1978). The C57 Bl/6j strain of mice, which show a hyperglycaemic response to glucose challenge, has been shown to prefer alcohol to water and consume an average of 82% of their total daily fluid as alcohol. Moreover, alcohol has been shown to produce a greater hypoglycaemic effect in these animals (Connelly & Taberner, 1981). Although a causal relationship cannot be demonstrated from a single inbred strain of mice, such findings suggest that a metabolic dysfunction such as glucose intolerance may well be a predisposing factor for alcohol intake. It is well recognized that chronic alcohol consumption produces a variety of metabolic disturbances including hypoglycaemia in fasted animals (Singh & Patel, 1976; Singh et al., 1976) and humans (Tramill et al., 1981) and abnormally high glucose tolerance (Myrhed, 1975; Phillips & Safrit, 1971).

In addition, prior administration of small amounts of alcohol to normal subjects can produce a potentiation of the plasma insulin response to administered glucose (Metz et al., 1969). Although there seems little doubt that biochemical disturbances may be consequential to chronic alcohol intake, the converse of this may also hold. That is, an altered metabolic status may influence the amount of alcohol consumed. This may be not only valid in the latter stages of alcoholism, but individual variations in glucose metabolism may be an important factor during initial alcohol exposures. For instance, an unexpectedly high percentage of human alcoholics displaying
abnormally high glucose tolerance (Phillips & Safrit, 1971; Myrhed, 1975) suggests the possibility that diabetogenic disturbances may function as predisposing factors, which if combined with appropriate environmental conditions, could produce a compelling stimulus for alcohol consumption and addiction (Goas et al., 1978).

In the previous experiments in which we looked at the effects of glucose injections and glucose utilization on alcohol intake, no significant effects were found. In the present study we have used a relatively common procedure (i.e. injections of streptozotocin, a pancreatic β cell toxin) to induce diabetes to test whether this endogenous hyperglycaemic condition is sufficient to produce changes in alcohol intake in rats. Furthermore, insulin injections will be given to the animals to examine their effects on alcohol consumption since insulin can exert a potent hypoglycemic action. The aims of the present work were therefore, first, to compare the effects of experimentally-induced diabetes on alcohol intake in rats which exhibit elevated blood glucose levels endogenously and, second, to manipulate the blood glucose levels by use of insulin in order to examine whether or not the changes in blood glucose levels would alter alcohol consumption.

5.2 Methods

5.2.1 Experimental Animals

Drug-naive male Wistar rats (Charles River, Montreal) weighing between 290-340 g served as subjects for this experiment. Upon receipt from the supplier animals were individually housed in hanging wire cages (reversed 12 hour light/dark cycle) where they received free access to water and food (Purina rodent chow) throughout the experiment. Following a one week acclimation period animals were randomly divided into three treatment groups (Streptozotocin 65 mg/kg, Streptozotocin
40 mg/kg, Control). A total of 24 rats (n=24) completed this experiment.

5.2.2 Drug Preparations

Alcohol solutions were prepared by mixing 95% ethanol w/v with tap water to a required concentration: 3% w/v during the initial training and 6% w/v for the remainder of the experiment.

Thiopental, 20 mg/kg, was administered i.p. as a tranquilizer for the rats before the administration of tail vein injections.

Streptozotocin (Sigma Chemical Co., St. Louis) in two doses of 40 mg/kg and 65 mg/kg were administered into the tail vein in physiological saline (0.9% w/v).

Slow releasing insulin zinc protamine (Eli Lilly, Indiana) in doses of 10 U/kg, 20 U/kg and 40 U/kg were administered s.c. in physiological saline (0.9% w/v).

5.2.3 Procedure

Rats were randomly assigned into three groups: group 1 (n=7) received an injection of 65 mg/kg streptozotocin, group 2 (n=7) received an injection of 40 mg/kg streptozotocin and group 3 (n=10) was given the saline control. Each group was then offered a choice of alcohol or water in separate drinking cages for a period of 40 minutes each day. Initially the animals were offered a choice between 3% w/v ethanol and water for 14 days and then a choice between 6% w/v ethanol and water for the remainder of the experiment.

This experiment started with a baseline phase, where the average intake of alcohol and water during the last six days of training with 6% ethanol was used as a baseline for the comparison to fluid intake during the phases where the drugs were administered. This phase lasted for six consecutive days.

Three treatment phases followed which were each 10 days in duration. In phase one, all
subjects received 10 U/kg insulin daily at 4:00 pm. It should be noted that since the limited access procedure was performed at 10:00 am every morning, there was a 16 hours lapse between the insulin injections and the limited access procedure. It has been demonstrated in a number of studies (Smith et al., 1987; Tucker et al., 1991; Nielsen et al., 1995) that insulin administrations given with this timing can normalize blood glucose levels while not affecting the animals' feeding behavior. The dose of insulin increased to 20 U/kg/day in phase two and during phase three, all animals received 40 U/kg/day insulin. The consumption of 6% ethanol and water was measured across 10 consecutive days.

5.2.4 Blood glucose measurements

Blood glucose levels were measured at the end of each phase using the Glucometer Elite Blood Glucose Meter (Bayer, Ontario, Canada). Two samples which were 30 minutes apart were obtained from each animal on a blood glucose day. The limited access procedure was not run and alcohol was not available during these measurements.

5.3 Results

The data displayed in figure 7, panel A are the mean alcohol consumption for each group over the course of the experiment. A two-way repeated measures analysis of variance was performed on the alcohol data and revealed a significant effect of phase \([F_{3,63}=4.63, P=0.0055]\). No effect of treatment \([F_{2,63}=0.90, P=n.s.]\) or treatment*phase interaction \([F_{6,63}=1.14, P=n.s.]\) was found on alcohol consumption. This suggests that while alcohol intake was modified over the course of experiment the effect did not appear to be dependent on treatment or on a specific phase of treatment.

One-way unweighted means analysis of variance of phase, with group (treatment) as the
Figure 7. Effect of experimentally-induced diabetes and insulin on alcohol or water intake.

Mean alcohol intake (panel A) or water intake (panel B) in the groups pretreated with either 65 mg/kg streptozotocin (group 1), 40 mg/kg streptozotocin (group 2), or sham operated (group 3). In phase 1, all groups received 10 Units/kg insulin per day. In phase 2, all groups received 20 Units/kg insulin per day. In phase 3, all groups received 40 Units/kg insulin per day. \( T \) bars represent the standard error of the mean.
A

ALCOHOL INTAKE mL/kg

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Phase 1 (Insulin 10)</th>
<th>Phase 2 (Insulin 20)</th>
<th>Phase 3 (Insulin 40)</th>
</tr>
</thead>
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B

WATER INTAKE mL/kg

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Phase 1 (Insulin 10)</th>
<th>Phase 2 (Insulin 20)</th>
<th>Phase 3 (Insulin 40)</th>
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within phase factor, was performed for alcohol intake in the baseline phase. No groups differed in alcohol intake \[F_{2,21}=0.49, P=\text{n.s.}\] and thus, all groups started with equal levels of alcohol intake. This indicates that experimentally-induced insulin dependent diabetes had no effect on alcohol acquisition during the early stages of alcohol exposure. A one-way randomized block analysis of variance of alcohol drinking in three treatment groups revealed no significant effects on alcohol drinking in each group, group 1 (65 mg/kg streptozotocin) \[F_{3,24}=2.01, P=\text{n.s.}\], group 2 (40 mg/kg streptozotocin) \[F_{3,24}=0.47, P=\text{n.s.}\] and group 3 (saline control) \[F_{3,20}=0.44, P=\text{n.s.}\]. Hence, although alcohol drinking appeared to increase slightly with increasing doses of insulin in particular to the 65 mg/kg streptozotocin pretreated group, the changes in alcohol intake were not significant.

Figure 7, panel B shows mean water consumption during the baseline and the three insulin treatment phases. Two-way repeated measures analysis of variance of the water data revealed significant effects of treatment \[F_{2,63}=8.77, P=0.0017\], phase \[F_{3,63}=6.34, P=0.0008\] and treatment*phase interaction \[F_{6,63}=4.44, P=0.0008\]. From the observation of the graph, this indicates that water intake was decreased over the course of the experiment in a manner dependent on the insulin doses.

One-way unweighted means analysis of phase, with group (treatment) as the within phase factor, was performed for water intake in the baseline phase and revealed a significant difference of groups within this phase \[F_{2,21}=17.85, P<0.00001\]. Post hoc Duncan's analysis \[P<0.05\] confirmed what is obvious from the graph that water intake in the 65 mg/kg streptozotocin treated group consumed more water than the rest of the subjects. A high level of water intake is characteristic of diabetic subjects because they would consume larger amounts of fluid to excrete the additional glucose present in their bodies. This phenomenon of diabetes is known as glycosuria and this also
serves as an independent confirmation that this group of animals were in fact diabetic. One-way unweighted means analysis of the 10 U/kg/day \([F_{2,21}=5.38, P=0.013]\) and 20 U/kg/day \([F_{2,21}=4.18, P=0.0296]\) insulin treatment phases revealed that water consumption was higher than the rest of the subjects. However in phase 3 of the experiment, when 40 U/kg of insulin was administered, no significant effects on water intake were found among the three groups \([F_{2,21}=1.67, P=n.s.}\). This indicates that insulin administered at the highest dose, 40 U/kg/day, appeared to be effective in correcting the diabetogenic disturbances and reversed the glycosuria as evidenced by a decrease in water intake.

Figure 8 illustrates the blood glucose levels recorded after each phase of the experiment. A two-way repeated measures analysis of variance revealed significant effects of treatment \([F_{2,63}=472.35, P<0.00001]\), phase \([F_{3,63}=3.84, P=0.0137]\) and treatment*phase interaction \([F_{6,63}=3.17, P=0.0088}\). Post hoc Duncan's tests \([P<0.05]\) showed that blood glucose levels of the 65 mg/kg streptozotocin treated group were higher than the other two groups across all phases. One-way randomized block analysis of variance was performed for each group with phase as the within group factor. This was done to examine any changes in blood glucose levels across phases in each group. No effects of blood glucose levels were found for the 40 mg/kg streptozotocin treated \([F_{3,24}=0.76, P=n.s.}\) or the saline control group \([F_{3,33}=2.39, P=n.s.}\). Although it seemed to produce a slight decrease in blood glucose levels for the 65 mg/kg streptozotocin treated group, no significant effects were found \([F_{3,24}=0.91, P=n.s.}\). Taken together, these findings indicated that the daily injections of insulin at various doses had no effects on decreasing the blood glucose levels in the diabetic rats at the interval tested.
Figure 8. Effect of experimentally-induced diabetes and insulin on blood glucose level.

Mean blood glucose levels in the groups pretreated with either 65 mg/kg streptozotocin (group 1), 40 mg/kg streptozotocin (group 2), or sham operated (group 3). In phase 1, all groups received 10 Units/kg insulin per day. In phase 2, all groups received 20 Units/kg insulin per day. In phase 3, all groups received 40 Units/kg insulin per day. Bars represent the standard error of the mean.
5.4 Conclusion

From the baseline phase of alcohol intake, our findings suggested that an endogenous elevated blood glucose level did not have any effects on the acquisition of alcohol drinking. This indicated once again that a high blood glucose level per se was not sufficient to impair or prohibit alcohol intake.

When insulin was administered to the diabetic rats, alcohol consumption appeared to increase slightly but the increase was not statistically significant. No effects of alcohol consumption were observed in the other two groups of animals during the insulin administration phases. Since insulin was administered as a daily maintenance dose at 4 pm, the short-term hyperphagic response induced by insulin was not observed in these animals tested. Thus, alcohol intake remained not affected and stayed relatively stable across the treatment phases.

Although insulin was shown to be able to decrease the water consumption in the diabetic rats, no effects on the blood glucose levels were found. On the other hand, in the literature, these doses of insulin have been demonstrated to be effective in reducing the blood glucose levels in these streptozotocin-induced diabetic rats (Smith et al., 1987; Tucker et al., 1991; Nielsen et al., 1995). The lack of effect observed in the blood glucose levels in this experiment is therefore puzzling. However, when we examined the protocol of this experiment, insulin injections were administered daily at 4:00 pm while the sampling of blood glucose took place at 10:00 am the following day. Since there was a 18 hr time lapse between the insulin injections and blood glucose measurements, the lack of effect as seen in blood glucose could be due to the timing of the injection. If the timing of insulin injections had been brought closer to the time of blood glucose measurements, we might have observed an effect of insulin on blood glucose.
6. EXPERIMENT FOUR

THE EFFECTS OF TRIFLUOPERAZINE AND 2,4-DINITROPHENOL ON THE INHIBITORY EFFECT OF NALTREXONE ON ALCOHOL INTAKE

6.1 Rationale

Evidence suggests that alcohol-induced activation of the endogenous opioid system may be part of a neurobiological mechanism that is involved in mediating alcohol reinforcement and excessive drinking behaviour. For example, alcohol consumption in animals is enhanced by opiate agonists (Reid & Hunter, 1984; Hubbell & Reid, 1990) and reduced by opiate antagonists such as naltrexone (Hubbell et al, 1988; Linseman, 1989). Although naltrexone (REVIA®) has been approved for the treatment of alcoholism in humans, its mechanism of action is not clear. One hypothesis suggests that alcohol use leads to an increase in endogenous opioid activity, which then promotes alcohol's reinforcing effects (see review by Swift, 1995; Froehlich and Li, 1994). This hypothesis is supported in part by the demonstration that opioids stimulate the release of dopamine, a neurotransmitter implicated in reinforcement. Therefore, researchers have hypothesized that a medication that blocks opioid activity may block the reinforcement aspects of alcohol (Swift, 1995).

On the other hand, with regard to consummatory behaviour, it has been repeatedly demonstrated that the nonselective opiate receptor antagonists such as naloxone or naltrexone have a potent inhibitory effect on food intake (Sanger, 1981; Apfelbaum & Mandenoff, 1981; Brands et al., 1979). Furthermore, since opiate agonists were found to stimulate food intake (Grandison & Guidotti, 1977) while opiate antagonists inhibit feeding, the close parallel between the effects of opiates on feeding and alcohol drinking raises the possibility of a similar underlying mechanism.
McLaughlin and her colleagues (1986) examined the effects of an opiate antagonist, nalmefene on energy balance and glucose regulation in rats. They found that both acute and chronic nalmefene treatment were associated with increased glucose concentrations and decreased food intake. This indicated that glucose concentrations might play a part in the regulation of food intake and similarly, glucose concentrations might also influence consumption of alcohol. Therefore, in the first part of the present experiment, we will assess the effects of pretreatment with various doses of trifluoperazine (TFP), a glycogenolytic inhibitor, on alcohol intake and on the inhibitory effects of naltrexone on alcohol intake.

In a preliminary experiment, in which TFP was co-administered with angiotensin II to rats in the 40 minutes limited access procedure, TFP attenuated the reduction of alcohol intake produced by ANG II (unpublished observations). The mechanism of ANG II-induced inhibition of alcohol consumption is not known, but a major function of ANG II in the liver is to stimulate glycogenolysis (Ma et al., 1979). Since satiety is correlated with elevated glucose availability, ANG II may reduce alcohol intake by stimulating glycogenolysis and satiety. The fact that TFP has the ability to inhibit the activation of glycogenolysis by ANG II through a calmodulin blocking (i.e. a Ca** dependent) mechanism (Koide et al., 1982) has prompted our interests in this drug as it may imply a possible influence of glucoregulatory control of voluntary alcohol consumption.

In the second part of the experiment, an oxidative phosphorylation uncoupler, 2,4-dinitrophenol (Kessler, 1976) will be examined together with naltrexone. As hypothesized by Tordoff and Friedman (1993), an ATP satiety signal may be responsible in the control of feeding behaviour. Since the metabolic control of glucose is closely linked to cellular energetics (i.e. the ATP level), the implication of cellular ATP in glucostatic control of feeding and alcohol intake
appears to be possible. Unlike l-malate, which is an ATP-generating substrate, 2,4-dinitrophenol depletes cellular ATP (Slater, 1967). In a morphine-antinociceptive study carried out by Singh et al. (1983), l-malate synergized with glucose injections to enhance the attenuation of morphine-induced analgesia whereas 2,4-dinitrophenol attenuated the actions of glucose in morphine-analgesia. Hence, we may expect that when 2,4-dinitrophenol is co-administered with naltrexone to the rats, there may be an attenuation of the inhibition on alcohol intake produced by naltrexone. If so, this would suggest that alcohol drinking might be modulated by a cellular energy linked mechanism.

6.2 Methods

6.2.1 Experimental Animals

The subjects were male Wistar rats (Charles River, Montreal) weighing between 260 and 320 g at the start of the experiment. The animals were individually housed and maintained on a reverse 12 hour/12 hour light/dark cycle with lights off at 7 am. Purina rat chow and tap water were available in home cages ad libitum. No food was available during the drinking sessions. The experiment was conducted seven days a week, between 10 am and 1 pm, during the dark portion of the cycle. A total of 16 rats \(n=16\) completed the whole experiment.

6.2.2 Drug Preparations

Alcohol solution was prepared by diluting 95% ethanol w/v with tap water to a required concentration: 3% w/v during the initial training and 6% w/v for the remainder of the experiment. Trifluoperazine (RBI) was dissolved in physiological saline and injected intraperitoneally at doses of 6.25, 12.5 and 25 \(\mu g/kg\) 30 minutes prior to alcohol access.

2,4-dinitrophenol was purchased from Sigma Chemical Co. and dissolved in physiological
saline. The resultant solutions were adjusted to pH 7.2-7.5 by dropwise addition of sodium hydroxide. Injections were administered in doses of 5 and 10 mg/kg i.p. 30 minutes prior to alcohol access (Singh et al., 1983).

Naltrexone (Sigma Chemical Co.) was dissolved in physiological saline and injections were administered in doses of 1.5, 2 and 3 mg/kg i.p. 10 minutes prior to alcohol access.

6.2.3 Procedure

The experiment was divided into two phases, one testing the effect of trifluoperazine on the action of naltrexone, and the other testing the effect of 2,4-dinitrophenol on the action of naltrexone. The second phase of the experiment was run immediately after the first phase using the same animals.

40 Minutes Limited Access

Please refer to the GENERAL METHODS section for details.

Treatment:

Phase 1: Four different drug treatment combinations were administered to all rats on successive treatment days. These combinations were: 1) saline followed by naltrexone; 2) TFP followed by naltrexone; 3) TFP followed by saline; 4) saline followed by saline. The first and second injections were separated by 20 minutes and the animals were placed into the drinking cages 5 minutes following the second injection. Successive drug testing days were separated by an intervening washout day to allow the animals to recover to their normal drinking levels. The saline vehicle was administered by the same route and schedule as the TFP or naltrexone injections.

Phase 2: Four different drug treatment combinations were administered to all rats on successive treatment days as in the previous phase. These combinations were: 1) 2,4-dinitrophenol followed
by naltrexone; 2) 2,4-dinitrophenol followed by saline; 3) saline followed by saline; 4) saline followed by naltrexone. The first and second injections were separated by 20 minutes and the animals were placed into the drinking cages 5 minutes following the second injection. Successive treatment days were separated by an intervening washout day, during which the limited access procedure was run but no injections were given.

6.2.4 Blood glucose measurements

At the end of phase one, blood glucose measurements were taken using the Glucometer Elite Blood Glucose Meter (Bayer, Ontario, Canada). On each of 4 days, three squads of four rats and one squad of three rats were allocated to one of the four drug treatment combinations in phase one such that by the end of the series, all rats received each treatment combination in a balanced order. The sequence and timing of the injections were identical to the treatment phase and a washout day intervened between each blood glucose day.

6.3 Results

**Phase 1: The effect of trifluoperazine on alcohol intake**

Figure 9 illustrates the mean alcohol consumption for the rats over phase one of the experiment. The values of bars denoted the average alcohol intake of the rats on the drug testing days and the linked dotted lines denoted the average alcohol consumption on the intervening non-drug days. A one-way repeated measures analysis of variance on the alcohol data showed a significant change in the alcohol consumption in this phase \( F_{25.364} = 11.11, P < 0.00001 \). Compared to alcohol consumption on the immediately preceding or following intervening days, the saline/naltrexone (2 and 3 mg/kg) treatments significantly reduced alcohol intake on the two trials
Figure 9. Effect of trifluoperazine on naltrexone-induced reduction in alcohol intake.

Mean daily intake of alcohol during the 40-minute limited access procedure. Rats were pretreated with either 6.25, 12.5 or 25 μg/kg trifluoperazine in combination with either 1.5, 2 or 3 mg/kg naltrexone. T-bars represent the standard error of the mean. Filled circles connected by solid lines represent the alcohol intake on intervening days.

TFP-Trifluoperazine
SAL-Saline
NALT-Naltrexone
in which they were presented. Pretreatment with 12.5 or 25 μg/kg TFP did not alter the 3 mg/kg naltrexone-induced reduction in alcohol intake in all trials. In subsequent trials, the dose of naltrexone was reduced to 1.5 and then to 2 mg/kg in order to decrease the action of the opiate antagonist and thereby unmask an effect of TFP. However, 1.5 mg/kg naltrexone did not attenuate alcohol intake significantly and we proceeded with 2 mg/kg of naltrexone. From the graph, a dose-response relationship was observed in the reduction of alcohol intake with increasing doses of naltrexone. Pretreatment with 12.5 μg/kg TFP on the first trial appeared to attenuate the 2 mg/kg naltrexone-induced reduction in alcohol intake but the increase in alcohol drinking was not significant. A second trial of this combination showed that TFP pretreatment did not alter alcohol intake when compared to the saline/naltrexone combination. Although the range of doses of TFP used were below those at which TFP acts as a dopaminergic antagonist (Baldessarini, 1997), nevertheless we reduced the dose of TFP and examined its effect of this lower dose on naltrexone. In fact, decreasing dose of TFP did not antagonize the action of naltrexone when compared to the 12.5 μg/kg TFP/naltrexone combination. The 12.5 μg/kg TFP/saline combination had no effect on alcohol intake when compared to the intervening days. Finally, intake on the saline/saline trial was equivalent to that on both the preceding and following intervening days, indicating the injection procedures did not affect alcohol consumption. Moreover, alcohol intake on intervening days remained at the same level indicating little or no carry-over effect from the days in which treatment was given.

**Phase 1: The effect of trifluoperazine on water intake**

Figure 10 illustrates the effect of different treatment combinations on water intake. Water intake was very low throughout the entire experiment ranging from 0 to 3 ml/kg on the treatment and
Figure 10. Effect of trifluoperazine/naltrexone combination on water intake.

Mean daily intake of water during the 40-minute limited access procedure. Rats were pretreated with either 6.25, 12.5 or 25 µg/kg trifluoperazine in combination with either 1.5, 2 or 3 mg/kg naltrexone. *T* bars represent the standard error of the mean. *Filled circles connected by solid lines* represent the water intake on intervening days.

TFP-Trifluoperazine  
SAL-Saline  
NALT-Naltrexone
WATER INTAKE ml/kg

NALT-Naltrexone
TFP-Trifluoperazine
SAL-Saline
intervening days. No systematic changes related to the different drug treatment combinations were observed \([F_{2,364}=0.92, P=n.s.}\).  

**Phase 1: The effect of drug treatment combinations on blood glucose levels**  

Figure 11 illustrates the effect of the different treatment combinations on blood glucose levels at times corresponding to 10, 20 and 30 minutes into the limited access procedure. A two-way repeated measures analysis of variance showed significant effect of Time \([F_{2,112}=5.44, P=0.0056}\) and interaction of Treatment*Time \([F_{6,112}=2.66, P=0.019]\) but the effect of Treatment was not significant \([F_{3,112}=0.95, P=n.s.}\). Post hoc analysis revealed significant treatment differences in blood glucose levels only at the 10-minute interval. Naltrexone (2 mg/kg) either given on its own or in combination with TFP significantly increased blood glucose levels at the 10-minute point when compared to the saline/saline group but recovered to normal levels afterwards. No effect was observed in blood glucose levels produced by TFP (12.5 μg/kg) when compared to the saline control.  

**Phase 2: The effect of 2,4-dinitrophenol on alcohol intake**  

Figure 12 illustrates the mean alcohol consumption for all rats in the second phase of the experiment. A one-way repeated measures analysis of variance revealed a significant change in alcohol intake \([F_{19,280}=10.06, P<0.00001]\). On the first and second drug treatment days, 2,4-dinitrophenol pretreatment did not alter the reduction of alcohol intake produced by 3 mg/kg of naltrexone. When 2,4-dinitrophenol was administered on its own, it had no effect on alcohol intake. On the remaining trials, the dose of naltrexone was decreased to 2 mg/kg. Pretreatment with 5 mg/kg 2,4-dinitrophenol did not alter the naltrexone-induced reduction in alcohol intake on any of the three trials in which it was given. Increasing the dose of 2,4-dinitrophenol to 10 mg/kg had no observable effects on alcohol intake on its own and it did not attenuate the inhibition of alcohol
Figure 11. Effect of trifluoperazine/naltrexone combination on mean blood glucose level.

Trifluoperazine was administered in a dose of 12.5 µg/kg and naltrexone at a dose of 2 mg/kg. T10, T20, T30 are the blood glucose sampling times and correspond to 10, 20, 30 min into the limited access procedure.
Figure 12. Effect of 2,4-dinitrophenol and naltrexone on alcohol intake.

Mean daily intake of alcohol during the 40-minute limited access procedure. Rats were pretreated with either 5 or 10 mg/kg 2,4-dinitrophenol in combination with either 2 or 3 mg/kg naltrexone. T bars represent the standard error of the mean. Filled circles connected by solid lines represent the alcohol intake on intervening days.

DNP-2,4-dinitrophenol

SAL-Saline

NALT-Naltrexone

Numbers underneath represent doses in mg/kg.
consumption produced by naltrexone. Intake on the saline/saline trial was comparable to the level on the intervening days indicating no carry-over effects of any treatment.

Phase 2: The effect of 2,4-dinitrophenol on water intake

Figure 13 illustrates the mean water consumption for all rats in the second phase of the experiment. One-way repeated measures analysis of variance revealed that water intake was not modified by any treatments administered during the whole phase of experiment \( F_{19,280} = 0.71, p = \text{n.s.} \).

6.4 Conclusion

Naltrexone at a dose of 2 mg/kg reduced alcohol intake with a concomitant increase in blood glucose levels. The glycogenolytic inhibitor, trifluoperazine, however did not alter the naltrexone-induced reduction of alcohol intake. This indicates that the effects produced by naltrexone are mediated by a mechanism unrelated to changes in blood glucose levels. With respect to the blood glucose levels produced by the TFP/Naltrexone combination, TFP did not suppress the increase in blood glucose produced by naltrexone suggesting glycogenolysis was not inhibited. Since glycogenolysis can be mediated by at least 2 second messenger systems (e.g. Ca\(^{++}\) for angiotensin, Blackmore et al., 1978; cAMP for isoproterenol, Tamir, 1993), a plausible explanation for the inability of TFP to inhibit glycogenolysis by naltrexone is that naltrexone's action on blood glucose is mediated by the cAMP messenger system instead of Ca\(^{++}\). In hindsight, a review of the opioid receptor literature revealed that the mechanism of action of naltrexone is in part mediated through a cAMP-dependent process (Guitart & Nestler, 1989; Rasmussen et al., 1990; Carter & Medzihradsky, 1993). Hence, these results indicate the possibility that cAMP may be involved in the generation of glucose produced by naltrexone and TFP will not be able to inhibit glycogenolysis.
Figure 13. Effect of 2,4-dinitrophenol and naltrexone on water intake.

Mean daily intake of water during the 40-minute limited access procedure. Rats were pretreated with either 5 or 10 mg/kg 2,4-dinitrophenol in combination with either 2 or 3 mg/kg naltrexone. Bars represent the standard error of the mean. Filled circles connected by solid lines represent the water intake on intervening days.

DNP-2,4-dinitrophenol

SAL-Saline

NALT-Naltrexone

Numbers underneath represent doses in mg/kg.
through the Ca\textsuperscript{2+} second messenger pathway.

In phase 2 of the experiment, 2,4-dinitrophenol was administered in order to interfere with the cellular generation of ATP. Tordoff and Friedman (1993) suggested that ATP may be the satiety signal that turns off feeding. The inability of 2,4-dinitrophenol to enhance alcohol consumption on its own or to attenuate naltrexone effect on alcohol intake suggests that the regulation of alcohol consumption may not be influenced by the satiety signal generated by ATP.
7. EXPERIMENT FIVE

THE EFFECTS OF THE $\alpha_2$-ADRENOCEPTOR ANTAGONISTS IDAZOXAN AND METHOXY-IDAZOXAN ON ALCOHOL INTAKE AND BLOOD GLUCOSE LEVELS

7.1 Rationale

Noradrenaline has been implicated in the control of alcohol intake in several studies. Neurochemical lesions of noradrenergic neurons (Brown & Amit, 1977) and inhibition of noradrenaline synthesis (Davis et al., 1978) were found to suppress voluntary alcohol consumption. In addition, the acute administration of a single-dose of alcohol is associated with increases in noradrenaline turnover (Pohorecky & Jaffe, 1975; Karoum et al., 1976) and release (Sun, 1976) in rats. The co-administration of $\alpha_2$-adrenoceptor antagonists with alcohol to rodents reduces some of the effects of acute alcohol administration (e.g. ataxia and hypothermia; Durcan et al., 1989a,b; Lister et al., 1989). In addition, dopamine is converted to noradrenaline by dopamine-$\beta$-hydroxylase. The depletion of noradrenaline by dopamine-$\beta$-hydroxylase inhibitors FLA-63 and FLA-57 have been shown to attenuate voluntary alcohol consumption in rats (Amit et al., 1977; Amit & Stern, 1971; Brown et al., 1977). These reports suggested that the decrease in consumption was a result of an attenuation of the reinforcing properties of alcohol brought about by the depletion of noradrenaline (Amit et al., 1977; Amit & Stern, 1971). Consistent with this suggestion, FLA-57 was shown to attenuate intragastric alcohol self-administration and block the development of a secondary reinforcer to signalled alcohol infusions (Davis et al., 1979).

In the present experiment, the effects on alcohol consumption and blood glucose levels of
Two \( \alpha_2 \)-adrenoceptor antagonists, idazoxan and methoxy-idazoxan, are studied. Methoxy-idazoxan is an \( \alpha_1 \)-adrenoceptor antagonist which is devoid of any affinity for the non-adrenergic idazoxan (imidazoline) binding sites and the net effect of this compound is an increase in levels of noradrenaline resulting in glycogenolysis and hyperglycaemia (Antonis et al., 1967). It has been reported that methoxy-idazoxan and other non-imidazoline binding \( \alpha_1 \)-antagonists such as yohimbine decreased alcohol consumption in rats (Davis et al., 1978; Reid et al., 1994). On the other hand, idazoxan is an \( \alpha_2 \)-adrenergic antagonist which has been shown to bind to the imidazoline receptors (Michel & Insel, 1989) and produces a significant decrease in blood glucose levels. In addition, idazoxan has also been found to be able to induce alcohol consumption in rats with a concurrent decrease in blood glucose levels (Grupp et al., 1997). Prior to this discovery, it has been demonstrated that the \( \alpha_2 \)-adrenoceptor antagonist idazoxan increased food intake in freely-feeding rats (Jackson et al., 1991). The exact mechanisms underlying the idazoxan-induced food intake are still uncertain. Given this, the first objective of the present experiment was therefore to compare the effects of the two drugs on alcohol consumption and blood glucose levels and secondly, to assess the possibility of an inverse relationship between alcohol intake and blood glucose levels.

Based on these findings, our hypothesis that glucoregulatory processes are salient to voluntary alcohol consumption would be supported if an \( \alpha_2 \)-adrenoceptor antagonist which increased blood glucose levels produced opposite effects on alcohol consumption to an \( \alpha_2 \)-adrenoceptor antagonist which decreased blood glucose levels. Although correlating results from a single class of drugs cannot establish a causal relationship, such findings would suggest that some glucoregulatory processes may well be a contributing factor to the control of alcohol intake. Therefore, this experiment was conducted to assess the possible divergent effects of methoxy-
idazoxan and idazoxan with respect to alcohol consumption and blood glucose levels.

7.2 Methods

7.2.1 Subjects

The subjects were male Wistar rats (Charles River, Montreal) weighing between 270 and 330 g at the start of the experiment. The animals were individually housed and maintained on a reverse 12 hour/12 hour light/dark cycle with lights off at 7 am. Purina rat chow and tap water were available in home cages ad libitum. No food was available during the drinking sessions. The experiment was conducted seven days a week, between 10 am and 1 pm, during the dark portion of the cycle.

7.2.2 Drug Preparations

Alcohol solution was prepared by diluting 95% ethanol w/v with tap water to a required concentration: 3% w/v during the initial training and 6% w/v for the remainder of the experiment.

Idazoxan (4 and 8 mg/kg; Sigma Chemical Co., St. Louis, Mo., USA) was dissolved in 0.9% saline and administered subcutaneously 30 minutes prior to the drinking sessions.

Methoxy-idazoxan was purchased from Research Biochemicals Inc. (Natick, Mass., USA) and dissolved in isotonic saline. Injections (3 and 6 mg/kg) were given s.c. 30 minutes prior to alcohol access.

7.2.3 Procedure

40 Minutes Limited Access

Please refer to the GENERAL METHODS section for details.

Treatment:
Six different drug treatment were administered to all rats on successive treatment days. These treatments were: 1) idazoxan at doses of 4 and 8 mg/kg; 2) methoxy-idazoxan at doses of 3 and 6 mg/kg; 3) saline s.c., control for idazoxan; 4) saline i.p., control for methoxy-idazoxan. Successive treatment days were separated by an intervening washout day to allow the animals to recover to their normal drinking levels. Except for the lower doses of idazoxan (4 mg/kg) and methoxy-idazoxan (3 mg/kg) treatments, each treatment was repeated at least twice during the study.

7.2.4 Blood glucose measurements

At the end of the drinking experiment blood glucose measurements were taken using the Glucometer Elite Blood Glucose Meter (Bayer, Ontario, Canada). On each of 4 days, four squads of four rats were allocated to one of the four treatments such that by the end of the series, all rats received each treatment in a balanced order. The sequence and timing of injections were identical to the drinking phase and a washout day intervened between each glucose day. Blood glucose measurements were performed for the 8 mg/kg idazoxan and 6 mg/kg methoxy-idazoxan doses and their corresponding controls.

7.3 Results

**Effect of methoxy-idazoxan and idazoxan on alcohol intake**

Figure 14 illustrates the mean alcohol intake of all rats over the course of the experiment. The data as plotted follow the exact temporal sequence of treatments over the entire experiment. A one-way repeated measures analysis of variance revealed a highly significant change in alcohol intake \([F_{19,400}=4.31, P<0.00001]\). Despite the fact that idazoxan increased alcohol intake on three trials, only the first trial of the 8 mg/kg dose of idazoxan produced a significantly higher intake when
Figure 14. Effect of idazoxan and methoxy-idazoxan on alcohol intake.

Mean daily intake of alcohol during the 40-minute limited access procedure. Rats were pretreated with either idazoxan (4 or 8 mg/kg), methoxy-idazoxan (3 or 6 mg/kg) or their corresponding vehicle injections. T bars represent the standard error of the mean. Filled circles connected by solid lines represent the alcohol intake on intervening days.

IDAZ-Idazoxan
mIDAZ-Methoxy-idazoxan
SAL-Saline

Numbers underneath represent doses in mg/kg.
compared to alcohol consumption on the immediately preceding or following intervening days. Although idazoxan at the 4 mg/kg dose and the second trial at 8 mg/kg dose did not produce a significant increase in alcohol intake, alcohol consumption was found to increase in most of the subjects during these days. On the other hand, 3 mg/kg of methoxy-idazoxan seemed to attenuate alcohol drinking on the first trial but the decrease in intake was not substantial and was not statistically significant. On its second and third trial, however, when the dose of methoxy-idazoxan was doubled to 6 mg/kg, it produced a significant attenuation of alcohol consumption (>50% reduction) when compared to the intervening days and the saline injection days. Saline injections administered in two different routes had no effect on alcohol intake on all trials and no changes in alcohol consumption were observed on the intervening days across the whole experiment.

**Effect of methoxy-idazoxan and idazoxan on water intake**

Figure 15 shows the mean water intake of all animals over the course of the experiment. Considerable fluctuations in water consumption were observed during the course of the experiment with intake ranging from 1 to 9 ml/kg. One-way repeated measures analysis of variance revealed a significant effect of drug treatment on water intake \([F_{19,400}=1.84, P=0.0171]\). Post hoc Duncan's tests revealed that only the 8 mg/kg dose of idazoxan on its second trial increased water intake when compared to the intervening days. No effects on water intake were observed from the administration of methoxy-idazoxan.

**Effect of treatment combinations on blood glucose levels**

Figure 16 illustrates the effect of the different treatment combinations on blood glucose levels at times corresponding to 10, 20 and 30 minutes into the limited access procedure. A two-way repeated measures analysis of variance showed significant effects of Treatment
Figure 15. Effect of idazoxan and methoxy-idazoxan on water intake.

Mean daily intake of water during the 40-minute limited access procedure. Rats were pretreated with either idazoxan (4 or 8 mg/kg), methoxy-idazoxan (3 or 6 mg/kg) or their corresponding vehicle injections. $T$ bars represent the standard error of the mean. Filled circles connected by solid lines represent the water intake on intervening days.

IDAZ-Idazoxan

mIDAZ-Methoxy-idazoxan

SAL-Saline

Numbers underneath represent doses in mg/kg.
Figure 16. Effect of idazoxan and methoxy-idazoxan on blood glucose level.

Mean blood glucose levels in idazoxan (8 mg/kg), methoxy-idazoxan (6 mg/kg) or vehicle treated rats. T10, T20, T30 are the blood sampling times and correspond to 10, 20, 30 min into the limited access procedure. T bars represent the standard error of the mean.
[F_{3,160}=7.37, P=0.0002], Time [F_{2,160}=5.85, P=0.0035] and interaction of Treatment*Time
[F_{6,160}=4.74, P<0.0002]. Post hoc Duncan's tests revealed significant treatment differences in blood
glucose levels at the 10- and 20-minute intervals but not at the 30-minute interval. Idazoxan (8
mg/kg) significantly reduced blood glucose levels at both the 10- and 20-minute intervals compared
to saline control. A paired t-test analysis revealed that methoxy-idazoxan on its own produced a
significant increase in blood glucose levels at the 20-minute interval when compared to the saline
vehicle injection [T=, P<0.05].

7.4 Conclusion

In this experiment, we showed that idazoxan increased alcohol consumption while decreasing
blood glucose levels and methoxy-idazoxan decreased alcohol consumption with a concomitant
increase in blood glucose levels. On the other hand, the effect of idazoxan at the 8 mg/kg dose on
alcohol consumption was equivocal in this experiment. Since 8 mg/kg of idazoxan did not increase
alcohol consumption significantly on the second trial, it would seem imprudent to draw any
conclusions as to the effect of idazoxan on alcohol consumption at least in this experiment. In an
attempt to resolve this inconsistency, a plausible explanation for the lack of significant effect on the
second trial would be the result of the peripheral actions of idazoxan. One major difference between
the two α₂-adrenoceptor antagonists is the structure (Hoffman & Lefkowitz, 1997) and therefore
their abilities to penetrate the blood brain barrier. Methoxy-idazoxan has a substituted ring structure
and hence possesses a lower partition coefficient whereas idazoxan has a much higher partition
coefficient and thus has a higher bioavailability in the brain. It has been suggested that the binding
of idazoxan in the brain has been concentrated in sites associated with control of food intake
(Hudson et al., 1991). Given this, the effects of idazoxan-induced alcohol consumption may also be mediated by similar mechanisms in the brain. Since other non-imidazoline binding $\alpha_2$-adrenoceptor antagonists have been shown to attenuate voluntary alcohol consumption by a peripheral mechanism, this suggested that idazoxan might have similar peripheral actions when given at higher doses. As a consequence, the increase in alcohol consumption produced by idazoxan centrally might be partially blocked by its opposite actions peripherally. Compared to our previous study (Grupp et al., 1997) in which we showed idazoxan increased alcohol consumption, the dose (2 mg/kg) used in that experiment was indeed lower than the dose employed here (8 mg/kg). However, despite the inconsistency of the results from idazoxan, the opposite effects of alcohol intake and blood glucose seen with methoxy-idazoxan indicated that there was an inverse relationship between consumption of alcohol and blood glucose levels. This was an encouraging finding that should prompt the replication of this experiment with the additional assessment of a larger range of doses for both drugs.
8. GENERAL DISCUSSION

One of the goals of basic research in the field of alcohol addiction is to understand the factors which contribute to the control of alcohol consumption behaviour. However, the ultimate goal and inspiration for such work is the development of more effective pharmacological agents to treat the problem of alcoholism in humans. Since alcoholism is a multifaceted and complex problem, treatment of alcoholism may best be directed by a multidisciplinary approach. This thesis has focused on the possible role of glucoregulatory mechanisms in alcohol consumption. In the following discussion, we will evaluate the potential merits of the studies in glucoregulatory processes pertaining to the control of alcohol consumption.

8.1 Why glucoregulatory mechanisms?

8.1.1 Effects of glucose and saccharin on alcohol intake

Several lines of evidence suggested that glucose and other sweet tasting substances might be implicated in the control of consumption of alcohol. In previous studies, alcohol intake has been shown to correlate with preference for sweet tasting solutions in rodents. For example, three lines/strains of rats (the alcohol-preferring AA, FH and P rats and alcohol-nonpreferring ANA, FRL and NP rats) selected for high or low alcohol preference, show correspondingly high and low preferences for sweet saccharin solutions (Overstreet et al., 1997). It has also been reported that rats which showed the greatest intake of a saccharin solution also consumed the greatest amount of alcohol (Gosnell & Krahn, 1992; Overstreet et al., 1993; Bell et al., 1993).
Building on the research of these alcohol-preferring inbred lines of rats, scientists have used animals to find genes that influence responses to alcohol by looking for areas on chromosomes called quantitative trait loci (QTL). Recent advances in transgenic technology and recombinant inbred strains now make feasible the positional cloning of QTLs that influence sensitivity to drugs of abuse (Crabbe et al., 1994). Once identified, QTL in animals can be used as a guide to examine probable locations for the genes in humans (Plomin & McClearn, 1993). Previous QTL analyses revealed that several genetic markers were associated with high alcohol consumption levels, including markers for the D₂ dopamine receptor (see review by Agarwal, 1997; Plomin & McClearn, 1993). More recently, QTL analyses of saccharin and sweetened alcoholic beverages identified the sac locus, which is thought to determine the ability to detect saccharin or sweet-tasting substance (Phillips et al., 1994). Hence, the relationship of sweet preferences and glucose regulation may be an important variable in the processes that regulate alcohol consumption.

From a clinical viewpoint, physicians who treat alcoholics have long noted that many of the newly sober patients develop a preference for carbohydrates. Some of them begin eating a large amount of cake, ice cream, chocolate and candies which they have never liked before. Given this, these observations suggest that the alcohol drinking behaviour in humans may be altered by nutritional interventions. Furthermore, the fellowship of Alcoholic Anonymous traditionally encourages new members to carry candies with them to help suppress the urge to drink. These observations, though interesting, lack scientific documentation and thorough studies. In a previous report, Hasunen et al. (1976) found that people in Finland who took in more than 30 g of alcohol a day ate diets that were lower in carbohydrates compared to those who drank less alcohol. In a more recent study (Yung et al., 1983), 64 newly sober outpatients were interviewed by nutritionists. Those
who stayed sober longer chose diets containing twice as much sugar added to beverages and more overall carbohydrates. On the other hand, no significant difference was observed in the consumption of other dietary components in the two groups of outpatients. Whether the increased length of sobriety resulted from a higher carbohydrate intake or the converse is true is not known. However, the fact that a significant difference in carbohydrate consumption was found at the time of the first interview but not at the time of last interview suggests that higher carbohydrate intake may contribute to sobriety.

In conclusion, all of the above findings raise the possibility that the preference for 'sweets' may be related to the mechanism regulating alcohol consumption in both animals and humans. However, more detailed studies are required to determine the exact biological mechanism involved and to assess whether dietary approaches have implications for alcoholism treatment.

8.1.2 Behavioural actions of glucose

To further support the use of glucose in our study of its role on alcohol intake, previous studies have demonstrated that glucose injections can alter some of the behavioural actions of other drugs of abuse. For example, the alteration of glucose levels in vivo by diabetes or acute glucose loading can lead to significant changes in the analgesic potency of morphine (Simon & Dewey, 1981; Simon et al., 1981; Singh et al., 1983). Lux et al. (1988) reported that the antinociceptive effect of either subcutaneously or intrathecally administered morphine could be reduced by intraperitoneal injections of glucose. Furthermore, a relationship between blood glucose level and analgesia was further confirmed by Brase and Dewey (1988) in mice and by Raz et al. (1988) and Lee & McCarty (1990) in rats. Aside from its effects on morphine analgesia, Gold and colleagues (1992) found that
glucose injections could also attenuate memory impairments in rodents caused by opiate agonists such as morphine.

Taken together, these findings suggest that glucose injections can exert behavioural actions in different areas. In particular, the work with morphine is especially relevant since it raises the possibility that the functional properties of addictive drugs such as morphine and alcohol might be modified by glucose manipulations.

8.1.3 Alcohol as a food

As noted earlier, alcohol is unique when compared to other drugs of abuse in that alcohol is a source of calories. Thus, it is reasonable to suspect that factors influencing alcohol intake and diet regulation may have some degree of overlap. Specifically, according to the glucostatic theory of food intake, a high level of blood glucose may inhibit the subsequent ingestion of alcohol. Conversely, agents which can decrease blood glucose levels may be able to induce alcohol consumption via a compensatory glucostatic satiety mechanism.

In addition, the glucostatic theory of food intake also recognized the importance of glucose utilization in the regulation of food intake. 2-deoxy-D-glucose (2-DG) is a competitive inhibitor of glycolysis at the phosphohexoisomerase step (Wick et al., 1957). Studies have shown that administration of 2-DG elicited feeding (Granneman & Friedman, 1983; Miselis & Epstein, 1975). The principal finding of these experiments is that pharmacological inhibition of glucose utilization by 2-DG robustly produces a significant increase in food intake. Given this, we hypothesized that when glucose utilization was altered by food deprivation, alcohol intake might also be influenced. In this case, animals which were food-deprived might consume less alcohol when glucose injections
were administered because the glucose injected would be utilized and a satiety signal might be generated to suppress the ingestion of alcohol.

Apart from the glucostatic theory on the control of food intake, it has been demonstrated that glucose and fat metabolism interact in some coordinated fashion to control food intake (Friedman & Tordoff, 1986). Since ATP (i.e. energy) production is the common product subsequent to the oxidation of glucose and fatty acid, it is reasonable to assume that ATP availability or utilization can have direct effects on food intake. Given this, we also hypothesized that when ATP production was increased, alcohol intake might be inhibited due to the ATP-generated satiety signal. In contrast, if ATP production was inhibited by other pharmacological agents, we hypothesized that alcohol intake might be augmented because of the compensatory energostatic mechanism.

To summarize, studies of the metabolic control of eating behaviour indicate that the metabolism of fuels in liver may provide a satiety signal that modulates energy intake. Evidence points to changes in hepatic ATP as the source of such a signal and suggests that this information is transmitted to the brain via vagal afferent neurons. Such actions of the satiety signal are important in the subconscious regulation of appetite and ingestive behaviour, an aspect important regarding consumption of alcohol since alcohol is a source of calories. If the energy from alcohol is handled by the body in the same manner as is energy from other foods, the caloristatic hypothesis predicts a compensatory mechanism in which ingestion of more alcohol might displace food intake whereas more food (or energy) might displace alcohol intake. Based on this assumption, this thesis set out to answer the possible role of a glucoregulatory mechanism in the control of alcohol intake.
8.2 Summary and Discussion

In experiment one we examined the effects of parenteral glucose injections on alcohol intake. Despite a significant increase in blood glucose levels produced by glucose injections, alcohol consumption was not changed. In the second part of the experiment, L-malate was used to determine whether an increase in energy input would influence alcohol intake. In this case, we hypothesized that the increased ATP production induced by L-malate might provide a satiety signal to inhibit alcohol intake. However, the results showed that L-malate injections, at various doses which had been demonstrated to have an effect on food intake or morphine-analgesia (Langhans et al., 1985; Singh et al., 1983), did not alter alcohol intake. In this experiment, the ATP levels were not recorded after L-malate injections. Therefore, this study could have been further improved by taking actual measurements of ATP levels after L-malate injections. In conclusion, from this experiment, we found that changes in plasma glucose levels did not contribute to the regulation of alcohol intake. Similarly, increases in energy input by enhancing ATP production also did not decrease alcohol intake. Taken together, these data indicated that the glucostatic hypothesis of satiety did not seem to apply to the regulation of alcohol intake.

Experiments two and three examined the possible role of glucoregulatory processes on alcohol intake from a different perspective. Experiment two took advantage of the increased glucose utilization produced by food-deprived conditions (Issad et al., 1987). This experiment was designed to address the fact that glucose injected in the first experiment might not have been utilized since the rats were sated and therefore unlikely to utilize the injected glucose. If this was the case then no satiety signal would have been generated. As a result, the effects of two food.deprivational states on alcohol consumption were tested. Before testing the effects of food deprivation on consumption
of alcohol, we first replicated our findings from experiment one that glucose injections did not alter alcohol intake in sated rats. In the second phase, glucose injections identical to the first experiment did not alter alcohol intake in rats which were 24 hour food-deprived. Interestingly, alcohol intake on the intervening days in this phase was dramatically reduced after the first day of 24 hr food-deprivation and this reduction did not recover in the following intervening days when the animals were refed. We concluded this was due to the significant amount of alcohol absorbed by the rat which had an empty stomach. This resulted in a rapid absorption of alcohol into the central nervous system and consequently a very high level of intoxication that might lead to the development of many unpleasant pharmacological effects. As a result, the rats avoided the alcohol when they were sated. In the third phase, rats were chronically food-deprived until their weights were reduced to 80-85% of their free-feeding weights. We confirmed an increase in glucose utilization in chronically food-deprived rats in that glucose injections produced a significant lower blood glucose level compared to sated rats. Nevertheless, a direct measurement of glucose utilization for example by measuring the hepatic arteriolar and venous blood glucose differences would have added further confirmation of a change in glucose utilization. Increases in alcohol consumption were found in this phase when compared to the food-satiation and acute food-deprivation phase. This finding was in line with the literature in which food deprivation consistently increased consumption of other drugs of abuse (Carroll & Meisch, 1984). In spite of the increased glucose utilization in chronic food-deprivation, glucose injections did not decrease alcohol intake. Once again, these results suggested that increased glucose utilization did not contribute to the control of alcohol intake.

In experiment one an increase in blood glucose level was achieved by direct injection. This would result in a significant but short-lived rise in blood glucose level. Experiment three was
conducted to examine the effects of a chronic and endogenously elevated level of blood glucose on alcohol intake. According to our hypothesis, animals made diabetic by injections of streptozotocin might consume less alcohol because of their high and enduring blood glucose levels. Streptozotocin is a pancreatic β cell toxin and produces a permanent insulin-dependent diabetes state in subjects unless they are corrected by injections of insulin. Blood glucose levels confirmed that the streptozotocin treated (65 mg/kg) rats were made diabetic. We found that diabetic and sham controls drank similar amounts of alcohol indicating that even when blood glucose levels were raised, it was unable to inhibit alcohol intake in the diabetic rats. A daily injection of insulin was given every afternoon at 4:00 pm and this procedure has been shown to be effective in normalizing blood glucose levels in diabetic rats in several experiments (Smith et al., 1987; Tucker et al., 1991). When insulin was administered to the rats, it did not alter alcohol intake but water intake was reduced. However, the blood glucose measurement results from this experiment were puzzling. Although water intake was reduced in the group of diabetic animals, suggesting insulin was effective in rectifying glycosuria which is a prominent symptom of diabetes, their blood glucose levels did not decrease at any dose of insulin administered. We hypothesized that this could be due to the timing of the insulin injections because there was a long time lapse (18 hrs) between the administration of insulin and the measurements of blood glucose. If the injections could be brought closer to the time of blood glucose measurements, insulin might have produced a stronger effect on blood glucose levels. As a result, we could only conclude from this experiment that blood glucose levels alone could not determine alcohol consumption. A high blood glucose level per se does not necessarily imply a low level of alcohol intake.

In experiment four we examined the possible role of glucoregulatory processes in the action
of the opiate antagonist naltrexone which has been shown to decrease alcohol intake and is currently
used to treat alcoholics. Naltrexone has been shown to be able to potentiate hyperglycaemia
(McCubbin et al., 1989). In addition, another opiate antagonist nalmefene has been shown to
decrease food intake with a corresponding increase in blood glucose (McLaughlin et al., 1986). To
test the hypothesis that naltrexone-induced attenuation in alcohol intake was mediated by a
 gluoregulatory mechanism, two drugs acting on different mechanisms were used in an attempt to
block its actions on alcohol intake. Since the mode of actions of naltrexone is unclear and given the
fact that naltrexone can produce a significant elevation of plasma glucose in a time frame matching
its actions on alcohol intake (unpublished observations), we postulated that naltrexone's effects on
alcohol might at least in part be mediated by a gluoregulatory mechanism. Trifluoperazine is a
glycogenolytic inhibitor which may inhibit the increase in blood glucose levels produced by
 naltrexone. Therefore, we hypothesized that trifluoperazine might inhibit the attenuation in alcohol
intake produced by naltrexone through the opposite actions of trifluoperazine on blood glucose. On
the other hand, 2,4-dinitrophenol is an inhibitor of ATP synthesis and will decrease ATP levels and
thus energy output. In this case, we hypothesized that the decrease in energy production would lead
to the generation of a hunger signal and hence reversed the satiety signal produced by naltrexone.
Nevertheless, both trifluoperazine and 2,4-dinitrophenol failed to inhibit naltrexone's actions in
 attenuating alcohol intake. Although trifluoperazine did not inhibit the elevation of blood glucose
produced by naltrexone, this was thought to be due to the different second messenger systems
 involved with the two drugs. Thus, this experiment indicated that naltrexone's actions on alcohol
intake were not mediated by a gluoregulatory mechanism.

Lastly, in experiment five we attempted to examine the effects of drug-induced alterations
in blood glucose levels on the consumption of alcohol. Two \( \alpha_2 \)-adrenergic receptor antagonists, idazoxan and methoxy-idazoxan, were used to compare their effects on blood glucose levels and alcohol consumption. Idazoxan has been shown to increase alcohol intake with a concomitant decrease in blood glucose levels (Grupp et al., 1997). Methoxy-idazoxan, however, is known to increase blood glucose levels due to the release of epinephrine and has been shown to decrease alcohol consumption in a separate study (Reid et al., 1994). Therefore, if we could show that when one \( \alpha_2 \)-adrenergic antagonist which decreased blood glucose levels produced an increase in alcohol intake whereas another \( \alpha_2 \)-adrenergic antagonist which increased blood glucose levels also decreased alcohol intake, these correlating results would support a possible role of glucoregulatory processes in the regulation of alcohol intake. In conclusion, methoxy-idazoxan at a dose of 6 mg/kg decreased alcohol intake with a corresponding significant increase in blood glucose. Unfortunately, results from the idazoxan treatments were not as clear. Although idazoxan produced an elevation in alcohol consumption in all of the three trials administered, only one trial produced a significant increase in alcohol intake. Since the effect of 8 mg/kg of idazoxan on alcohol intake were equivocal in two of the trials administered, definitive conclusions cannot be made as to the effect of idazoxan on alcohol intake. Nevertheless, blood glucose measurements confirmed that idazoxan at a dose of 8 mg/kg produced a significant reduction in blood glucose level. Given this, the effects of idazoxan and methoxy-idazoxan on alcohol consumption and blood glucose should be reexamined to see if there was an inverse relationship. Moreover, a larger range of doses for both drugs is recommended if the experiment should be repeated.

In conclusion, five experiments were conducted to examine the role of glucoregulatory processes in alcohol consumption. In this study, each experiment undertakes to address the question
in different and independent perspective. This thesis, however, does not attempt to provide comprehensive coverage of all glucoregulatory mechanisms to assess their effects on alcohol consumption. Rather, it focuses on peripheral injections of glucose, glucose utilization, experimentally-induced diabetes and the ATP satiety signal in order to add further information about aspects which are known only sketchily at this time. Taken together, the results of the present experiments reject a possible role of glucoregulatory mechanisms in the control of alcohol intake at least in the areas tested in this thesis.

8.3 Suggestions for future work

Even though the research discussed in this thesis has not answered some of the questions regarding the possible role of glucoregulatory mechanisms in controlling alcohol consumption in experimental animals, further study into other areas of glucoregulatory processes or manipulations using different strategy is recommended. For example, 2-deoxyglucose can be used in the limited access procedure to determine whether the effects of its glucoprivic (i.e. inhibition of glucose utilization) actions will influence alcohol intake by a glucostatic mechanism. Furthermore, the effect of 2-DG on naltrexone should also be investigated because it has been demonstrated that 2-DG can attenuate the reduction of food intake caused by naltrexone (Koch & Bodnar, 1994; Schaefer et al., 1994).

To better determine the role of glucoregulatory mechanisms in alcohol consumption, systemic injections of fructose could be used in replacement of glucose. In a study carried out by Tordoff and Friedman (1988), they showed that when jugular infusions of glucose failed to decrease
the food intake in rats, jugular infusion of fructose increased systemic plasma glucose and decreased food intake reliably. The divergent effects of glucose and fructose resides in the fact that the tissue utilizaion of these two fuels are different (Park et al., 1957; Van den Berghe, 1978). Since glucose can be easily utilized throughout the body, fructose is only poorly utilized by brain and peripheral tissues. Hence, it is likely that larger quantities of infused fructose than of infused glucose would reach the liver, thereby producing a satiety signal to suppress feeding. In fact, fructose has been demonstrated to be more potent than glucose in other behavioural actions such as a lower dose of fructose has been shown to have a higher ability to attenuate the morphine-induced analgesia than glucose (Lux et al., 1988).

In order to complete our assessment of the glucostatic mechanism on alcohol consumption parallel to food intake, the effects of hepatic portal glucose infusions should also be evaluated. Furthermore, as glucose intolerant rats have been shown to consume more alcohol than normal animals and several lines of alcohol-prefering rats are glucose intolerant, it will be of particular interest to study the responses of some of the alcohol-preferring lines of rats to the hepatic portal glucose infusions.
References:


APPENDIX: STATISTICS

EXPERIMENT ONE: THE EFFECTS OF GLUCOSE AND L-MALATE ON ALCOHOL INTAKE

A) ONE WAY REPEATED MEASURES ANOVA OF THE ALCOHOL INTAKE DATA

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B) ONE WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA

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EXPERIMENT TWO: THE EFFECTS OF FOOD DEPRIVATION AND GLUCOSE INJECTIONS ON ALCOHOL INTAKE

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F) TWO WAY REPEATED MEASURES ANOVA OF THE BLOOD GLUCOSE LEVEL DATA

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EXPERIMENT THREE: THE EFFECTS OF EXPERIMENTALLY-INDUCED DIABETES ON ALCOHOL INTAKE

A) TWO WAY REPEATED MEASURES ANOVA OF THE ALCOHOL INTAKE DATA

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<td>70.36004</td>
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<td>3</td>
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B) TWO WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA

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C) ONE WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA (BASELINE)

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D) ONE WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA (PHASE 1)

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<td>A</td>
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<td>64.84148</td>
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<td>253.066</td>
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E) ONE WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA (PHASE 2)

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<tbody>
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<td>A</td>
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<td>24.19619</td>
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F) ONE WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA (PHASE 3)

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<td>6.636177</td>
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G) TWO WAY REPEATED MEASURES ANOVA OF THE BLOOD GLUCOSE LEVEL DATA

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EXPERIMENT FOUR: THE EFFECTS OF TRIFLUOPERAZINE AND 2,4-DINITROPHENOL ON THE INHIBITORY EFFECT OF NALTREXONE ON ALCOHOL INTAKE

A) ONE WAY REPEATED MEASURES ANOVA OF THE ALCOHOL INTAKE DATA
(Trifluoperazine/Naltrexone)

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<td>8988.511</td>
<td>24.69371</td>
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B) ONE WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA
(Trifluoperazine/Naltrexone)

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<th>PROB&gt;F</th>
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<td>A</td>
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<td>90.27773</td>
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C) TWO WAY REPEATED MEASURES ANOVA OF THE BLOOD GLUCOSE LEVEL DATA
(Trifluoperazine/Naltrexone)

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D) ONE WAY REPEATED MEASURES ANOVA OF THE ALCOHOL INTAKE DATA
(2,4-dinitrophenol/Naltrexone)

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E) ONE WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA
(2,4-dinitrophenol/Naltrexone)

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EXPERIMENT FIVE: THE EFFECT OF IDAZOXAN AND METHOXY-IDAZOXAN ON ALCOHOL INTAKE

A) ONE WAY REPEATED MEASURES ANOVA OF THE ALCOHOL INTAKE DATA

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B) ONE WAY REPEATED MEASURES ANOVA OF THE WATER INTAKE DATA

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C) TWO WAY REPEATED MEASURES ANOVA OF THE BLOOD GLUCOSE LEVEL DATA

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