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The Effects of Opiate Antagonists on Voluntary Ethanol Consumption in Rats: Studies on the Mechanism of Action

Dena Davidson

A Thesis

in

The Department

of

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Presented in Partial Fulfillment of the Requirements
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ABSTRACT

The Effects of Opiate Antagonists on Voluntary
Ethanol Consumption in Rats:
Studies on the Mechanism of Action

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Concordia University, 1994

Clinical trials with the opiate antagonist, naltrexone (NTX) have found reductions in the voluntary ethanol intake of alcoholics. The mechanism of action of this compound is not well understood, however, and it has been suggested that the drug may have decreased the motivation to drink ethanol. Using an animal model of voluntary oral ethanol self-administration, the experiments contained in this thesis attempted to determine the mechanism by which opiate antagonists are mediating their reductions in ethanol drinking.

In experiment 1 rats were provided with 24 hr free-access to ethanol and water. There was no effect of NTX on voluntary oral ethanol intake at any of several doses administered. Experiment 2 was an attempt to examine more directly, the hypothesis that NTX has the capacity to block ethanol-mediated reinforcement. In that experiment ethanol self-administration was not extinguished during 20 days of forced-choice ethanol exposure and treatment with NTX. Although the experiment did not support the notion that NTX was acting by blocking ethanol-reinforcement, a direct, unconditioned suppression of ethanol intake was observed 4 hr following drug treatment. Thus Experiment 3 was designed to test the hypothesis that NTX's direct suppressant effects may be mediated through pre-ingestional mechanisms. In that experiment, NTX was administered during ethanol acquisition where pre-ingestional mechanisms are thought to be more important in mediating ethanol intake. Both 2.5 and 10 mg of NTX decreased ethanol intake at each concentration of ethanol presented and the acquisition

of ethanol drinking at 8% ethanol was also blocked. The data from this experiment were interpreted as evidence for the capacity of NTX to affect pre- and post-ingestional components of ethanol intake. Specifically, decreases in ethanol intake following NTX treatment may have been mediated through the induction of a conditioned taste aversion (CTA) at high doses (post-ingestional effects) and possibly through shifts in taste sensitivity at low doses (pre-injectional effects). Experiment 4 utilized a CTA paradigm. It was found that 10 mg of naloxone (NAL) induced a CTA to ethanol. Pre-exposing rats to NAL in experiment 5 blocked the development of a NAL-induced CTA to ethanol. Finally in experiment 6 it was demonstrated that the capacity of NAL to induce a CTA was also observed when a 0.1% saccharin solution was paired with NAL-treatment.

Taken together, the studies conducted in this thesis found no evidence that opiate antagonists have the capacity to block ethanol-mediated reinforcement. Two other mechanisms for NTX's action's were proposed: 1) opiate antagonists may induce their suppression on voluntary oral ethanol intake through their actions on pre-ingestional mechanisms such as shifts in the taste sensitivity for ethanol and, 2) depending on dose, opiate antagonists may decrease voluntary ethanol intake by inducing a CTA. Although both mechanisms result in the desired reduction in ethanol intake, a drug that mediates its effects through either mechanism would be expected to hold limited clinical value.

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I would like to dedicate this thesis to my son Tristan because in many ways, his sacrifice was greater than mine.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Ethanol is the most widely consumed recreational drug in Western society and an unfortunate consequence of its popularity is that ethanol dependence also constitutes the most prevalent form of drug dependence (Roach, 1971; Lieber, 1976). Alcoholism has serious consequences for society in terms of medical costs, absenteeism from work, auto- and work-related accidents, and family violence (Chick, 1984; Le Dain, 1973). It is, therefore, in the interest of health professionals who work with alcoholics to develop new and more efficacious methods for the prevention and treatment of alcoholism. One way of achieving this goal may be through the identification of factors that motivate and sustain ethanol self-administration.

Animal models of voluntary ethanol intake have provided compelling evidence that ethanol, like other drugs of abuse, is consumed for its positive reinforcing properties (Griffiths, Bigelow & Henningfield, 1980; Johanson, 1978; Kelleher & Goldberg, 1975). Support for this notion came from studies that have shown that animals will work to obtain access to ethanol (Meisch & Thompson, 1973; Woods, Ikomi & Winger, 1971). Ethanol, for example, can serve as a positive reinforcer in non-dependent animals under a variety of schedules of reinforcement when presented orally (Henningfield & Meisch, 1978; Meisch & Beardsley, 1975; Henningfield & Meisch, 1976), intravenously (Amit & Stern, 1969; Smith, Werner & Davis, 1976; Altshuler & Talley, 1977) or through the intragastric route of administration (Amit & Stern, 1969; Smith, Werner & Davis, 1976; Smith & Davis, 1974). The findings from these studies suggest that the reinforcing consequences of ethanol drinking play a significant role in maintaining ethanol self-administration (Griffiths, Bigelow & Henningfield, 1980; Johanson, 1978; Kelleher & Goldberg, 1975). By blocking the reinforcement derived from the ingestion of ethanol, the motivation to drink should be attenuated. This, in turn, could assist alcoholics in their efforts to abstain from drinking ethanol.

The most recent pharmacological intervention thought to be promising in the treatment of alcoholism involves administration of the opiate antagonist, naltrexone

(NTX). Clinical trials, with this compound, have found that when compared to alcoholics treated with placebo, those treated with NTX showed a decrease in the number of drinks consumed on days when ethanol was consumed, a decrease in the rate of relapse following ethanol consumption, and an increase in the length of abstinence periods (O'Malley, Jaffe, Chang, Schottenfeld, Meyer & Rounsaville, 1992; Volpicelli, Alterman, Hayshida & O'Brien, 1992). The reduction in relapse, observed in alcoholics that drank ethanol (Volpicelli et al., 1992), together with the decrease in the amount of ethanol consumed on drinking days (O'Malley et al., 1992; Volpicelli et al., 1992), suggested that the motivation to drink ethanol may have been attenuated in NTX-treated patients (Volpicelli et al., 1992). It is difficult, however, to defend the notion that the behaviors reported in these studies could have been mediated exclusively through the blockade of ethanol-mediated reinforcement by NTX and the subsequent extinction of ethanol-drinking behavior. If NTX were acting by blocking ethanol-mediated reinforcement, this mechanism would not explain the increase in periods of abstinence. Abstainers did not have the opportunity to learn that ethanol was no longer reinforcing because they did not drink ethanol while under medication or non-reinforcing conditions. Regardless of the mechanism by which NTX-treatment may be mediating its clinical effects, however, the results from preliminary clinical trials suggested that NTX may have some utility as a pharmacological adjunct to the treatment of alcoholism (Sinclair, 1990). The mechanism by which NTX is mediating its clinical effects, however, remains to be determined.

One approach to determine the mechanism by which NTX produced its clinical effects (i.e., decreased ethanol drinking and increased periods of abstinence) is to examine the effect of NTX administration on an animal model of ethanol self-administration. It was through this approach that the mechanism by which the serotonin uptake blocker, zimelidine, reduced voluntary ethanol consumption in rats was discovered (Gill & Amit, 1987). Zimelidine was a drug that was thought to block some

of the reinforcing properties of ethanol (Rockman, Amit, Carr, Brown & Ögren, 1979; Rockman, Amit, Carr & Ögren, 1979). Subsequent tests on an animal model of ethanol self-administration revealed, however, that zimelidine's suppressant effect was the result of the drug's capacity to enhance the onset of satiety rather then a specific capacity to block ethanol-mediated reinforcement (Gill & Amit, 1987). The mechanism underlying zimelidine's suppressant effects provides one example of how a drug can induce a reduction in ethanol intake without blocking ethanol-mediated reinforcement. This distinction between specific and non-specific interventions is important because clinicians are primarily interested in drugs that act by blocking ethanol-mediated reinforcement specifically, rather than those that act on all motivated behaviors. It is important, therefore, to understand the mechanisms through which a drug may mediate downward changes in ethanol intake because some of these mechanisms, as suggested above, may make the drug unsuitable for clinical application. Animal models that have been employed in the search for amethystic agents, have revealed a number of mechanisms by which ethanol intake can be reliably reduced, but which are not the result of an agent's capacity to block ethanol reinforcement (Amit, Gill & Ng Cheong Ton, 1991; Gill & Amit, 1987; Kline, Wren, Cooper, Varga & Canal, 1974; Sinclair, 1990). Some common examples of mechanisms that can mediate decreases in ethanol self-administration in rats, but that would have negligible clinical utility, will be discussed in the following section.

Issues Related to Decreases in Ethanol Self-administration: Behavioral Mechanisms

Conditioned Taste Aversions

When a drug is shown to decrease ethanol intake one cannot immediately conclude that the reduction was mediated by a blockade of ethanol-mediated reinforcement. It is equally possible that the reduction may be the result of a conditioned taste aversion (CTA) (e.g., Sirota & Boland, 1987; Gill, Amit & Ng Cheong Ton, 1991). A CTA is said to occur when animals, presented with a novel tasting fluid (the conditioned stimulus), paired with a drug or noxious physiological stimulus (the unconditioned stimulus), will avoid the fluid in future presentations even in the absence of the unconditioned stimulus (Hunt & Amit, 1987). This avoidance behavior studied pr..narily in the rat demonstrates that the animal has "learned" an association between the aversive effects of the unconditioned stimulus and the taste properties of the fluid that was paired with the unconditioned stimulus. For example, the dopamine-β-hydroxylase inhibitor. FLA-57, was initially believed to reduce voluntary ethanol consumption in rats by its capacity to block the reinforcing properties of ethanol (Amit, Brown, Levitan & Ögren, 1977; Davis, Werner & Smith, 1979). Further tests with FLA-57, revealed that when the drug was administered to rats in both forward and backward conditioning CTA paradigms, a CTA to ethanol was induced (Gill, Amit & Ng Cheong Ton, 1991). Interestingly, the CTA developed despite considerable prior exposure to ethanol suggesting that ethanol need not be a novel flavor in order for rats to develop a CTA. It is of relevance to this thesis that FLA-57 was initially thought to act by blocking some of the reinforcing properties of ethanol and thus, initially held substantial clinical appeal. Through systematic testing of this drug in animal models, it was revealed that the drug was producing its suppression in ethanol intake at least in part by inducing a CTA. The discovery of this mechanism was important because a drug that is producing a decrease in ethanol intake in this manner, would be limited in

its usefulness. Specifically, patient compliance would undoubtedly be poor because most individuals would not willingly follow a prescription that may be acting by inducing malaise. CTA's are often attributed to the capacity of the inducing agents to cause gastric distress or malaise in the organism (Garcia & Ervin, 1968). Interestingly, aversive effects of NTX have been reported in non-drug-dependent human volunteers treated with NTX (Hollister, Johnson, Boukhabza & Gillespie, 1981). Furthermore, clinical tests of NTX have found that nausea was a recurrent side-effect reported by some subjects. These side-effects were severe enough for some subjects to withdraw from treatment (O'Malley et al., 1992; Swift, Whelihan & Kuznelsov, 1993; Volpicelli et al., 1992). It is conceivable that clinical reports of NTX-mediated decreases in the amount of ethanol consumed on drinking days, as well as reductions in relapse that followed ethanol drinking (Volpicelli et al., 1992) were the result of the drug's aversive effects and not of its capacity to block reinforcement. It is also conceivable that increased periods of abstinence from ethanol (O'Malley et al., 1992) may be the consequence of general feeling of malaise in patients treated with NTX. Consequently, alcoholics medicated with NTX, may not feel well enough to engage in the social activity that generally accompanies ethanol drinking and this may explain the increase in periods of abstinence that have been reported (O'Malley et al., 1992).

Taste Factors

Another mechanism capable of mediating a decrease in ethanol consumption, other than one acting on pharmacological processes of reinforcement, may be the alteration of taste sensitivity. Ethanol, unlike some other drugs of abuse, is consumed exclusively through the oral route. Consequently, a drug manipulation that alters the taste properties of ethanol could theoretically increase or decrease voluntary ethanol consumption without necessarily affecting the post-ingestional reinforcing effects of ethanol consumption. Support for this notion can be obtained from studies on the acquisition of

ethanol drinking behavior in both rats and humans. Naïve rats, for example, seldom drink high concentrations of laboratory ethanol (8% and higher) upon first exposure (Cicero & Myers, 1968; Richter & Campbell, 1940). The initial reluctance to drink high concentrations of ethanol is assumed to reflect the aversive taste properties of absolute ethanol diluted with tap water. Conversely, the majority of naïve rats seem to prefer low concentrations of ethanol over water (Gill, 1984; Kiefer, Lawrence & Metzler, 1987; Kulkosky, 1981). This observed preference would suggest that the taste properties of low concentrations of ethanol (4% and lower) may be perceived as more palatable. Furthermore, rats will voluntarily consume pharmacologically-meaningful levels of ethanol only when: 1) exposed to relatively high concentrations of ethanol over an extended period of time (Daoust, Saligaut, Lhuintre, Moore, Flipo & Boismare, 1987), 2) slowly introduced to high concentrations of ethanol following a period of acclimatization to lower, more palatable concentrations (Mendelson & Mello, 1964; Myers & Veale, 1972; Williams, 1949), or 3) when the taste of ethanol is adulterated with saccharin (Samson, 1986).

These different acquisition procedures, which are often employed in animal models of ethanol self-administration, demonstrate the significant role of taste factors in initiating ethanol drinking in rats. Likewise, the initiation of humans to ethanol drinking generally involves adulterating ethanol with flavors that appeal to the human palate. For example, humans generally do not voluntarily consume absolute ethanol, but instead almost universally prefer to drink flavored alcoholic beverages. Therefore, it would appear that when rats and humans are initially exposed to ethanol drinking, prior to experiencing the positive post-ingestional consequences of ethanol ingestion, taste plays a significant role in determining whether the behavior will be repeated.

Support for the notion that opiate antagonists might effect taste sensitivity has been provided by Levin, Murray, Kneip, Grace and Morley (1982). They found that flavor enhanced the antidipsogenic effects of NAL. Thus decreases in water intake, observed

during treatment with NAL, was greater if the water was adulterated with saline, sucrose or HCl. This enhancement of the antidipsogenic effects of opiate antagonists has also been observed in rats presented with bitter solutions (Le Magnen, Marfaing-Jallat, Miceli & Devos, 1980). On the basis of these observations, it has been suggested that opiate antagonists may act by decreasing sensitivity to sweet or preferred fluids and by increasing the aversive properties of bitter or nonpreferred fluids (Le Magnen et al., 1980). Therefore, it is possible that opiate antagonists may decrease ethanol consumption by enhancing the aversive taste properties of concentrated ethanol solutions and by decreasing preference for the taste of low concentrations of ethanol. The mechanism underlying the decrease in ethanol intake measures, following treatment with opiate antagonists, therefore, could be related to a pre-ingestional shift in the palatability of ethanol, rather than the capacity of NAL to block the post-ingestional reinforcing effects of ethanol. The clinical utility of a drug that induced a decrease in ethanol intake by shifting palatability would be doubtful, because its effect on taste would likely effect all ingested substances and not just ethanol.

Motivation/Reinforcement

The current thinking on drug dependence is that self-administration of drugs of abuse is motivated and maintained by the positive reinforcing consequences of their ingestion (Spealman & Golberg, 1978). Ethanol, like other drugs of abuse, can serve as a positive reinforcer in non-dependent animals under various schedules of reinforcement and following several routes of administration (Amit & Stern, 1969; Henningfield & Meisch, 1978; Smith & Davis, 1974). Furthermore, neurobiological systems that are thought to mediate the reinforcement underlying natural incentive events, such as feeding and sexual behavior, might also mediate the reinforcement that is thought to sustain self-administration of ethanol (Stein & Belluzi, 1987; Wise, 1980). The identification of such systems, could in theory, allow for the discovery of

pharmacological treatments that would block reinforcement and thus over time, assist in extinguishing the drug-taking behavior.

One neurotransmitter system thought to be involved in reinforcement of a variety of motivated behaviors [e.g., sexual (Bilsky, Hubbell, Delconte & Reid, 1991; Leyton & Stewart, 1991), consummatory (Badiani, Rodaros & Stewart, 1991; Morley, Levine, Yim & Lowy, 1983] and opiate-intake (Bozarth, 1988) is the opioid system. Following the discovery of endogenous opioid receptors in the brain, it was also demonstrated that areas of the brain, believed to mediate reward, contained naturally occurring opioid-like substances such as enkephalins and endorphins which could occupy these receptors (Pert & Snyder, 1973). When these receptors are occupied by these endogenous opioids an individual experiences feelings of well-being or euphoria (van Ree & de Wied, 1985) and craving for ethanol should not develop (Trachtenberg & Blum, 1987). Indeed, clinicians have often found that opiate-dependent patients often have a history of heavy ethanol use which has led some clinicians to suggest that alcoholism may be an etiological factor in the development of opiate dependence and there may be a biological reciprocity mediating these two forms of dependence (Green & Jaffe, 1977). Some of the correlational evidence in support of a behavioral reciprocity between ethanol and opiate self-administration will now be reviewed.

Correlational Evidence for an Ethanol-Opioid Interaction

Clinical Studies

Epidemiological studies carried out on populations of opiate users have found that they often have a history of cross-dependence on both opiates and ethanol (Kolb, 1962). For example, Brown, Kozel, Myers and Dupont (1973) found that past ethanol consumption in their sample of heroin users was significantly higher than that of a normal control group. This finding is in agreement with Kolb (1962) who reported that in his sample, opiate users periodically drank ethanol and many suffered serious ethanol-related problems prior to becoming dependent on narcotics. Consistent with these reports of cross-dependence between opiates and ethanol, are observations that opiate addicts often substitute ethanol for opiates during periods of abstinence from opiates (Kolb, 1962) and that opiate users often reinstate excessive ethanol consumption following treatment for their dependence on opiates (Kreek, 1976; O'Donnell, 1964). Although the data from epidemiology studies find evidence for a reciprocity between the use of both ethanol and opiates, it is entirely possible that what has been interpreted as cross-dependence is merely co-occurrence of dependencies. Seevers (1970), for example, cautioned that cross-dependence observed in users needs to be distinguished from nonspecific attempts to attenuate withdrawal symptoms of opiates which might account for the use of both drugs. For example, ethanol is a drug that can cause depression of the central nervous system and therefore, it is possible that opiate addicts withdrawing from opiates, are self-medicating their withdrawal symptoms by self-administering ethanol rather than using ethanol as a substitute reinforcer.

Animal Studies

The propensity to self-administer both ethanol and opiates has also been observed in animals. Rats, bred to self-administer large amounts of morphine have also been shown to self-administer large amounts of ethanol (Smith, Werner & Davis, 1981). The reverse was also observed, rats that failed to self-administered morphine also failed to self-administered ethanol (Smith, Werner & Davis, 1981). Gelfand and Amit (1976), examined the effects of intraperitoneal injections of ethanol on oral morphine self-administration in rats and failed to observed any change in morphine preference following the administration of ethanol. They concluded that if an interaction between opiates and ethanol does exist, it could not be a simple symmetric relationship.

In conclusion, the evidence for reciprocity between opiates and ethanol self-administration among both animal and human users, have lead some investigators to suggest that both forms of dependence may share a common underlying biological mechanism (Green & Jaffe, 1977). Furthermore, it is possible that this mechanism may involve endogenous opioid systems (Blum, Briggs, Elston, Hirst, Hamilton & Vereby, 1980). In view of the experimental and correlational observations suggesting the presence of such a reciprocal relationship. some investigators have proposed possible mechanisms that may underlie both forms of dependence. Three of these theories will be discussed below.

Theories on the Relationship between the Endogenous Opioid Systems and Ethanol Self-administration

Opioid Deficiency Hypothesis

The assumption underlying the opioid deficiency hypothesis is that alcoholics suffer from a deficiency in central opioidergic activity which gets corrected when the alcoholic drinks ethanol (Blum, Briggs, Elston, Hirst, Hamilton & Verebey, 1980). This hypothesis further assumes that when ethanol is consumed it has the direct or indirect effect of activating endogenous opioid systems. Therefore, it follows from this hypothesis, that opiate antagonists reduce ethanol intake by blocking the actions of ethanol on central endogenous opioid receptors and this blockade in turn eliminates the motivation to drink.

Despite the behavioral evidence for some limited reciprocity between ethanol and opiate self-administration (Blum, Briggs, Elston, Hirst, Hamilton & Verebey, 1980; Green & Jaffe, 1977), it is presently not known how the activation of endogenous opioid systems can result in a behavior as complicated as alcoholism or even ethanol self-administration. Furthermore, the opioid deficiency hypothesis is also incongruent with the body of research that finds that opiate antagonists decrease ethanol self-administration. Presumably, opiate antagonists should artificially induce a deficit in opioid activity and according to the opioid deficiency hypothesis, this should increase ethanol self-administration, rather than decrease it.

The Opioid Surfeit Hypothesis

The premise of the opioid surfeit hypothesis (Reid, Delconte, Nichols, Bilsky & Hubbell, 1991), is also based on the assumption that an opioid-related, biological imbalance may exist in alcoholics. The form of this imbalance, however, is nearly the opposite to that suggested by the opioid deficiency hypothesis (Reid et al., 1991). The

opioid surfeit hypothesis proposes that the propensity to drink excessive amounts of ethanol is related to a biological surfeit or overabundance of activity in endogenous opioid systems (Reid et al., 1991). Support for this hypothesis rests on studies reporting that small doses of morphine (1.0 to 2.5 mg) administered to rats, artificially induces a surfeit condition in the brain, that results in an increase in ethanol intake (Reid et al., 1991). Furthermore, injections of NAL (10 mg/kg), which presumably prevents a surfeit condition, results in a decrease in ethanol intake (Hubbell, Czirr, Hunter, Beaman, Le-Cann & Reid, 1986; Reid & Hunter, 1984). The major proponent of the surfeit hypothesis, Reid (1991), contends that the changes produced in the functioning of endogenous opioid systems by ethanol, alter affective processes in the brain in such a manner that ingestive behaviors are reinforced more strongly under relatively high (surfeit) opioidergic activity as compared to periods of relatively low opioidergic activity (induced by opiate antagonists). Therefore, this putative underlying biological imbalance may result in alcoholics experiencing ethanol as more reinforcing compared to nonalcoholics.

It should be noted that Reid and his associates are the only investigators that have been able to demonstrate that the administration of low doses of morphine increase ethanol intake. Furthermore, it is possible that Reid's finding (Reid et al., 1991) may be an artifact of his method for getting animals to drink ethanol. Reid's protocol for training rats to drink ethanol includes sweetening the ethanol with sucrose. It has been demonstrated that low doses of morphine increase the intake of sweet substances (Calcagnetti & Reid, 1983). It is, therefore, difficult to determine whether the increase in ethanol intake observed by Reid was due to an increased preference for ethanol or an increased preference for sapid fluids in general.

"Link" Hypothesis

The impetus for this hypothesis came from experiments conducted in two independent laboratories. Davis and Walsh (1970) demonstrated, using brain stem homogenates, that tetrahydropapaveroline could form from dopamine and that this conversion was enhanced by the presence of ethanol and its primary metabolite acetaldehyde. In the same year, Cohen and Collens (1970) demonstrated that tetrahydroisoquinolines (TIQs) could also be formed *in vivo* from the condensation of catecholamines with the ethanol metabolite acetaldehyde. The significance of these findings is that these same alkaloids are precursors to the biosynthesis of morphine in the poppy plant. The possible synthesis of these alkaloids in mammalian tissues provided a potential mechanism by which ethanol ingestion, and the subsequent hypothesized formation of these morphine-like alkaloids, could act as false-neurotransmitters which could stimulate activity in endogenous opioid systems. On the basis of these reports, Blum, Hamilton, Hirst and Wallace (1978) proposed the "Link" hypothesis which claims that ethanol-mediated reinforcement, in similar fashion to opiate-mediated reinforcement, was supported through stimulation of endogenous opioid systems, which was achieved following the biosynthesis of these false-neurotransmitters derived from the ingestion of ethanol. It is important to note that since the discovery of these alkaloid compounds, considerable uncertainty exists as to whether TIQs are actually formed in human brain tissue. Furthermore, it has never been demonstrated that these compounds play a role in human ethanol drinking (Smith, Brown, & Amit, 1980).

In summary, although each of these hypotheses suggests an interaction between ethanol and opiates, it needs to be established that these two distinct classes of drugs can interact with the same neurotransmitter system. The biochemical evidence in support of this proposition will be reviewed in the following section, beginning with a brief review of the functioning and the neuroanatomy of endogenous opioid systems.

Endogenous Opioid Systems

The peptides that comprise the endogenous opioid family include the endorphins, enkephalins and dynorphins (Akil, Bronstein & Mansour, 1988; Simon, 1985). Each of these peptides consists of a unique chain of proteins that is derived from a protein precursor (Simon, 1985). For example, pro-opiomelanocortin (POMC) is the common precursor for the peptides, adrenocorticotropin (ACTH), α-MSH, β-lipotropin and β-endorphin (Akil, Bronstein & Mansour, 1988; Cooper, Bloom & Roth, 1986; Nakanishi, Inoue, Kita, Nukamura & Chung 1979; Simon, 1985). Pro-enkephalin is the precursor for the enkephalins; met-enkephalin (methionine enkephalin) and leu-enkephalin (leucine enkephalin) (Akil, Bronstein & Mansour, 1988; Noda, Furutani, Takahashi, Toyosato, Hirose, Inayama, Nakanishi & Numa, 1982; Simon, 1985). The precursor, pro-dynorphin, is cleaved into a-neo-endorphin, β-neo-endorphin, dynorphin 1-18 and dynorphin 1-17 as well as two larger dynorphins (Akil, Bronstein & Mansour, 1988; Kakidani, Furutani, Takeashi, Noda, Morimoto, Hirone, Asai, Inayama, Nakanishi & Numa, 1982; Simon, 1985).

Receptor binding studies (Paterson, Robson & Kosterlitz, 1983) have suggested the presence of three types of opioid receptors, designated μ, κ and δ. Although, there is evidence for the existence of a fourth opioid receptor, σ, (Schulz, Fause, Wuster & Herz, 1979; Wuster, Schulz, Herz, 1978), there is no convincing evidence that it exists in the brain (Akil, Bronstein & Mansour, 1988). Furthermore, this receptor subtype may not be a true opioid receptor because behavioral effects mediated by the stimulation of these receptors are not reversed by naloxone (NAL). Reversibility by NAL is the criterion used by pharmacologists to identify opioid receptors (Simon, 1985). Because each of the protein precursors for the three opioid peptides originate from the same ancestral gene (Simon, 1985), there are structural similarities between each of the peptides. These structural similarities allow these peptides to interact with all of the

opioid receptor subtypes to different degrees of efficacy, and this capacity for interaction makes endogenous opioid systems remarkably flexible (Akil, Bronstein & Mansour, 1988).

β-Endorphin

β-endorphin is the most potent and stable of the opioid peptides. It is concentrated in the anterior and intermediate lobe of the pituitary, and parts of the hypothalamus (Cooper, Bloom & Roth, 1986). This peptide is stored in neurons with long projection systems that extend rostrally to the amygdala, septum, nucleus accumbens, and caudally through the medial thalamus to the periaqueductal gray and locus coeruleus (Akil, Bronstein & Mansour, 1988; Copper, Bloom & Roth, 1986). In the anterior pituitary, POMC is processed largely to corticotropin and to an opioid inactive form of β-endorphin, whereas in the neurointermediate lobe cells of the pituitary and hypothalamus, the same precursor is processed into α-MSH and the opioid active form of β-endorphin. β-endorphin recognizes μ and δ receptors with equal affinity and has only a negligible effect at κ (Akil, Hewlett, Barchas & Li, 1980; Paterson, Robson & Kosterlitz, 1983; Wood, Charleson, Lane & Hudgin, 1981). This peptide has been studied most by researchers interested in the interaction between ethanol and endogenous opioid systems, and it is the endorphin peptide of primary interest in ethanol self-administration studies (see Gianoulakis, 1989).

Enkephalins

The enkephalins are much less stable than b-endorphin and therefore, once released are rapidly degraded. These peptides interact preferentially with d receptors however, both enkephalins can act on μ receptors. They have little effect at κ receptors (Paterson, Robson & Kosterlitz, 1983), which are the most selective of all the opioid receptors. Both met-enkephalin and leu-enkephalin are found in the corpus striatum,

hypothalamus, midbrain, hippocampus, cortex and cerebellum (Cooper, Bloom & Roth, 1986). The enkephalins are generally found in short, local neurocircuits, in the brain and are pervasively distributed throughout the peripheral nervous system (Cooper, Bloom & Roth, 1986). There is behavioral data suggesting that manipulations of central met-enkephalin levels can alter ethanol self-administration in rats (Froehlich, Zweifel, Harts, Lumeng & Li, 1991). For example, increasing levels of this peptide in the brain through the administration of an enkephalinase inhibitor, has been shown to increase voluntary oral ethanol self-administration in rats (Froehlich et al., 1991).

Thus far, dynorphins have not been implicated in contributing to the regulation of voluntary ethanol consumption and therefore will not receive further discussion in this thesis.

Naltrexone and Naloxone

The primary site of action of these opiate antagonists are the opioid receptor subtypes. Therefore, one approach to the understanding of the nature of the interaction between ethanol and endogenous opioid systems is by measuring the effect of opiate antagonists on ethanol self-administration, as well as measuring the concomitant effects of opiate antagonist treatment on endogenous opioid system functioning. NAL and NTX act as antagonists at all three opioid receptors. NTX is a much longer acting antagonist. They are equipotent their ability to reverse μ and δ opioid actions depending on the dose administered. At low doses (below 1.0 mg/kg) mostly μ receptors are filled (Childers, Creese, Snowman & Snyder, 1979; Chang & Cuatrecasas, 1981). When NAL is given in doses above 1.0 mg/kg, δ receptors are also bound (Chang & Cuatrecasas, 1981; Leander, 1983, Leander, Hart, Lochner, Hynes & Zimmerman, 1982). By comparison 20-30 times more NAL is necessary to antagonize opioid actions at κ receptors (Childers, Creese, Snowman & Snyder, 1979; Leander, 1983). Because

the doses of antagonists that show behavioral effects in voluntary drinking paradigms have been low, most research is aimed at examining the involvement of μ receptors.

Central Actions of Ethanol

Unlike other drugs of abuse (e.g., opiates), a specific central site of action has not been identified for ethanol (Goldstein, Chin & Lyon, 1982; Harris & Schroeder, 1981). Mammalian cell membranes consist of a lipid bilayer containing a number of proteins (Deitrich, Dunwiddie, Harris & Erwin, 1989). It is generally believed that ethanol acts on the hydrophobic portions of a neuron which alters the lipid bilayers and thus increases membrane fluidity (Charness, 1989; Deitrich, Dunwiddie, Harris & Erwin, 1989; Goldstein, Chin & Lyon, 1982; Harris & Schroeder, 1981). The precise hydrophobic site, however, has not yet been identified (Charness, 1989). By fluidizing the neuronal membrane, ethanol can disrupt normal neuronal activity which may lead to different behavioral effects depending on the brain region and cell-type affected (Charness, 1989; Deitrich, Dunwiddie, Harris & Erwin, 1989). Presumably, therefore, ethanol can interact with endogenous opioid systems by altering some aspects of membrane functioning of opioid neurons. This in turn may lead to a number of behavioral consequences such as intoxication and euphoria (Blum, 1983; Vereby & Blum, 1979). Several mechanisms have been proposed as potential sites of action for ethanol on endogenous opioid systems including: 1) changes in the release and synthesis of opioid peptides (Hiller, Angel & Simon, 1981; Seizinger, Bovermann, Maysinger, Höllt & Herz, 1983), 2) modifications in the binding properties of opioid receptors (Charness, Gordon & Diamond, 1983; Gianoulakis, 1983), and 3) production of certain ethanol metabolites, the isoquinolines, which can act as false neurotransmitters on opioid receptors (Davis & Walsh, 1970). The following section will review the effects of acute and chronic ethanol treatment on each of these processes.

Biochemical Evidence for an Interaction between Endogenous Opioid Systems and Ethanol

Ethanol effects on levels of endogenous opioid peptides

The acute and chronic effects of ethanol on levels of endogenous opioid peptides have been examined using both *in vivo* and *in vitro* techniques in rats (see Gianoulakis, 1989). Although it is beyond the scope of this thesis to critically analyze these studies on merit or to comment on the implications of the observed effects for a behavior as complicated as ethanol self-administration, the following section will examine the evidence that proposes a relationship between ethanol's pharmacological actions and the functioning of endogenous opioid systems.

In vivo studies conducted to examine the effects of acute peripheral injections of ethanol administered to rats on the content of central and peripheral content of β-endorphin, have found that ethanol can effect β-endorphin levels by interacting both at the level of the pituitary (Rivier, Bruhn & Vale, 1984) and the brain (Schulz, Wuster, Duka & Herz, 1980; Seizinger, Bovermann, Maysinger, Höllt & Herz, 1983). For example, peripheral injections of ethanol administered to rats, were shown to increase β-endorphin levels in the hypothalamus (Schulz, Wuster, Duka & Herz, 1980). Another investigator failed to replicate this finding but did observe an increase in β-endorphin in the plasma of rats, following the administration of a higher dose of ethanol (Seizinger, Bovermann, Maysinger, Höllt & Herz, 1983). The two studies cited above are typical of a group of studies where the results obtained in one laboratory are either the opposite to those reported by another, or yet another laboratory where no effects were measured (Gianoulakis, 1989). This state of affairs, however, is not surprising considering that the total content of β-endorphin measured in a particular tissue is affected by a variety of procedural differences included the time of sampling, the dose of ethanol, the route of administration of ethanol, as well as the target tissue

sampled (Gianoulakis, 1989). Furthermore, even when all procedures are held constant, the peptide content eventually measured also reflects the rate of biosynthesis, release and degradation of the final product. Each of these processes in turn, may be altered by ethanol. Despite the lack of consensus concerning the direction of ethanol's effects on total content of β-endorphin in target tissues, these same studies are nevertheless in agreement that ethanol can alter tissue content of b-endorphin. Furthermore, some mechanisms for the mediation of these processes by ethanol have been proposed.

The central release of β-endorphin is controlled mainly by corticotropin releasing factor (CRF) and by an inhibitory dopaminergic and stimulatory adrenergic system (Akil & Watson, 1986; Gibbs, Stewart, Vale, Rivier & Yen, 1982; Locafelli, Petraglia, Panalva & Pancrai, 1983)). CRF acts on the anterior lobe of the pituitary (Akil & Watson, 1986). The dopaminergic and adrenergic systems act on the neurointermediate lobe of the pituitary (Gibbs, Stewart, Vale, Rivier & Yen, 1982, Locafelli, Petraglia, Panalva & Panerai, 1983; Przewlocki, Höllt, Voight & Herz, 1979). Ethanol has been shown to affect the central dopaminergic systems (Vermes, Tilders & Stoof, 1985· Blum & Topel, 1986) and the release of CRF (Rivier, Bruhn & Vale, 1984). For example, at low doses, ethanol was shown to decrease the release of dopamine while high doses increase the release of dopamine (Kiianmaa & Tabakoff, 1983). Furthermore, both high and low doses of ethanol increase the synthesis of dopamine (Kiianmaa & Tabakoff, 1983). Thus the net effect of ethanol on the pituitary and brain β-endorphin level, may vary greatly depending on the dose of ethanol administered, and the time of sampling (Gianoulakis, 1989).

Ethanol has been shown to influence β-endorphin release through its actions on both CRF and ACTH (Keith, Crabb, Robertson & Kendall, 1986; Vermes, Tilders & Stoff, 1985). CRF regulates the release of ACTH, which is co-released with β-endorphin (Akil, Shiomi & Matthews, 1985, Guillemin, Vargo, Rossier, Minick, Ling, Rivier,

Vale & Bloom, 1977). Additionally, blocking the release of ACTH by the administration of an anti-serum to CRF, together with ethanol administration, abolished the release of ACTH and corticosterone that was observed when ethanol was administered alone (Vermes, Tilders & Stoff, 1985).

The effects of acute and chronic ethanol treatment on tissue content of the enkephalins in the rat brain have also been investigated (Schulz, Wuster, Duka & Herz, 1980; Seizinger, Bovermann, Maysinger, Höllt & Herz, 1983). The findings are similar to those in studies that examined the acute and chronic ethanol administration on the central tissue content of β-endorphin. In general, there is no consensus on ethanol's effect on total tissue content and the results seem to depend on the brain area examined. For example, acute administration of 2.5 g of ethanol to rats, did not change met-enkephalin content in the hypothalamus, pons/medulla, or mid brain when measurements were taken 20 and 60 min post-ethanol treatment (Renugopalakrishnan, Huang & Rapaka, 1987). In the same study, however, an increase in met-enkephalin content was measured in the striatum when the measurements were taken 60 min post-ethanol (Renugopalakrishnan, Huang & Rapaka, 1987). In a similar study, using the same dose of ethanol, significant increases in met-enkephalin in the hypothalamus, striatum, and midbrain but not the hippocampus have observe when measurements were taken 60 min post-ethanol treatment (Richelson, Stenstrom, Forray, Enloe & Pfenning, 1986). Chronic exposure to 20% ethanol decreased met-enkephalin content in the striatum, pons/medulla, midbrain but not in the hypothalamus of rats (Renugopalakrishnan, Huang, & Rapaka. 1987). When chronic exposure to ethanol was provided in the form of an ethanol-containing liquid diet to rats, a significant decrease in met enkephalin content in the hypothalamus, striatum, but not the midbrain or hippocampus was observed (Renugopalakrishnan, Huang & Rapaka, 1987). Thus, it appears that acute exposure to ethanol can increase tissue content of met-enkephalin, whereas chronic ethanol exposure decreases tissue content of this opioid peptide.

Whether or not this effect could be measured, however, would depend on the tissue examined, as well as the laboratory procedures followed.

In conclusion, the effect of ethanol exposure on brain tissue is different with each opioid peptide, even when tested in the same tissue. Furthermore, the effect of ethanol on the same opioid peptide will depend on the tissue sampled (Gianoulakis, 1989). Although there is no consensus on the direction of ethanol's effect on peptide content, there is agreement that ethanol can effect tissue content of endogenous opioids.

Ethanol effects on endogenous opioid receptors

The effects of ethanol administration on the binding of opioid peptides to their receptors have also been examined. Hiller, Angel and Simon (1981) demonstrated that acute *in vitro* exposure of brain tissue to low concentrations of ethanol selectively inhibited the binding of tritiated D-Ala-Leu-enkephalin. Because enkephalin binds preferentially to δ receptors (Paterson, Robson & Kosterlitz, 1983), Hiller et al. (1981) suggested that d receptors may be more sensitive to the actions of ethanol than other opioid receptor subtypes. In a subsequent study, the same investigators replicated their original findings, and demonstrated that μ and κ opiate receptor subtypes were insensitive to inhibition by ethanol (Hiller, Angel & Simon, 1983). In addition, they found that the mechanism by which ethanol reduced enkephalin binding to δ receptors was by decreasing receptor affinity for the ligand (Hiller, Angel & Simon, 1983). The inhibitory effect of ethanol on d receptors has been replicated by others (Tabakoff & Hoffman, 1983; Hynes, Lochner, Bemis & Hymson, 1983). Unlike the findings by Hiller et al., (1981) however, Tabakoff and Hoffman (1983) did find μ receptor binding sensitive to the effects of acute ethanol exposure *in vitro*, although in agreement with Hiller's group, they found the disruption of enkephalin binding to d was twice as sensitive. It would appear therefore, that acute ethanol administration inhibits the binding of opioid peptides to both δ and μ receptor subtypes, and the

difference between the findings of these two studies may be the result of differences in experimental conditions.

Chronic, *in vivo* exposure of tissue to ethanol has also been shown to affect the binding of μ receptors. The binding of ^{3}H-dihydromorphine to μ receptors was decreased following chronic ethanol treatment, whereas little effect of chronic ethanol treatment on the binding of enkephalin to δ receptors was observed (Pfieffer, Seizinger & Herz, 1981). This finding has been confirmed by other laboratories (Hynes, Lochner, Bemis & Hymson, 1983; Lucchi, Rius, Uzumaki, Govoni & Trabucchi, 1984). The decrease in the binding of ^{3}H-dihydromorphine to μ receptor is believed to be the result of a down-regulation of μ receptors induced by chronic ethanol exposure (see Gianoulakis, 1983; Creese & Sibley, 1981). Another laboratory, however, did measure an effect of chronic exposure to ethanol on δ receptor binding, but the direction of the effect was to increase rather than decrease binding (Lucchi, Rius, Uzumaki, Govoni & Trabucchi, 1984). The increase in binding of enkephalins to δ receptors, was thought to be an up-regulation of these receptors in the presence of ethanol (Gianoulakis, 1983).

Thus, ethanol may interact with opiate receptors and the direction of ethanol's effects depend on the duration of exposure to ethanol and the opioid receptor subtype measured. Acute exposure to ethanol may produce a down-regulation of both μ and δ receptor subtypes. Chronic exposure to ethanol, however, produces a down-regulation of μ receptors and an up-regulation of δ receptors. The mechanism by which ethanol induces these changes in receptor binding, is most likely through ethanol's fluidizing effects on cellular membranes (Deitrich, Dunwiddie, Harris & Erwin, 1989).

Ethanol and Tetrahydroisoquinolines

As mentioned previously, Davis and Walsh (1970) demonstrated the formation of tetrahydropapaveroline (THP) from dopamine in rat brain stem homogenates in the

presence of ethanol and its primary metabolite acetaldehyde. The formation of similar morphine-like condensation products, the tetrahydroisoquinolines (TIQ), produced through the condensation of catecholamines and acetaldehyde in cow adrenal glands were also discovered (Cohen & Collins, 1970). Subsequently, two laboratories reported that following ethanol administration, TIQs could be detected in the brains of laboratory animals and in the urine of Parkinsonian patients treated with L-DOPA (Turner, Baker, Algeri, Trigenio & Garattini, 1974; Sandler, Carter, Hunter & Stern, 1973). This provided the necessary demonstration that these alkaloids could be synthesized in mammalian tissue.

Subsequent studies examining TIQ alkaloids, found that these compounds may have the capacity to act as false neurotransmitters. Furthermore, they could be taken up and stored in catecholamine neurons and consequently inhibit the reuptake of naturally occurring catecholamines. These compounds were also shown to stimulate catecholaminergic receptors and to bind competitively to enzyme systems that synthesize or limit the actions of catecholamines. Because some of these TIQ alkaloids had previously been shown to be intermediate precursors of morphine in the poppy plant (Battersby, 1961; Shamma, 1972), it was suggested that TIQs may contribute to the regulation of some aspects of the pharmacological consequences of ethanol ingestion and may provide the "link" (Blum et al., 1978) which will explain how TIQs formed following ethanol ingestion can function as opiates.

It is important to note, however, that considerable uncertainty exists as to whether TIQs are actually formed in human brain tissue, and therefore, whether they play any role in human ethanol drinking. Furthermore, although Myers and his co-workers have repeatedly measured marked increases in ethanol intake in rats following intraventricular infusions of a variety of TIQ compounds, their findings have not been replicated by an independent laboratory (Sinclair & Myers, 1982; Smith, Brown & Amit, 1980) so there are also questions about a role for TIQs in animal intake of

ethanol. Although the discovery of these alkaloids initially produced an interesting putative mechanism by which ethanol could act on endogenous opioid systems, since their discovery increases in ethanol intake following TIQ infusions have not been replicated. Furthermore, the evidence would suggest that TIQs may simply be a by-product of consumed ethanol without any mediational role that would be of relevance to ethanol drinking (Smith, Brown & Amit, 1980).

In conclusion, there is considerable biochemical and neurophysiological evidence that ethanol can interact with endogenous opioid systems (see Gianoulakis, 1989, Olson, Olson & Kastin, 1992). The fluidizing effect of ethanol on membrane lipids may in turn influence the activity of proteins which reside within the lipid environment of the membranes (receptors, enzymes) as well as the rate of release of certain cellular products. With the discovery of the endogenous opioids it seems reasonable to suggest that ethanol may exert some of its effects, such as reinforcement, through actions on the endogenous opioid systems. Ethanol may interact with the endogenous opioid systems: a) by altering the release, synthesis and post-translational processing of endogenous opiate peptides; b) by altering the binding properties of opiate receptors and c) through the production of certain ethanol metabolites the isoquinolines which bind to opiate receptors.

Although the precise mechanism by which ethanol interacts with endogenous opioid systems is still unknown, behavioral studies that have been conducted to examine the effects of opiate antagonists on ethanol drinking, with few exceptions (Samson & Doyle, 1985), generally agree with the observation that opiate antagonists decreased ethanol intake (Altshuler, Phillips & Feinhandler, 1980; Froehlich, Harts, Lumeng & Li, 1990; Kornet, Goosen & van Ree, 1991, Sinclair, 1990). What is still unknown at the present, is the behavioral process through which opiate antagonists are mediating the observed reduction in ethanol intake. In humans, ethanol is consumed exclusively through the oral route. It follows then, that both pre- and post-absorptive feedback cues

may contribute to the initiation and maintenance of ethanol drinking and that opiate antagonist may suppress ethanol intake in rats by acting on either, or both of these mechanisms. Pre-absorptive factors can include the palatability of the ingested substance. Post-absorptive variables may include such factors as the blockade of the pharmacological or reinforcing properties of the ingested substance or the induction of a conditioned taste aversion. Consequently, investigators interested in the identification of agents which decrease ethanol intake by blocking reinforcement have employed a number of innovative paradigms designed to differentiate between the unique contribution of each of these variables. These studies will now be reviewed.

Behavioral Evidence for an Interaction between Endogenous Opioid Systems and Ethanol Self-administration

Post-Ingestional Effects

Altshuler, Phillips and Feinhandler (1980) were the first group to examine the effects of an opiate antagonist on the reinforcing properties of ethanol. In that study, 3 doses of Naltrexone (NTX) (1.0, 3.0, and 5.0 mg/kg) were administered to rhesus monkeys that were trained to self-administer a 10% concentration of ethanol through the intravenous route. They found that chronic pre-treatment with NTX initially increased responding for ethanol which was then followed by a significant decrease in responding. When saline was administered between periods of NTX treatment, responding for ethanol returned to baseline levels. The initial increase in responding observed during NTX treatment, was interpreted by the authors as demonstrating the extinction of the previously "learned" ethanol self-administration behavior. Specifically, the antagonism of opioid receptors by NTX was assumed to have blocked the reinforcement previously supported by ethanol self-administration. Thus, the monkeys initially increased their rates of responding, presumably to compensate for the loss of reinforcement. Once they "learned" that the reinforcing effects of ethanol were now absent, responding for ethanol began to extinguish.

This study has been cited frequently as providing evidence that opiate antagonists decrease ethanol self-administration by blocking ethanol-mediated reinforcement (Myers & Critcher, 1982; Samson & Doyle, 1985; Sinden, Marfaing-Jallat & Le Magnen, 1983; Weiss, Mitchiner, Bloom & Koob, 1990). There are, however, several problems with this study which may allow for alternative interpretations. First, water and food intake levels were not reported by authors. Without adequate controls for the effects of chronic NTX-administration on other consummatory behaviors, the issue of NTX's specificity to ethanol-mediated reinforcement remains unanswered. For

example, zimelidine is a drug that was once believed to decrease ethanol intake by blocking some of the reinforcing properties of ethanol (Gill & Amit, 1987; 1989). Subsequent tests, however, revealed that zimelidine's suppressant effects were not specific for ethanol self-administration, but rather were the result of the drugs capacity to enhance the onset of satiety (Gill & Amit, 1987; 1989). Second, chronic dosing with NTX has been shown to sensitize a variety of behavioral responses (Snell, Feller, Bylund & Harris, 1982; Tang & Collins, 1978; Young & Woods, 1982). The decrease in responding for ethanol reported in this study was only observed after 15 days of NAL administration. It is possible that the decrease in responding for ethanol did not reflect an extinction of ethanol self-administration behavior but may have been mediated by a direct, mechanism, that did not require learning. Therefore, what was interpreted as extinction may have been the result of an increased sensitivity to NTX, or an accumulation of the chronically administrated drug. Moreover, no replication of these data have ever been reported either from independent investigators or Altshuler's own laboratory.

In summary, the data described in Altshuler et al. study (1980) are not sufficient to conclude that NTX decreased responding for ethanol by blocking the reinforcing properties of ethanol. Furthermore, this study does not demonstrate that the observed suppressant effects of NTX observed were specific to ethanol.

Recently, the effects of NTX on voluntary oral ethanol intake were again tested in rhesus monkeys by Kornet, Goosen, and van Ree (1991). In that study monkeys were provided with one year of free-access to a 16% and 32% (v/v) solution of ethanol, along with food and water. One of six doses of NTX (0.02, 0.06, 0.17, 0.5, 1.0, and 1.5 mg/kg) was randomly administered to the monkeys over 12 consecutive trials, so that each monkey received a single injection of each dose. NTX-injections were paired with a placebo injection in a cross-over design. Following this experiment, an ethanol-abstinence period was imposed on the same monkeys by removing the ethanol for a

two day period. The authors have found that abstinence periods imposed on monkeys that were chronically self-administering ethanol, induced an increase in ethanol intake when access to ethanol was reinstated (Kornet, Goosen & van Ree, 1990). Following the abstinence period, NTX was again administered using the same dosing schedule described in experiment one. Thus, the effects of NTX were measured on chronic uninterrupted ethanol intake and on the abstinence-induced increase in ethanol intake. In both experiments, water and ethanol intake was measured for a 2-hr period, 30 min following afternoon injections, and again the following morning.

In the first experiment a dose-dependent decrease in ethanol intake was observed 30 min following NTX injections, and the suppression was still observed the following morning. Water intake was also reduced during the 30 min measurement period, but unlike ethanol intake, the reduction was no longer observed the following morning. Preference for ethanol was not effected by NTX treatment. The 32% ethanol solution was always the least preferred fluid, and the reduction in ethanol intake which followed NTX treatment, was most pronounced for the 16% ethanol solution.

In the second experiment the two day abstinence period produced the expected increase in ethanol intake. This increase in ethanol intake was observed when monkeys were injected with saline, 0.02 or 0.06 mg of NTX following ethanol-abstinence. Thus, the two lowest doses of NTX had no suppressant effect on ethanol intake. The abstinence-induced increase in ethanol intake was blocked in monkeys that received 0.17 mg of NTX, however, it was not blocked when they received the highest dose (1.5 mg). Although, the abstinence-induced increase in ethanol was still observed following treatment with 1.5 mg of NTX, the increase remained significantly lower than the increase measured following saline injections. Unlike experiment one, suppression of ethanol intake was only observed during the first 2-hr measurement period, and was no longer seen 24-hr after drug injections. There was no effect of NTX on water intake. Because the suppression of abstinence-induced ethanol intake

did not increase with increasing dose, the authors suggested that the 0.17 mg dose of NTX induced the maximal suppressant effect. In this experiment, ethanol-abstinence also led to a higher preference for ethanol over water, although the 16% solution was always preferred to the 32% solution. During NTX-treatment, this preference for the 16% solution was decreased.

The authors suggested that the NTX-induced decrease in ethanol intake observed in both experiments indicate that endogenous opioid systems were involved in chronic ethanol drinking in experiment 1, as well as drinking that follows an imposed period of ethanol-abstinence, in experiment 2. Furthermore, because preference for 16% ethanol was greater than for 32% ethanol and because the NTX-induced shift in preference observed in experiment 2 was greater for the 16% solution, they suggested NTX's suppressant effects were greater for the more preferred and thus more reinforcing concentration of ethanol.

The study by Kornet, Goosen and van Ree (1991) is consistent with studies conducted by other investigators that find antidipsogenic effects of NTX on ethanol intake (Sinclair, 1990; Froehlich et al., 1991). Although these data do suggest that endogenous opioid systems may be involved in ethanol drinking, it remains unclear whether the suppression of ethanol intake that followed NTX treatment was mediated through post- or pre-ingestional mechanisms. The authors interpreted the monkeys' shift in preference to the more preferred 16% solution as indicating that the more reinforcing fluid was also more effected by NTX. Thus, they interpreted this finding as a demonstration that NTX's suppressant effects were mediated by a blockade of ethanol reinforcement. It is also possible, however, that NTX acted by shifting taste sensitivity for ethanol. If NTX shifted taste sensitivity for ethanol, one would expect the suppression of the more preferred 16% solution to be greater compared to the suppression of the least preferred, 32% solution. Indeed, experiments specifically designed to measure the effect of opiate antagonists on palatability have found that,

when aversive bitter fluids were presented to rats, their reluctance to consume the fluid was not further enhanced by drug treatment (Levin et al., 1982). This was most probably because aversion was already maximal (Levin et al., 1982) Therefore, the shift in preference observed in this study may have little to do with the pharmacological consequences that followed the ingestion of either ethanol solutions. Furthermore, despite the suppression of the ethanol-abstinence-induced increase in the monkeys treated with 1.5 mg of NTX, compared to ethanol consumption during paired saline injections, an increase in ethanol intake was nevertheless observed. Assuming the abstinence-induced increase in ethanol intake reflects the organisms motivation to drink ethanol, it would appear that the highest dose of NTX did not abolish this motivation.

Myers and Critcher (1982), tested the effects of NAL on voluntary oral ethanol drinking in rats given 24 hr, free access to either an 8% or 16% concentration of ethanol. One of several doses of NAL (1.5-3.0 mg/kg), was administered to rats, 2-6 times per day for a period of 3 days. In addition, the ethanol intake of half of the group of animals was enhanced through intracerebroventricular infusions of THP (tetrahydropapaveroline); the remaining rats were not infused with THP. Previously Myers had shown that intracerebroventricular infusions of THP, an opiate-like bi-product, can increase ethanol intake in rats (Myers & Melchior, 1977; Myers & Oblinger, 1977). The authors did not explain their rational for comparing the effects of NAL on the drinking of rats infused with THP and non-THP-infused animals.

Both THP- and non-THP-infused rats decreased their ethanol intake during NAL treatment and the magnitude and duration of the suppression depended on the dose, frequency of drug administration and the rats individual basal levels of ethanol intake. Specifically, suppression of ethanol intake was greatest at the highest dose of NAL, when it was administered 6 times per day. Thus, it appeared that the drug regime that maintained the most consistent blood levels of NAL, was the most effective at suppressing ethanol intake. Additionally, the suppression of ethanol intake by NAL

was greater for the 16% concentration of ethanol than for the 8% concentration. Food and water intake were only decreased in the group that received the highest dose of NAL, 6 times a day. These authors concluded that their results supported the notion of an opioid receptor link in the pathogenesis and maintenance of aberrant drinking of ethanol. Furthermore, they suggested that the mechanism underlying NAL's suppressant effects may involve the endogenous action of an amine-aldehyde condensation products in the brain that followed ethanol metabolism, and in turn was blocked by the administration of the opiate antagonist.

Despite the suppression of ethanol intake by NAL observed in this experiment, rats drinking ethanol at levels above 5.0 g/kg prior to NAL treatment, continued to drink approximately 3.5 g/kg of ethanol during NAL treatment. Furthermore, rats that were drinking levels of ethanol below 5.0 g/kg, continued to drink approximately 2.8 g/kg of ethanol during NAL-treatment. Ethanol consumption within this dose range (2.8 to 3.5 g/kg) is sufficient to produce pharmacologically-relevant levels of ethanol in blood and brain (Gill, 1989). Therefore, although the reduction in ethanol intake reported in this study was statistically significant, these rats were still consuming sufficient amounts of ethanol to induce intoxication. This would suggest that it is unlikely that the mechanism behind the reduction in ethanol intake was due to a blockade of ethanol-mediated reinforcement. In addition, the authors noticed a rebound effect on ethanol intake following the initial suppression by NAL. This would suggest that the suppressant effect of NAL that was observed, was transitory. This pattern of transientory suppression is not generally associated with drugs thought to act by inducing extinction. Instead it may reflect a direct effect of the antagonist that was abolished once the drug was eliminated from the system. This might explain why the most effective dose regime for suppressing ethanol intake in Myers study (1982) was frequent injections of the highest dose of NAL. Finally, it was surprising that the authors did not report differential suppressant effects of NAL on THP-infused rats compared to rats

which were not THP-infused. If NAL was mediating the observed decreases in ethanol intake, by its capacity to block opiate receptors, then one should have expected this effect to be greater for the THP-infused group, whose ethanol intake was presumably enhanced by the infusion of these opiate metabolites.

An alternative explanation for the findings of Myers and Critcher (1982) may be that NAL exerted its suppression of ethanol intake through pre- rather than post-ingestional mechanisms, such as a shift in the taste sensitivity of ethanol. For example, the taste of ethanol may have been rendered aversive during NAL administration and this could explain the rapid decrease in ethanol intake immediately following NAL treatment. Alterations in the palatability of ethanol could also explain the author's observations that the antidipsogenic effects of NAL on ethanol intake were more pronounced in animals consuming a 16% concentration of ethanol rather than in animals consuming a 8% concentration of ethanol. One would expect the aversive taste properties of ethanol to be greater at higher and therefore, less palatable concentrations of ethanol. Indeed, NAL has been shown to accentuate taste aversions to a quinine solution and to suppress taste preferences for sweetened substances (Le Magnen et al., 1980). In conclusion, the pharmacologically-relevant amounts of ethanol consumed by rats, despite treatment with NAL, together with the rapid and transient duration of the suppression on ethanol intake (Myers & Critcher, 1982), makes it unlikely that NAL blocked ethanol reinforcement.

Myers and Critcher (1982) examined the effects of NAL on heterogeneous rat strains; Sprague-Dawley and Long-Evans. There have been three studies which examined the effects of opiate antagonist on ethanol intake in selectively bred rat strains (Froehlich et al., 1990; Iso & Brush, 1991; Sinclair, 1990). For example, Froehlich et al. (1990) examined the effects of acute injections of NAL on ethanol intake in rats selectively bred for high ethanol preference. Once every 2 weeks, rats of the HAD (high ethanol drinking) line received injections of a randomly determined dose of NAL,

ranging from 0-18.0 mg/kg. During a 2-hr period, rats were provided with access to either a 10% concentration of ethanol, or water. This paradigm was used in a second experiment, except that rats were now provided with 2-hr access to water alone (Froehlich, Harts, Lunmeng & Li, 1990). Fluids were restricted during the remaining 22-hr period during both experiments.

They found that NAL suppressed water intake when water was presented as the sole source of fluid. In contrast, NAL produced a dose-dependent decrease in ethanol intake, without altering water intake, when both fluids were available. This selective suppression of ethanol consumption by NAL was not attributable to changes in blood ethanol concentrations or ethanol elimination rates following NAL treatment. The authors concluded that although NAL may attenuate the positive reinforcing properties of both ethanol and water, ethanol drinking may be a subset of consummatory behaviors that is particularly sensitive to opioid receptor blockade. Furthermore, because larger doses of NAL were more effective at suppressing intake than lower doses, it was argued that δ opiate receptors, which are blocked by larger doses of opiate antagonists, may be more important in the regulation of ethanol drinking than μ receptors.

The above experiment provided additional evidence that NAL has antidipsogenic effects on both water and ethanol intake (Froehlich, Harts, Lunmeng & Li, 1990). The selective decrease in ethanol intake observed when both fluids were presented, however, could be the result of 22-hr fluid deprivation during the remainder of the day rather than an affinity of NAL for blocking ethanol intake over water intake. This deprivation period would have dehydrated the animals and thus, it would not be surprising that during the 2 hr period of drinking, rats would prefer water over ethanol. In this experiment, total ethanol intake was reduced during NAL treatment, but preference for ethanol was also reduced. Preference ratios are not only sensitive to manipulations that decrease ethanol intake but are also sensitive to manipulations that

increase water intake. For example, it is possible for rats to drink a large quantity of ethanol (6 g/kg and greater) and to also significantly increase water intake so that while preference for ethanol decreases, ethanol intake levels actually remain high. In conclusion it is possible that what Froehlich et al. (1990) interpreted as a selective reduction in ethanol intake, was in fact an artifact of an increase in water intake due to the period of fluid restriction outside of the 2-hr measurement period and not the blockade of ethanol reinforcement.

Iso and Brush (1991) examined the effects of chronic exposure of a subcutaneous implant of NTX on ethanol intake in a selectively bred strain of rats. The Syracuse high (SHA) and Syracuse low (SLA) avoidance rats were selectively bred from Long-Evans stock according to a behavioral criterion of good or poor shuttle-box avoidance learning. These strain differences in avoidance performance have been interpreted as inbred differences in emotional reactivity. The SLA rats were thought to be more emotionally reactive because they showed a lower number of intertrial responses during avoidance training, and because they defecated more during avoidance training, and in the open-field, as compared to SHA animals (Brush, Baron, Froehlich, Ison, Pellegrino, Phillips, Sakellaris & Williams, 1985). Rats from both strains were offered free-access to water and 10% (v/v) ethanol for two, 8 day periods that were separated by a 4 days of forced-choice exposure to ethanol (Iso & Brush, 1990). Baseline ethanol intake levels were not recorded, and rats were not matched on ethanol intake prior to their assignment to treatment groups. Both SHA and SLA rats were implanted with either a 30 mg pellet of NTX or a placebo pellet. Twenty-four hr water intake, was significantly reduced in both strains treated with NTX, however, the reduction was greater for the SLA group than for the SHA group. During the first free-access period, ethanol intake was suppressed in both SLA-NTX and SHA-NTX groups compared to controls. The SLA-NTX group, however, maintained low levels of ethanol intake throughout this period, whereas the SHA-NTX group recovered from the initial

suppression, so that by the end of this period they drank levels of ethanol above ethanol levels consumed by both control groups. The forced-choice period, resulted in increased intake in all groups compared to their levels of intake measured during the initial free-access period. This increase was greatest in the SHA-NTX group. During the second free-access period following the forced-choice period, the SLA-NTX group increased their ethanol intake to match the level of intake measured in the two control groups. The SHA-NTX group, however, continued to drink ethanol above the levels measured in the other 3 groups. The authors concluded that chronic exposure to NTX produced bi-directional effects on ethanol intake; suppressing ethanol intake in the SLA group and enhancing ethanol intake in the SHA group compared to controls. Furthermore, Iso and Brush (1990) argued that the increase in ethanol intake observed in the SHA-NTX group was the result of a compensation process against the loss of the reinforcing effects of ethanol by NAL, and therefore, ethanol reinforcement was of greater value to SHA rats, than SLA rats.

This is the only paper, in the literature, reporting an enhancement of ethanol intake following NTX-treatment (Iso & Brush, 1990). Although, the suppression in ethanol intake found with the SLA-NTX group is consistent with reports that find an inhibitory effect of NTX on ethanol intake (Altshuler, Phillips & Feinhandler, 1980; Sinclair, 1990), the authors seem to be overstating their data when they claim that ethanol intake was enhanced in the SHA-NTX group. The absence of measured baseline levels of ethanol intake at the start of this experiment presents a confound because ethanol intake in the SHA control group remained consistently higher than ethanol intake in the SLA control group. This observation may suggest that SHA rats may not only be bred for emotional reactivity but may have also inherited a preference for ethanol or that one leads to the other. Furthermore, suppression of ethanol intake by NTX was of shorter duration in the first free-choice period in the SHA-NTX group compared to the SLA-NTX group. The preference for ethanol demonstrated by the SHA control group,

together with the transient suppressant effect of NTX on ethanol intake in this group, could be interpreted as an insensitivity to the antidipsogenic effect of the antagonist rather than an increase in ethanol intake. Therefore, what was reported as an increase in ethanol intake in SHA-NTX treated groups may have been in fact, an artifact of assigning ethanol preferring rats to this group, their natural tendency for ethanol intake to increase over time as well as an insensitivity to the antidipsogenic effects of NAL.

The final study reviewed here was conducted by Sinclair (1990). Long-Evans and the AA strains of rats, the latter selectively bred for high ethanol drinking, were trained to drink a 10% concentration of ethanol during a 1-hr limited access (LA) schedule. Sinclair found that daily injections of 10 mg/kg NAL significantly decreased ethanol intake in both strains of rats. It was unlikely, however, that the suppression was due to a direct effect of NAL since the decrease in ethanol intake was not seen until the second day of injections. He argued that the delayed suppression could be explained in terms of the extinction of ethanol-drinking behavior. By the 4th day of NAL administration, ethanol intake was significantly lower in these animals compared to intake levels seen in saline control animals. It was also significantly lower than the animals own baseline intake. Sinclair (1990) suggested that the mechanism underlying this decrease was mediated by the capacity of NAL to block the reinforcement derived from drinking ethanol which over days extinguished the "learned" drinking response

An alternative explanation of Sinclair's data (1990), however, could be that the reduction in ethanol intake measured following NAL administration was caused by NAL's known capacity for inducing conditioned taste aversions (CTA) in rats (Van Der Kooy and Phillips, 1977; Le Blanc & Cappell, 1975, Stolerman, Pilcher & D'Mello, 1978). Opioid antagonists were reported to induce CTA's in rats that were exposed to novel sapid fluids such as ethanol and saccharin (Le Blanc & Cappell, 1975; Van Der Kooy & Phillips, 1977) at the same dose range as that administered by Sinclair (1990). Therefore, on the basis of existing data it would be difficult to determine if the decrease

in ethanol intake observed in Sinclair's study (1990) was the result of the extinction of drinking behavior previously supported by the positive reinforcing properties of ethanol, or whether it was the result of a CTA. It is worth noting here that when rats develop a CTA, it cannot be measured until the second pairing of the CTA-inducing subs e and the novel tasting fluid. Thus, the pattern of the decrease in ethanol intake found in Sinclair's study, would also be expected if these rats had been injected with an agent known to produce CTA's.

It has been suggested that the enkephalins might be the opioids involved in mediating the reinforcing effects of ethanol (Froehlich, Harts, Lumeng & Li, 1990). Froehlich, Zweifel, Harts, Lumeng & Li, (1991) tested this hypothesis by injecting HAD rats with an antagonist, ICI 174864, which is selective for d receptors, and either NAL or thiorphan. Thiorphan is an enkephalinase inhibitor which would be expected to act by increasing brain levels of enkephalins. The authors found that both antagonists were effective at decreasing ethanol intake, however, the selective antagonist ICI 174864, produced a larger reduction which suggested that the enkephalins might be of greater importance to the regulation of ethanol drinking. Interestingly, the effect of thiorphan was to increase ethanol intake. Taken together, these data suggested that the activation of the endogenous opioid systems may be an important mechanism which serves to maintain continued ethanol drinking.

The only investigators to find no effects of NAL on ethanol drinking in rats were Samson and Doyle (1985). They examined the effects of 3 doses of NAL (5.0, 10.0 or 20.0 mg/kg) on rat's operant responding for oral access to a dipper filled with 5% concentration of ethanol or a dipper filled with water. They also repeated the experiment and tested these same doses of NAL on responding for a 1% sucrose solution or water. In the first experiment, they found that only the highest dose of NAL produced a decrease in responding for ethanol. Responding for water was also decreased, but only at the 5 mg dose of NAL. In the second experiment, there was no effect of NAL on

responding for sucrose or water. They concluded that since only relatively large doses of NAL affected ethanol responding, in marked contrast to the effects of narcotic antagonists on morphine self administration, it seems unlikely that direct receptor activity by ethanol or its metabolites on endogenous opioid systems is involved in the maintenance of oral ethanol self-administration in rats.

It is possible that the negative results of Samson and Doyle's experiment (1985) stems from the fact that their paradigm is very different from oral drinking paradigms, or operant responding for intravenous self-administration of ethanol. Furthermore, the rats in this experiment were provided with access to a low concentration of ethanol and the testing period was only 30 min in length. In order to examine the effects of a drug on ethanol-mediated reinforcement, it would seem necessary for the organism to ingest sufficient quantities of ethanol in order to "learn" that reinforcement was being blocked. In this paradigm, however, responding was dramatically reduced when the 20 mg dose was administered, before the rats could have ingested a pharmacologically-relevant amount of ethanol. It could be argued that large doses of NAL inhibit the approach or anticipatory response to ethanol, however, it is unlikely that the high dose of NAL was effecting motor behavior since no detrimental effects were found in responding for sucrose. Samson and Doyle (1985) did not find any effects on responding for sucrose. It is possible that lever-pressing as opposed to drinking, recruits different motivational processes. For example, rats that are shown to ingest large amounts of ethanol in 24-hr two-bottle choice paradigm often fail to lever-press for ethanol reinforcement (Gill, 1984).

Pre-Ingestional Effects

Evidence for the idea that opiate antagonists effect taste sensitivity was provided in a study by Marfaing-Jallat, Miceli and Le Magnen (1983). They investigated the effect of acute injections of 1.0 mg NAL on oral intake of either an 8% or 10% concentration of

ethanol in either naïve rats or rats that they assumed were "behaviorally" dependent on ethanol. In order to induce the behavioral dependency, rats received chronic intragastric administration of a 10% ethanol solution. Thus, this experiment attempted to test the effects of NAL on rats that preferred ethanol, and on naïve rats, that generally show a spontaneous taste aversion to high concentrations of ethanol.

Naïve rats that consumed low levels of ethanol during a 30 min period, further reduced their level of ethanol intake during NAL injections. Ethanol intake in behaviorally dependent rats, provided with 8 hr access to ethanol, was also reduced following NAL injections. The authors interpreted the data to mean that the reduction in the intake of ethanol of both non-preferring naïve rats and ethanol-preferring dependent rats was the result of shifts in their taste sensitivity for ethanol. Ethanol-preferring rats were said to shift their preference for ethanol, and ethanol-nonpreferring rats, to find ethanol even more aversive. The problem with their interpretation is that the levels of ethanol intake by the putatively "behaviorally" dependent rats were not as high as those of rats that were indeed dependent on ethanol or over those that merely preferred ethanol over water. Mean baseline intake levels during 8-hr access, for the period of 6 days in that study was approximately 14 mls which means that in g/kg intake, these rats were drinking approximately 2.9 g/kg/day. High drinking genetic strains of rats, generally drink a mean of 6 g/kg of ethanol per day, without undergoing procedures to produce dependence (Froehlich, Harts, Lumeng & Li, 1990; Sinclair, 1991) Therefore, it is possible that the authors were actually testing spontaneously-induced aversion to ethanol in both groups of animals.

Support for the idea that opiate antagonists act on pre-ingestional mechanisms was also supported by a study by Sandi, Borrell and Guaza, (1988). The latter authors, provided naïve rats with 15 min access to a weak, 2.5% ethanol solution. These authors had previously shown that rats demonstrated stable ethanol preference at this low concentration of ethanol (Guaza, Borrell & Borrell (1986). Injections of 1.0 or 5.0

gm/kg NAL produced a dose-dependent decrease in ethanol intake that was no longer observed once injections were terminated. The authors (Sandi, Borrell & Guaza, 1988) concluded that NAL may have mediated its suppressant effect by blocking opiate receptors involved in gustatory learning, either by interfering with the taste of ethanol or possibly by decreasing the reward value of ethanol.

Although the authors (Sandi, Borrell & Guaza, 1988) demonstrated that NAL abolished the spontaneous preference for this weak ethanol solution, the concentration of ethanol was probably too low, and the duration of exposure to ethanol too brief for the rats to have consumed pharmacologically-relevant amounts. Therefore, the low concentration of ethanol used in this experiment does not allow any conclusions concerning NAL's putative effect on the reward value of ethanol. It is, however, likely that NAL reduced ethanol intake by interfering with the taste of ethanol, possibly by rendering the preferred 2.5% ethanol solution less palatable.

Sinden, Marfaing-Jallat and Le Magnen (1983) attempted to attenuate the influence of pre-ingestional cues associated with the oral intake of ethanol, by administering NAL to rats that were trained to level press for intragastric infusions of a 10% concentration of ethanol. Acute injections of 1.25 and 2.5 mg/kg NAL significantly inhibited lever pressing for ethanol during the initial 2-hr measurement period following treatment. Following this period, however, there was a rebound effect on lever pressing for ethanol measured for the 2.5 dose of NAL but not for the lower dose. It was unlikely that the suppression of lever pressing observed in that study (Sinden, Marfaing-Jallat & Le Magnen, 1983) was the result of a motor deficit since at the doses of NAL tested, deficits in motor activity in rats has not been reported (Amir, Solomon & Amit, 1979; Carey, Ross & Enns, 1981). There were also no effects of NAL on 24-hr measurements of ethanol, food, or water intake. The authors (Sinden, Marfaing-Jallat & Le Magnen, 1983) concluded that even when the taste and smell of ethanol was minimized, the effect of NAL was still capable of inhibiting lever pressing for ethanol.

Additionally, the initial decrease in bar pressing suggested that NAL's inhibition of ethanol intake does not solely depend on a transient post-absorptive component because ethanol in this study was not consumed through the oral route. It seems unlikely that the suppression in bar pressing for ethanol could have been mediated by NTX's blockade of ethanol reinforcement, however, because no ethanol was consumed to have allowed the rats to experience the blockade. Furthermore, since the net levels of ethanol intake remained high throughout the experiment (6.9 to 8.7 g/kg), it is unlikely that the suppression could be explained by a suppression in post-ingestional reinforcement derived from ethanol.

In conclusion, with the exception of Samson and Doyle (1985), animal studies conducted thus far, do show that opiate antagonists inhibit ethanol intake in a variety of species (e.g., monkeys; Kornet, Goosen & van Ree, 1991; rats; Sinclair, 1990; humans; Volpicelli et al., 1992), and through at least two routes of administration (i.v., Altshuler, Phillips & Feinhander, 1990; oral intake; Sandi, Borrell & Guaza, 1988). A comparison of the studies reviewed in this section can be seen in Tables 1-3. The evidence also points to a role for endogenous opioid systems in the mediation of NAL-induced decreases in ethanol self-administration (Froehlich, Harts, Lumeng & Li, 1990). Furthermore, biochemical studies have demonstrated that ethanol has pharmacological effects on different aspects of endogenous opioid system functioning (see Gianoulakis, 1989). The mechanisms underlying these decreases in ethanol self-administration, however, are still unidentified. The behavioral studies presented in this thesis (Altshuler et al., 1980; Sandi, Borrell & Guaza, 1988; Sinclair, 1990; Marfaing-Jallat, Miceli & Le Magnen, 1983) allude to at least two mechanisms for how opiate antagonists may decrease ethanol self-administration. They may decrease ethanol intake through their actions on post-ingestional mechanisms such as the blockade of reinforcement, or possibly the induction of a CTA to ethanol. Alternatively, the suppression induced by opiate antagonists, may be mediated through pre-ingestional

mechanisms such as shifts in taste sensitivity. One approach for differentiating between pre- vs post-ingestional mechanisms may involve examining the latency between the time of drug injection and suppression of ethanol intake. It seems reasonable to suggest that drug effects mediated through post-ingestional mechanisms would emerge more slowly. A period of time would be necessary to allow for the absorption of ingested ethanol and to "learn" from this experience, whether or not reinforcement was pharmacologically-blocked, or to experience an aversive physiological state, which generally follows the induction of a CTA (Garcia & Ervin, 1968). Decreases by opiate antagonists, mediated through pre-ingestional mechanisms, on the other hand, might be expected to produce a rapid reduction in ethanol intake upon the initial taste of ethanol intake.

Table 1

Comparison of Voluntary Ethanol (EtOH) Self-administration Paradigms: Concentration of Ethanol above 7% and Chronic Treatment with Opiate Antagonists (mg/kg)

Researchers	Species	Drug/Dose	Measurement Interval	Route of administration	Results
Altschuler et al. (1980)	monkey	NALT 1.0, 3.0, 5.0	4 hr	Intravenous	Initial increase in responding for EtOH, followed by a decrease in responding
Myers & Critcher (1982)	rat	Nal 1.5-3.0	24 hr	Oral	Decreases in EtOH intake; magnitudeof decrease related to dose and injection regime
Iso & Brush (1991)	rat	NALT 30 mg pellet	24 hr	Oral	Increases & decreases in EtOH intake; depending on strain
Sinclair (1990)	rat	NAL 10.0	1 hr	Oral	Gradual and sustained decrease in EtOH intake

Table 2

Comparison of Voluntary Ethanol (EtOH) Self-administration Paradigms: Concentration of Ethanol above 7% and Acute Treatment with Opiate Antagonists (mg/kg)

Researchers	Species	Drug/Dose	Measurement Interval	Route of administration	Results
Kornet, Goosen & van Ree (1991)	monkey	NAL 0.02, 0.06, 0.17. 0.5, 1.0 &1.5	2 hr & 24 hr	Oral	Decreases in EtOH intake following chronic EtOH drinking; decreases in EtOH intake following abstinence-induced increase in EtOH intake
Froehlich et al. (1990)	rat	Nal 0.05, 0.075, 0.1, 3.0, 6.0, 12.0 & 18.0	2 hr	Oral	Decreases in EtOH intake; *** fluid restriction outside of measurement phase

Table 3

Comparison of Voluntary Ethanol (EtOH) Self-administration Paradigms: Concentration of Ethanol below 7% and Acute Treatment with Opiate Antagonists (mg/kg)

Researchers	Species	Drug/Dose	Measurement Interval	Route of administration	Results
Marfaing-Jallat, Miceli & Le Magnen (1983)	rat	NAL 1.0	30 min / 8 hr	Oral	Decreases in EtOH intake in naive and behaviorally dependent rats
Sandi, Borrell & Guaza (1988)	rat	Nal 1.0, 5.0	15 min	Oral	Decreases in EtOH intake on very low concentration of EtOH (2.5%)
Sinden, Marfaing-Jallat & Le Magnan (1983)	rat	NAL	24 hr	Bar pressing for intragastric infusions	No decrease in 24 hr EtOH intake but initial reduction in bar pressing
Samson & Doyle	rat	NAL 5.0, 10.0 or 20.0	30 min	Lever pressing for oral access	Decrease only for 20 mg; decreases not found with saccharin

Review of Operant Conditioning Principles

With the exception of the study by Sinclair (1990) and the study by Altshuler et al. (1980), almost all of the studies that have examined the effects of opiate antagonists on the intake of ethanol have reported immediate and transitory suppressant effects (Froehlich, Harts, Lumeng & Li, 1990; Iso & Brush, 1991; Kornet, Goosen & van Ree, 1990; Marfaing-Jallat, Miceli & Le Magnen, 1983; Myers & Critcher, 1992; Sandi, Borrell & Guaza, 1988). Sinclair (1990) and Altshuler et al. (1980) have argued that the suppression of ethanol intake by opiate antagonists may reflect the blockade of ethanol-mediated reinforcement and, therefore, the decrease in ethanol self-administration observed in their studies reflected the extinction of ethanol drinking behavior. This interpretation is borrowed from operant conditioning principles which generally define extinction as a decrease in the frequency of a behavior due to nonreinforcement. For example, food can be used as a reinforcer to maintain lever-pressing behavior in food deprived animals (Skinner, 1953). When the food no longer follows lever pressing, the behavior will eventually extinguish. Ethanol as a reinforcer is not directly comparable to food as a reinforcer.

In order to experience the pharmacologically-reinforcing properties of ethanol, an organism must ingest a sufficient quantity of ethanol to achieve detectable blood or brain levels of ethanol. Only then could ethanol begin to support ethanol-oriented behavior. As mentioned previously the taste of ethanol at concentrations sufficient to have pharmacological effects is usually aversive to animals, including humans. Therefore, unlike the presence or absence of a food reinforcer in an operant paradigm, the presence or absence of ethanol-mediated reinforcement requires the consumption of a liquid with or without ethanol and the absorption of the liquid in order for the organism to experience the presence or absence of reinforcement. Therefore, a period of time is necessary as the organism learns and re-learns the association between the

pre-ingestional cues of the vehicle (i.e., taste, smell, texture) and the post-ingestional consequences that follow absorption of the vehicle (pharmacological effects). When extinction conditions are instated, a new association is learned (ethanol is no longer experienced as reinforcing), the ethanol drinking behavior declines until it is extinguished. In the animal studies that have been reviewed here, there is no evidence to suggest that opiate antagonists produced a pattern of suppression of ethanol self-administration that would support the notion that the organism was "learning" over time, that ethanol was no longer reinforcing, during treatment with opiate antagonists. It is the contention of this thesis that until such evidence is found it cannot be said that decreases in ethanol intake that follow a drug treatment are the result of the blockade of reinforcement and the subsequent extinction of the drinking response.

The Present Investigation

The purpose of the following experiments was to explore the possible mechanisms by which opiate antagonists mediate the suppression of voluntary ethanol self-administration in rats. Specifically, the experiments in this thesis were designed to test the idea that opiate antagonists mediate their antidipsogenic effects on oral ethanol intake through their capacity to block ethanol-mediated reinforcement. The possibility that the suppression of ethanol intake induced by opiate antagonists may be mediated through other post-ingestional factors, such as the induction of a conditioned taste aversion (CTA) or through pre-ingestional factors such as shifts in taste sensitivity for ethanol were also examined.

EXPERIMENT 1

On the basis of animal studies that have found antidipsogenic effects of opiate antagonists on ethanol self-administration it has been suggested that opiate antagonists mediate their suppressant effects by their capacity to block opioid receptors (Myers & Critcher, 1982; Froehlich, Harts, Lumeng & Li, 1990). It should follow, therefore, that the suppression of ethanol self-administration, induced by opiate antagonists, should be dose-dependent. This hypothesis was tested in experiment 1, using a drinking paradigm of 24 hr, free-access to ethanol and water.

Method

Subjects

Thirty-four naïve, male, Long-Evans rats (Charles River, Canada) weighing 125-150 g at the start of the experiment, were individually housed in standard stainless steel cages. They were maintained in a temperature and humidity controlled environment on a 12 hr reverse dark/light cycle (lights off at 11:00 AM). Fluids were presented in two glass Richter-type tubes, mounted on the front of the cage. Food and water were available ad libitum.

Procedure

Acquisition: Following a period of 2 weeks acclimatization to the reverse dark-light cycle, rats were presented with free-access to an ascending series of ethanol concentrations in one drinking tube, and water in the other on an alternate-day schedule. Each concentration of ethanol was obtained by diluting 95% ethyl alcohol with tap water. The concentrations of ethanol presented were 2%, 4%, 6% and 8% ethanol (v/v) and each concentration was presented twice. On intervening days, both tubes were refilled with water, thus the acquisition period lasted 16 days. The position

of the tubes was alternated with each ethanol presentation in order to screen for animals that demonstrated a strong position bias. Baseline intake was recorded following the 2nd presentation of 8% ethanol.

Baseline Maintenance: A period of five consecutive days of free-access to ethanol and water, and began on the day following the 2nd presentation of 8% (v/v) ethanol during acquisition on the basis of which animals were selected for inclusion in the study. Rats were eliminated from the study on the basis of 3 exclusion criteria; rats that demonstrated strong side preferences were excluded because the ethanol intake of such animals reflected, in large measure, their preference for drinking from a tube on a specific side of the cage rather than a preference for ethanol. Rats, whose mean ethanol intake during baseline was less than 1 g/kg ethanol, were also excluded from the study because it was considered unlikely that these animals could be consuming pharmacologically meaningful amounts of ethanol over the 24-hr period of measurement. Finally, rats were also discarded if they showed erratic fluctuations in daily ethanol intake (irregular fluctuations in g/kg intake that were 2 g/kg above or below the group mean). The remaining 27 drinkers were ranked according to their g/kg ethanol intake. The highest 5 drinkers were randomly assigned to 1 of 5 groups, followed by the next 5 highest drinkers so that each group was matched on baseline ethanol intake prior to the start of the treatment.

Treatment: NTX injections began on the morning after the fifth day of baseline measurements. Naltrexone hydrochloride (Research Biochemical Inc., Natick, MA) was dissolved in physiological saline and administered to rats intraperitoneally in a volume of 1 ml. Injections were administered 1 hr before the start of the 11:00 AM dark cycle. Groups 1 to 4 received 1 of 4 doses of NTX; 0.05, 0.1, 5.0, or 10.0 mg/kg respectively. Group 5 received the saline vehicle. Tubes were measured and refilled just prior to injections.

Results

Ethanol intake in the present and subsequent experiments was calculated both in terms of daily ethanol preference (ratio of ingested ethanol to total fluid intake) and in terms of daily absolute ethanol ingested (g/kg). A split-plot analysis of variance, with dose as the between factor and days as the within factor was carried out on 24-hr absolute ethanol intake and on ethanol preference.

There was no decrease in ethanol intake, $F(8,116)=1.47$, $p>.017$ (see Figure 1), or ethanol preference, $F(8,116)=0.55$, $p>0.82$ (see Figure 2), during NTX-treatment at any of the doses administered compared to the same measures taken during baseline. A split plot analysis of variance (dose x days) on 24 hr water intake also failed to find an effect of NTX ($F(20,110)=0.70$,$p>.82$) (see Figure 3).

Discussion

The data obtained in this experiment suggested that NTX-administration had no effect on ethanol intake at any of the doses administered. This lack of suppression was surprising given the numerous reports that opiate antagonists have antidipsogenic actions on ethanol self-administration (e.g., Myers & Critcher, 1982; Marfaing-Jallat, Micelli & Le Magnen, 1983; Sandi, Borrell & Guaza, 1988; Froehlich, Harts, Lumeng & Li, 1990; Sinclair, 1990). It is possible that this lack of evidence for suppression of ethanol intake resulted from the duration of the measurement period used in this experiment as compared to the duration of measurement used by those investigators that have measured suppressant effects. Researchers that have found antidipsogenic effects of opioid antagonists have all employed limited access (LA) schedules of ethanol presentation (Marfaing-Jallat, Micelli & Le Magnen, 1983; Sandi, Borrell & Guaza, 1988; Froehlich, Harts, Lumeng & Li, 1990; Sinclair, 1990). For example, Sinclair

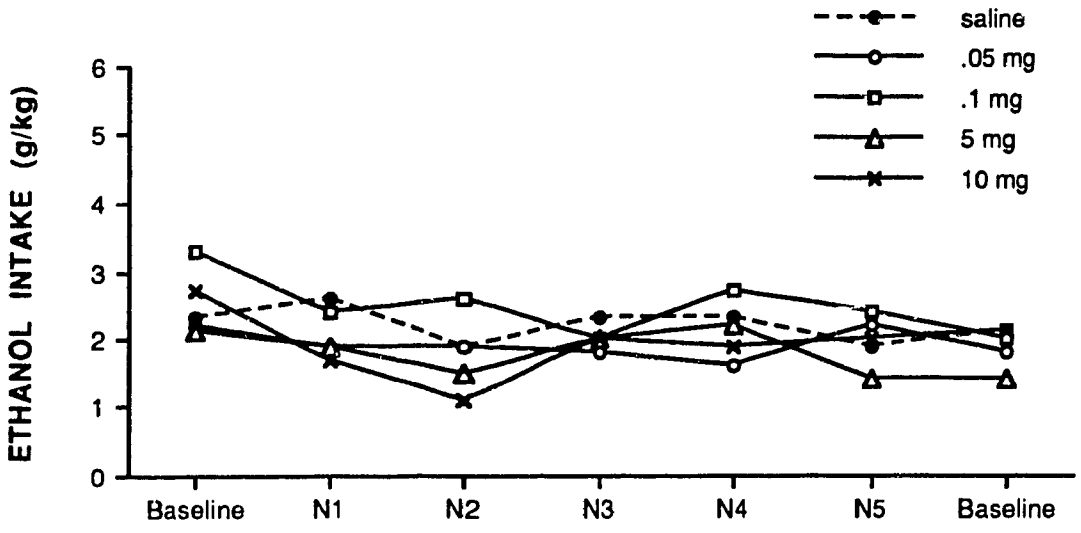

Figure 1. Mean absolute ethanol intake (8%). The last day of baseline, 5 days of injections with NTX and the day following termination of NTX-injections.

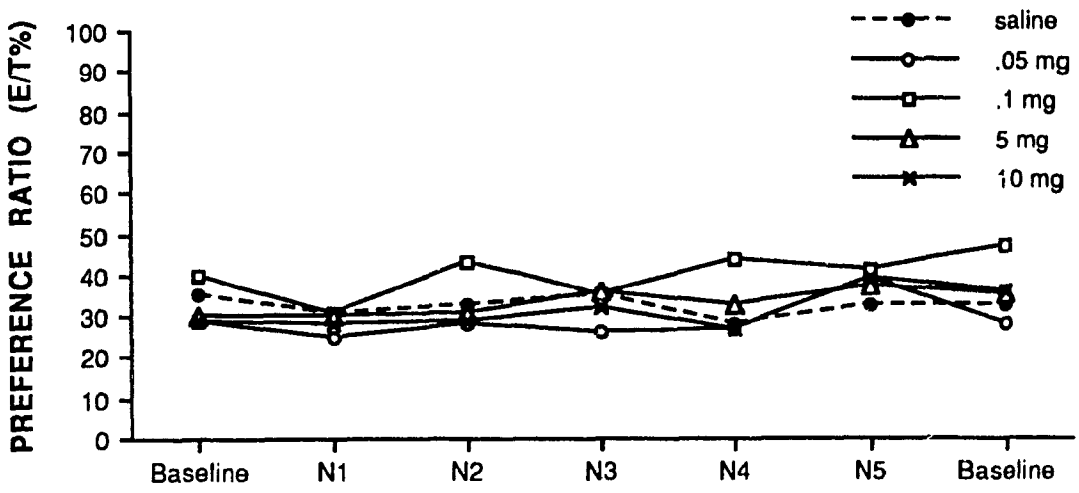

Figure 2. Mean ethanol preference ratios. The last day of baseline, 5 days of injections with NTX, and the day following termination of NTX-injections.

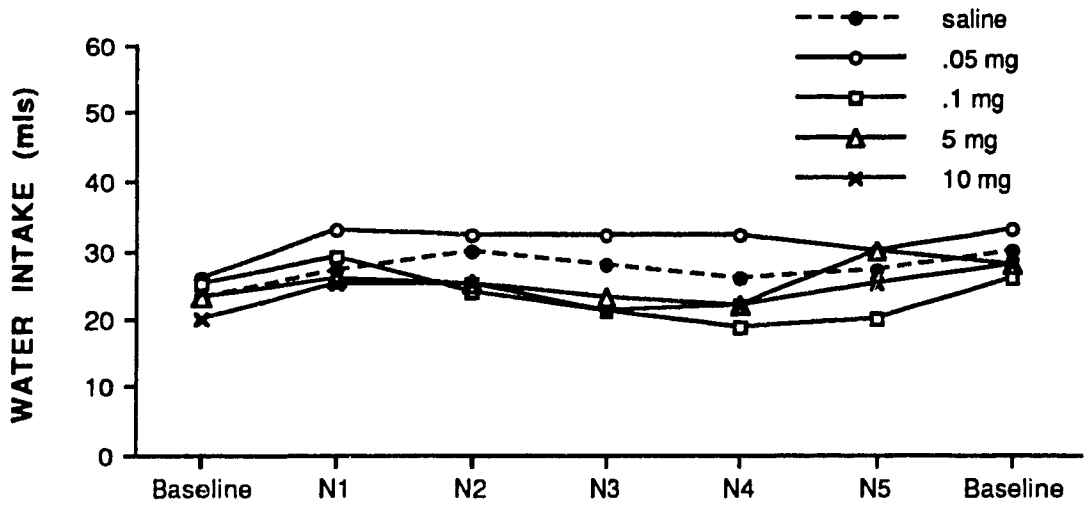

Figure 3. Mean water intake. The last day of baseline, 5 days of injections with NTX, and the day following termination of NTX-injections.

(1990), provided rats with 1-hr LA to ethanol and water, whereas Sandi, Borrell and Guaza (1988), provided rats with 15 min LA to ethanol and water; both studies found decreases in ethanol intake during NAL administration. In the present study, drinking was measured over a 24-hr period. The potential relevance of the length of the measurement period is also supported by two studies that did find decreases in ethanol intake by NTX in rats, when measurements were taken over a 24-hr period. Myers and Critcher (1982) reported a decrease in ethanol consumption, using a 24-hr measurement period, but only when NTX was administered several times each day. Their findings suggested that the suppressant effect of NTX on ethanol consumption was mediated by a direct effect of the antagonist, and was abolished once the drug was eliminated from the system. Iko and Brush (1991) also found decreases in ethanol intake in rats when measurements were taken over a 24-hr period. In their experiment, however, NTX was administered through subcutaneous implants. Subcutaneous implants allow for continuous release of the drug over the entire course of the experiment. Thus, the decrease in ethanol intake measured in 24-hr access paradigms by both Myers and Critcher (1982), and Iko and Brush (1991) would suggest that constant blood levels of the antagonist may be necessary for suppression of ethanol intake to be observed.

Micro-structural analysis of patterns of ethanol intake in rats, over 24-hr periods, has shown that rats consume only negligible amounts of ethanol during the course of an 12-hr light cycle, when rats are typically sleeping (Gill, 1994). Despite the 10-hr half-life of NTX, which would have covered the active dark cycle when rats initiate the majority of their drinking, no reduction in ethanol intake was observed in experiment 1. Therefore, it is unlikely that the rats in this experiment could have compensated for any suppression by NTX during the remaining hours of the experiment when the drug had been eliminated from the system. It was possible, however, that any suppressant effects of NTX would have been observed had ethanol intake been measured closer to the time of drug administration.

EXPERIMENT 2

Experiment 1 failed to demonstrate any evidence for a suppression of ethanol intake by NTX. Even if a decrease in ethanol intake had been observed in experiment 1, the mechanism underlying the decrease would not have been revealed. The purpose of experiment 2 was to test the notion, more directly, that opiate antagonists decrease ethanol intake in rats, by their capacity to block ethanol-mediated reinforcement.

The most effective method of suppressing or extinguishing a learned behavior that was previously supported by a reinforcer is to maintain that behavior for a period of time in the absence of the reinforcer (Skinner, 1938). Voluntary consumption of ethanol is commonly viewed as a learned behavior, therefore, it should be governed by the general laws of learning. Thus, ethanol-preferring rats, forced to drink ethanol while undergoing treatment with NTX should, over a period of trials, "learn" that ethanol is no longer reinforcing (extinction), if NTX was acting by blocking reinforcement. Consequently, the extinction of the ethanol drinking behavior should be apparent upon reinstatement of free-access drinking and termination of drug injections. This procedure has been successfully used in our laboratory when testing the capacity of zimelidine (a serotonin uptake blocker) to block ethanol self-administration (Rockman, Amit, Carr, Brown & Ögren, 1979; Rockman, Amit, Carr & Ögren, 1979).

Method

Subjects

Thirty-two male, Long-Evans rats (Charles River, Canada) weighed 125-150 g at the start of the experiment. Housing conditions were the same as described in Experiment 1.

Procedure

Acquisition: The acquisition procedure was identical to that described in Experiment 1.

Baseline: This period consisted of a 12 day, free-access to ethanol and water, alternate-day schedule where one drinking tube was filled with 8% (v/v) ethanol solution and the other with water. During the days that ethanol was presented, all animals were given injections of saline 30 min prior to the presentation of the drinking tubes. Ethanol intake was measured just prior to the start of the 11:00 AM dark cycle; on intervening days, both tubes were filled with water. Exclusion criteria were identical to those described in Experiment 1. Following baseline, animals were matched on g/kg ethanol intake according to the same criteria described in Experiment 1.

Forced Choice Exposure (FCE): This phase consisted of an alternate-day schedule of forced-choice presentations of 8% (v/v) ethanol which lasted for a period of 20 days. On the first day of FCE, both tubes were filled with ethanol; on alternate days, tubes were re-filled with water. Naltrexone hydrochloride (Research Biochemicals Inc, Natick, MA) was dissolved in physiological saline and administered intraperitoneally in a volume of 1 ml. NTX was administered 30 min before the start of the 11:00 AM dark cycle. Groups 1 and 2 received 2.5 and 5.0 mg/kg of NTX, respectively. Group 3 received the saline vehicle. Tubes were measured and refilled just prior to injections, and again 4 hr after the start of the night cycle.

Post-injection: During this period rats were returned to a free-access, alternate day schedule of ethanol presentation. Fluid intake was measured on ethanol days until all groups have returned to baseline levels of ethanol intake.

Results

The effects of the period of FCE exposure and NTX treatment on absolute ethanol intake, preference for ethanol over water and water intake was assessed by split-plot analysis of variance. The analysis was run with dose (0, 2.5 & 5.0) as the between factor and days as the within factor (last day of baseline fluid intake and fluid intake during the four post-injection days). Post hoc comparisons were made with Tukey's HSD.

There was no effect of treatment with NTX during FCE on absolute ethanol intake measured during the post-injection phase because ethanol intake levels, in all three groups, were not different from their baseline levels of intake, $F(2,29)=0.28$, $p>0.7$. There was, however, an effect of days, $F(4,116)=3.71$, $p<.007$. Post hoc comparisons found that absolute ethanol intake on the first day of the post-injection period was significantly lower for all three groups compared to ethanol intake measured on the last day of baseline (see Figure 4). The decrease in ethanol intake was likely the result of terminating the saline and NTX injections because there was no dose x drug interaction, $F(8,116)=1.47$, $p>0.17$.

There was also no effect of treatment with NTX and FCE on preference for ethanol during the post-injection phase because ethanol preference for all three groups was not different from preference measured during baseline, $F(2,29)=0.44$, $p>0.6$. There was, however, an effect of days, $F(4,116)=3.71$, $p<0.01$. Post hoc comparisons revealed that preference for ethanol increased during the last three post-injection days, for all three groups (see Figure 5). There was no dose x drug interaction, $F(8,116)=0.55$, $p>0.8$.

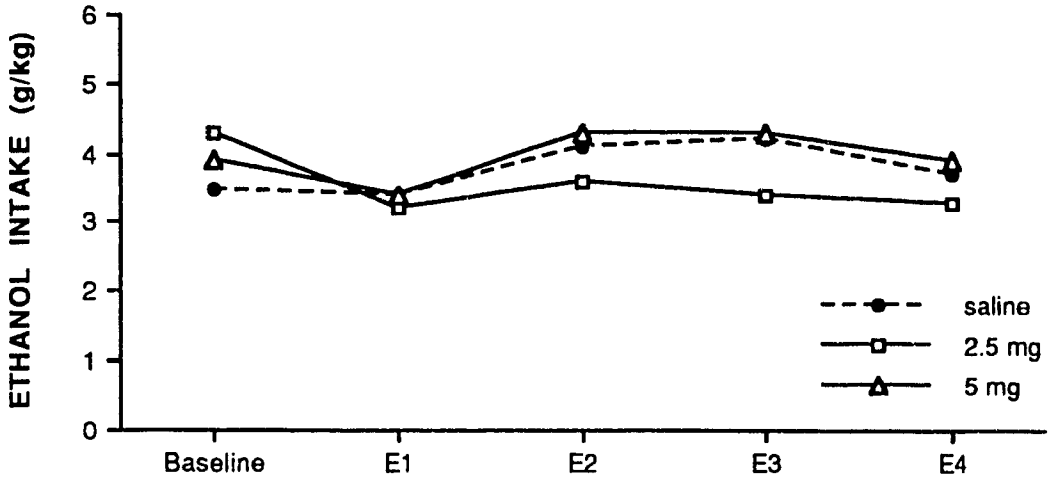

Figure 4. Mean ethanol intake. The last day of ethanol intake during baseline, compared to 4 days of ethanol intake after the termination of injections with NTX.

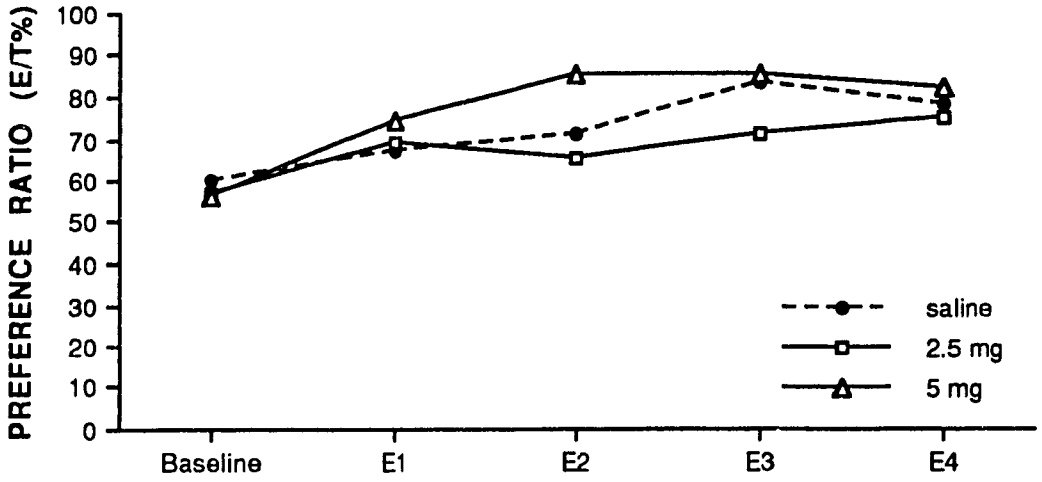

Figure 5. Mean preference ratio. Preference ratio for the last day of baseline, compared to the 4 days following the termination of injections with NTX. Preference significantly increased from E2 to E4.

The split-plot analysis of variance on water intake found no effect of treatment with NTX and FCE during the post-injection phase because water intake in all three groups was not different from water intake levels measured during baseline, $F(2,29)=0.60$, $p>0.5$. As with absolute ethanol intake and ethanol preference, there was an effect of days. Figure 6 illustrates the trend for all groups to decrease water intake during the last 3 post-injection days, although post hoc comparisons were not significant. The increase in preference for ethanol observed on the last three post-injections days was probably the result of the reduction in water intake observed during this period, rather than an actual preference for ethanol.

Absolute ethanol intake was also measured during the 20-day period of FCE. A split-plot analysis was run with dose as the between factor and the 10 days of ethanol measurement as the within factor. Ethanol intake measured 24-hr during the FCE period revealed no effect of dose, $F(2,29)=0.82$,$p>0.4$. There was a significant effect of days on ethanol intake, $F(9,261)=2.16$,$p<.02$ and Figure 7 illustrates the fluctation in ethanol intake across the 10 measurement days. There was no dose x days interaction, $F(18,261)=0.95$,$p>0.5$. Thus, NTX-treatment had no effect on 24-hr measurements of absolute ethanol intake.

Ethanol intake was also measured 4-hr following NTX administration during FCE. A split-plot analysis of variance revealed a main effect of dose, $F(2,12)=16.54p<.0004$ and of days, $F(18,108)=3.63$,$p<.0005$ but no interaction, $F(8,116)=0.66$,$p>0.7$. As can been seen from Figure 8, ethanol intake was lower in both NTX-treated groups compared to ethanol intake in the saline control group, however, both doses of NTX, produced similar levels of suppression.

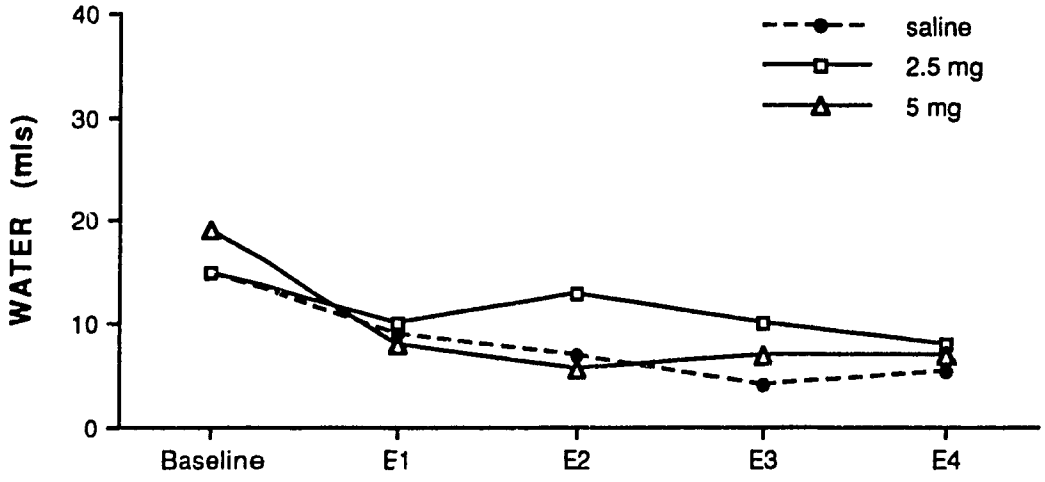

Figure 6. Mean water intake. The last day of water intake during baseline, compared to water intake for the 4 days following the termination of injections with NTX. Reductions in water intake was significant from E2 to E4.

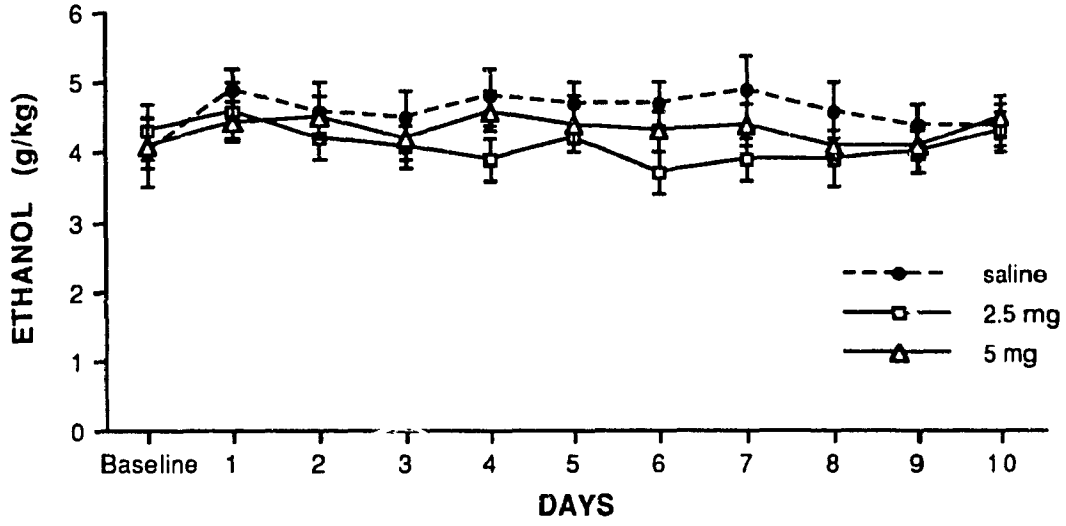

Figure 7. The last day of mean absolute ethanol intake (8%) measured during baseline, compared to ethanol intake during FCE and injections of NTX. Measurements were taken 24 hr post-injections.

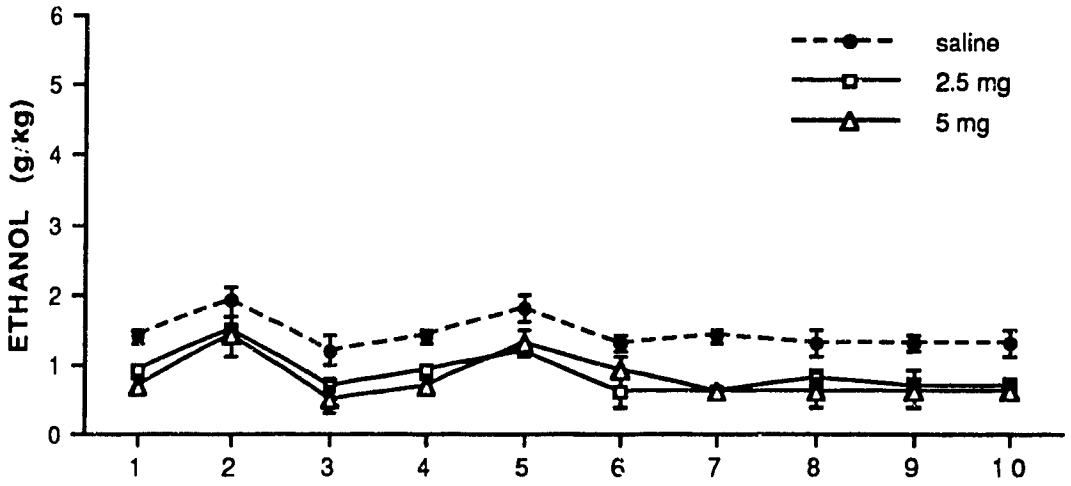

Figure 8. 8% ethanol intake during FCE and injections of NTX. Measurements taken 4 hr post-injections.

Discussion

The results of this experiment suggested that ethanol self-administration was not extinguished during the 20 day period of FCE and NTX-administration. Once free-access to ethanol and water was reinstated, ethanol intake in the NTX-treated groups was not different from their previous baseline levels of intake, or from intake levels measured in the control group. It is reasonable to hypothesize that during FCE, NTX-treated groups should have "learned" that ethanol was no longer reinforcing and ethanol intake should have slowly declined during this period, and remained suppressed during the post-injection period if NTX were decreasing ethanol intake by blocking ethanol reinforcement.

Measurements taken 4 hr after the onset of FCE, however, were consistent with reports that NTX has a direct, unconditioned, antidipsogenic effect on ethanol drinking (Marfaing-Jallat, Miceli & Le Magnen, 1983; Sandi, Borrell & Guaza, 1988). Ethanol intake was similarly reduced in both NTX-treated groups, despite the lack of suppression observed during 24 hr measurements. The limited duration of NTX's suppressant effects on ethanol intake suggested that the lack of decrease in 24 hr measurements observed in experiment 1, was most likely a function of the length of the time interval between drug injection and measurement of ethanol drinking. Of greater interest, however, was the short duration of NTX's effect despite the considerable half-life of this opiate antagonist (8-10 hr) (Goodman, Gilman, Rall & Murad, 1985). It would appear that the effect of NTX administration was to suppress ethanol drinking through some direct, unconditioned mechanism and that the NTX-treated groups recovered and compensated for this initial suppression in drinking at some point between the 4 and 24 hr measurement periods. Support for this finding was provided by a study conducted by Cooper (1980). He measured water intake over several hours in rats that were treated with the shorter acting opiate antagonist NAL. He found that

water intake was suppressed by NAL; however, rats had compensated for this suppression when intake levels were again recorded 8 hr later. It is unlikely that the decrease, that was measured during FCE, in the present experiment could have reflected a process whereby the animals "learned" that ethanol was no longer reinforcing because the suppression was immediate (within 4 hr) and short-lasting which is not typical of learned changes in behavior which typically follow a learning curve. Furthermore, despite having consumed significant levels of ethanol over 24 hr, while the drug was present in their system, no reduction in intake was seen once free-access was reinstated and injections terminated.

EXPERIMENT 3

The initiation and acquisition of ethanol self-administration behavior is thought to be mediated by factors other than those responsible for the maintenance of ethanol intake (Ng Cheong Ton, Brown, Michalakeas & Amit, 1983). This line of reasoning is supported by a variety of paradigms that investigators have employed to train animals to drink high, presumably less palatable concentrations of ethanol (for review see, Amit, Smith & Sutherland, 1987; Cicero, 1980; Deitrich & Melchoir, 1985). As an example, a few acquisition paradigms that have been employed by investigators include such procedures as exposure to high concentrations of ethanol over an extended period of time (Li, Sinclair), gradual increases in ethanol concentration following exposure to lower, more palatable concentrations of ethanol (Mendelson & Mello, 1964; Veale & Myers, 1969) and adulterating the taste of ethanol with dulcet flavoring (Grant & Samson, 1985; Samson, 1986). Each of these procedures would suggest that in order for rats to acquire ethanol drinking behavior, a period of acclimatization to the taste of ethanol may be necessary. Thus, it would appear that during acquisition, pre-absorptive components of ethanol drinking, such as taste, may play an even larger role in initiating ethanol drinking than post-absorptive components. Post-absorption components, such as the pharmacological effects of ethanol ingestion, are thought to be important to the maintenance of ethanol drinking behavior (Griffiths, Bigelow & Henningfield, 1980).

Data from experiment 2, suggested that NTX-treatment did not block ethanol-mediated reinforcement, but did induce a direct, unconditioned decrease in ethanol intake with measurements taken 4 hr post-injection during FCE. It is possible that this unconditioned effect might reflect a capacity of NTX to shift the rat's taste sensitivity for ethanol. The concept that a drug may alter taste perception is not new. It was first suggested in 1892 when it was demonstrated that gymenemic acid (an extract of leaves from Gymnema sylvestre) reduces sensitivity for sweet and bitter (Sanger & McCarthy,

1980). A drug acting by altering taste would be expected to reduce ethanol intake immediately following exposure to ethanol. This is in contrast to agents that are acting to block ethanol-mediated reinforcement, a process that should require a period of learning.

Richter (1941) was able to show that some rats drank more ethanol than water at concentrations ranging from 2% to 6%. Furthermore, Gill (1984) found that rats that initially preferred low concentrations of ethanol over water abruptly stopped drinking ethanol when the concentration exceeded 6%. It is possible that rats develop their preference for ethanol at low concentrations because they find the taste palatable. It appears that once the concentration of ethanol increases beyond 6%, rats previously drinking primarily for pre-ingestional factors, drop off consumption while rats that continue to drink, seem to do so for post-ingestional factors. Support of this hypothesis is provided by Keifer and Dopp (1989) who examined taste reactivity in rats to various concentrations of ethanol. Rats have been shown to display a characteristic set of orofacial and somatic reactions in response to pleasing or aversive flavors (Grill & Nogren, 1978). Keifer and Dopp (1989) found that although aversive responses to increasing concentrations of ethanol become significantly more frequent, consumption levels failed to change significantly. Their study suggested that rats that continue to consume high concentrations of ethanol, find the taste aversive and therefore their continued consumption may be accounted for by other factors, most probably, post-ingestional effects.

Experiment 3 examined the effects of NTX administration on acquisition of the ethanol drinking behavior. This was carried out in an attempt to differentiate between the actions of NTX on the post-ingestional components of ethanol intake and its actions on the pre-ingestional components of ethanol drinking. It was predicted that the administration of NTX, during acquisition, should induce a rapid suppression of ethanol drinking at low, more palatable concentrations of ethanol. Thus, NTX-

treatment during this period should prevent the acquisition of ethanol drinking at a higher, more pharmacologically-relevant concentration of ethanol.

Method

Subjects

32 naïve, male Long-Evans rats (Charles River, Canada) weighing 125-150 g at the start of the experiment, were individually housed in standard stainless steel cages. They were maintained in a temperature and humidity controlled environment on a 12 hr reverse dark/light cycle (lights off at 11:00 AM). Fluids were presented in two glass Richter-type tubes, mounted on the front of the cage. Food and water were available ad libitum.

Acquisition & Treatment: Following a period of 2 weeks acclimatization to the reverse dark-light cycle, rats were randomly assigned to one of four groups. These groups were presented with alternate-day, free-access to an ascending series of ethanol concentrations in one drinking tube, and water in the other. Ethanol concentrations were prepared by diluting 95% ethyl alcohol with tap water. The concentrations of ethanol presented were 2%, 4%, 6% and 8% ethanol (v/v) and each concentration was presented twice, consecutively. On intervening days, both tubes were refilled with water, thus the acquisition period lasted 16 days. The position of the tubes was alternated with each ethanol presentation.

Groups 1, 2 and 3, were given intraperitoneal injections of either 2.5, 5.0 or 10.0 mg/kg NTX, respectively, in a volume of 1.0 ml. Group 4 received injections of the saline vehicle. Injections were administered 30 min prior to the presentation of each concentration of ethanol. Following the 2nd presentation of 8% ethanol, injections were terminated. All groups continued to be provided with access to the 8% concentration of ethanol and water, and intake was recorded for 4 consecutive post-injection days.

Results

A split-plot analysis of variance with dose (0, 2.5, 5.0, 10.0) as the between factor and fluid intake, during the nine measurement days, as the within factor were performed. The analysis was carried out on 24 hr absolute ethanol intake, preference for ethanol over water and water intake. Post hoc analysis were preformed using Tukey's HSD.

There was a main effect of dose, $F(3,28) = 13.54$, $p<.0000$ and days, $F(8,224)=11.84$, $p<.0001$ and a dose x days interaction, $F(24,224)=2.33$, $p<.0007$ on absolute ethanol intake. Post hoc comparisons found that ethanol intake in the group receiving 2.5 mg of NTX was significantly different from saline controls beginning with the first presentation of 4% ethanol through to the last presentation of 8% ethanol during acquisition. This suppression was also observed in the post-injection period.

The 10.0 mg dose of NTX also significantly reduced ethanol intake compared to ethanol intake in the saline injected group, beginning with the second presentation of the 2% concentration of ethanol through to the final presentation of 8% ethanol measured during acquisition. This suppression was also observed during the post-injection period. Unexpectedly, 5.0 mg of NTX only reduced ethanol intake from intake levels measured in the saline control group on the first presentation of the 8% concentration of ethanol.

Figure 9 illustrates the pattern of the NTX-mediated reductions in ethanol intake. There was a trend for the pattern of reduction to differ between the group injected with the 2.5 mg dose and the group injected with the 10 mg dose, when the decreases in ethanol intake were compared for each concentration of ethanol. Specifically, in the group injected with 10 mg ethanol, the suppressant effects of NTX were more robust, and the suppression further enhanced after the second presentation of each new concentration of ethanol. In the group injected with 2.5 mg dose of NTX, the

suppression was less robust and there was also no pattern to the direction of the suppression within each concentration of ethanol presented. Post hoc comparisons revealed that the differences in amount of suppression between each of the same oncentrations for both the group receiving 2.5 mg of NTX and 10 mg of NTX was not significantly different.

A main effect of dose, $F(3,28)=13.95$, $p<.0000$, days, $F(8,224)=5.01$, $p<.0000$ and an interaction between dose and days, $F(24,224)=1.65$, $p<.03$ on preference for ethanol were also observed. Post hoc comparisons revealed that the 10 mg dose, significantly decreased preference for ethanol from the second presentation of 2% ethanol, through to the last presentation of 8% ethanol. This suppression was still observed in the post-injection period. In the group receiving the 2.5 dose of NTX, ethanol intake was significantly suppressed for both 4% presentations of ethanol, and the first presentation of 6% ethanol. This suppression was still observed in the post-injection period. These data are shown in Figure 10.

A repeated measures analysis of variance (dose x days) on the mean water intake, for the first day, revealed a main effect of dose, $F(3,28)=7.82$, $p<.0006$, and days, $F(4,224)=9.30$, $p<.0000$. Post hoc comparisons on NTX dose revealed that water intake was significantly increased in the group injected with 10 mg of NTX (see Figure 11). Post hoc comparison on days revealed that water intake was increased over the duration of the experiment, for all treatment groups.

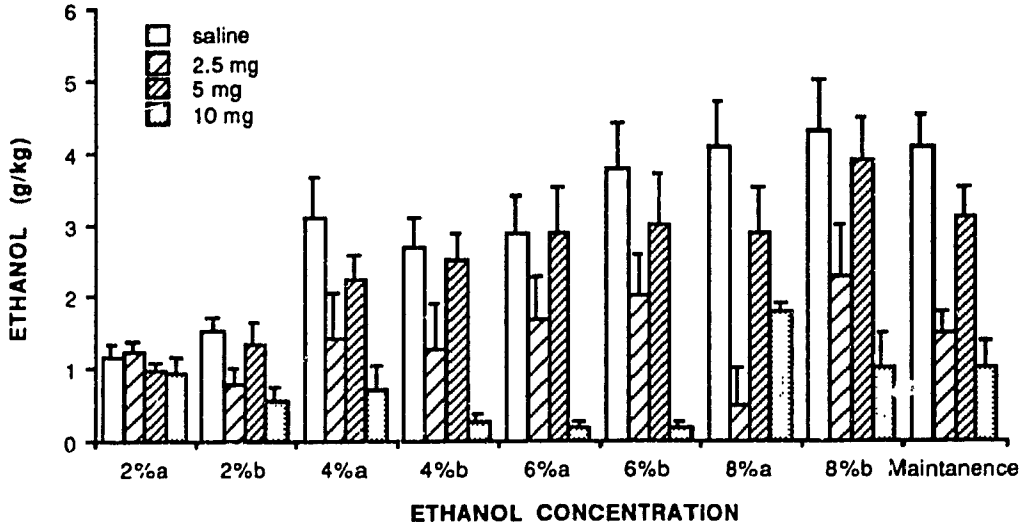

Figure 9. The mean ethanol intake at each same concentration of ethanol presented (i.e. a/b=1st presentation of 2% and 2nd presentation of 2% etoh) during acquisition, compared to 8% ethanol intake following acquisition and the termination of NTX-injections (Maintenance).

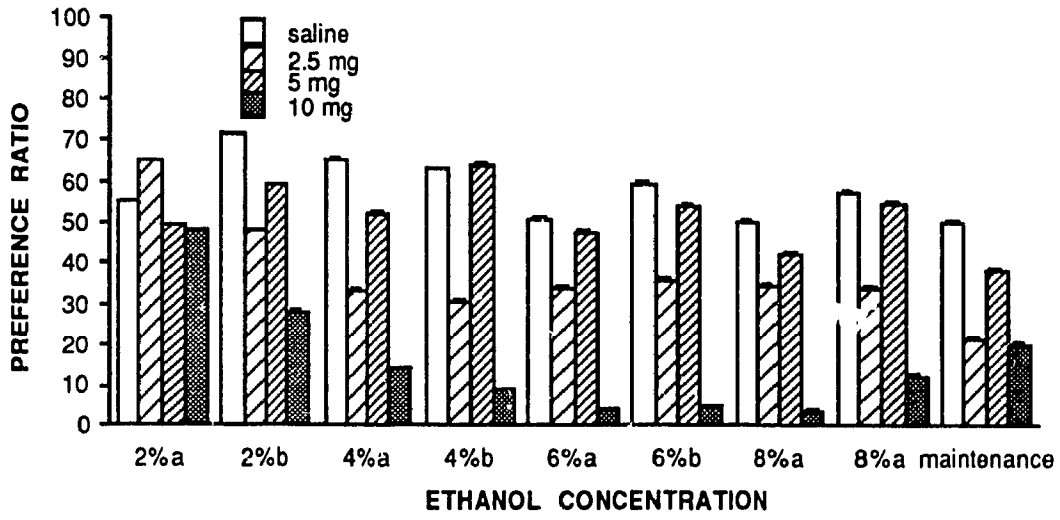

Figure 10. Mean preference ratio for each same concentration (i.e. a/b=2% 1st presentation and 2% 2nd presentation of etoh) of ethanol during acquistion, compared to preference for 8% ethanol following acquisition and the termination of NTX-injections (Maintanence).

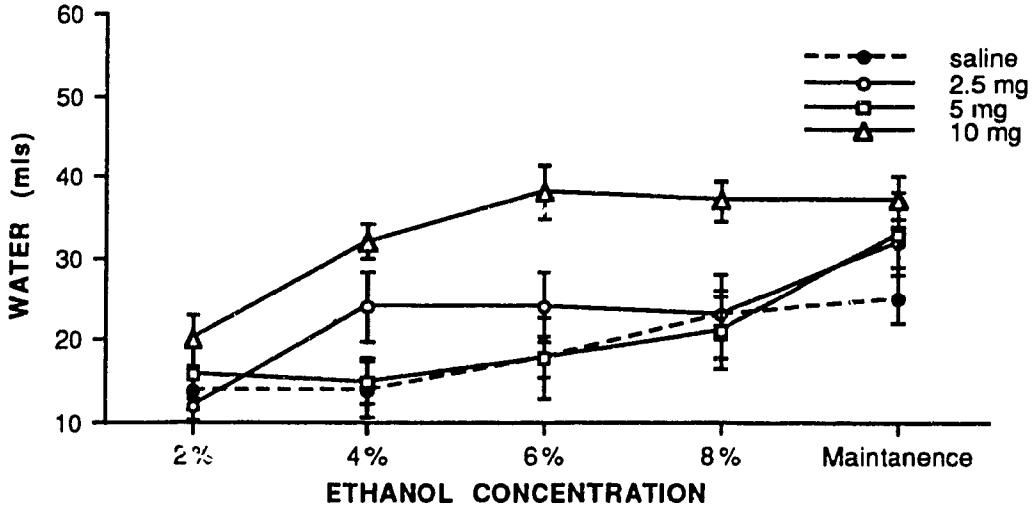

Figure 11. The mean water intake collapsed for each same concentration of ethanol during acquisition, compared to water intake measured following acquisition and the termination of NTX-injections.

Discussion

As was predicted, NTX administered during acquisition significantly blocked the acquisition of drinking the 8% concentration of ethanol in the groups that received 2.5 and 10 mg of NTX. At the end of the acquisition period, when injections were terminated, the saline-injected group was drinking approximately 3.8 g/kg ethanol, and showed a preference for ethanol above 50%. The groups injected with the 2.5 or 10 mg dose of NTX, did not acquire the ethanol self-administration behavior. Both groups were drinking approximately 1 g/kg ethanol at the end of acquisition, and preference for ethanol remained below 50%. On the basis of available data it is not possible to explain the lack of effect on ethanol intake observed in the group that received the 5 mg dose of NTX. Although, Kornet, Goosen and van Ree (1991) also found that 0.17 and 1.5 mg of NTX blocked an abstinence-induced increase in ethanol intake in monkeys, whereas no effect was seen at the 0.5 dose.

Although both the high and low dose of NTX, blocked the acquisition of drinking the 8% concentration of ethanol, differences in the pattern of suppression at each ethanol concentration, and differences in the suppression of water intake, may suggest that the suppression at these two doses was mediated by different mechanisms. For example, it could be argued that the lack of acquisition of 8% ethanol intake observed in the group receiving the 2.5 dose of NTX, could be due to a capacity of NTX to render the taste of ethanol less palatable. Consequently, this group did not acquire a preference for the 8% concentration of ethanol that was observed in the saline-injected group. This happened, presumably, because they did not consume sufficient amounts of ethanol to experience the reinforcing post-ingestional effects thought to be important in maintaining ethanol self-administration. The dramatic and sustained decrease in ethanol observed in the group receiving the 10 mg dose of NTX, however, and the enhancement of water intake, suggested that this group displayed a rather aversive

reaction to the taste of ethanol. Thus, the suppression in ethanol intake observed in this group may have been the result of a CTA. A CTA is thought to occur when rats are presented with a novel tasting fluid (the conditioned stimulus) paired with a drug or noxious physiological stimulus (the unconditioned stimulus) (Hunt & Amit, 1987). Rats then tend to avoid that fluid in future presentations even in the absence of the unconditioned stimulus (Kulkosky, Sickel & Riley, 1980; Lester, Nachman & Le Magnen, 1970: Hunt & Amit, 1987). The avoidance behavior of the rat seems to demonstrate that it has "learned" an association between the aversive effects of the unconditioned stimulus and the taste properties of the fluid that was paired with the unconditioned stimulus. More importantly, CTAs have been reported when high doses of NTX were used as the unconditioned stimulus (Le Blanc & Cappell, 1975; Van Der Kooy & Phillips, 1977). Although it is possible that the suppression of ethanol intake observed in the post-injection period in the group that received 2.5 mg NAL was also the result of a CTA, CTAs are not known to occur at this low dose of NAL (Sandi, Borrell & Guaza, 1986) and no suppression in ethanol intake was seen in the group receiving 5 mg of NAL. Furthermore, it is worth noting that in the group receiving the 10 mg dose of NTX, ethanol intake was not only dramatically suppressed but this suppression was always greater with the second presentation of the same concentration of ethanol. Each new concentration of ethanol, could be viewed as a novel taste and therefore, in essence, a CTA was developed to each new concentration presented and was enhanced with the second presentation of the novel taste. This pattern of step-wise suppression following the second presentation of each ethanol concentration was not observed in the group that received the 2.5 dose of NTX. Although ethanol intake was relatively low at all eight concentrations of ethanol compared to the intake of the control group, this suppression was not always further reduced following the second presentation of the same concentration of ethanol. For example, at the 2, 6 and 8% concentrations of ethanol, intake was slightly increased on second presentation

compared to intake recorded during the first presentation of the same concentration. In the group receiving 10 mg of NTX, the amount of ethanol consumed during the first presentation of 2, 4, 6, and 8% ethanol was almost double the amount consumed upon the 2nd presentation, suggesting the development of a CTA. Since it is unlikely that a CTA had developed in this group, the reduction in ethanol intake observed during acquisition may be the result of a shift in taste sensitivity for different concentrations of ethanol that abolished the acquisition of the 8% concentration of ethanol at the end of the acquisition period.

EXPERIMENT 4

The results of Experiment 3 suggested that one mechanism by which NTX may be suppressing ethanol intake is by inducing a CTA. Indeed, opioid antagonists are known to induce CTA's at the dose of NTX administered in this experiment (Le Blanc & Cappell, 1975; Van Der Kooy & Phillips, 1977). Furthermore, using a 1 hr limited access paradigm, Sinclair (1990), found that 10 mg of NAL had no effect on ethanol intake in rats on the first day of injections, however, a progressive decrease in ethanol intake was observed during the remaining 3 days of injections. This suppression persisted when injections were terminated. Sinclair (1990) suggested that this delay in the emergence of the suppression in ethanol intake reflected the extinction of ethanol-drinking behavior. Furthermore, he suggested that NAL had blocked the reinforcing effects of ethanol. It is possible, however, that the suppression of ethanol intake observed by Sinclair, was the result of a NAL-induced CTA. The design of Sinclair's experiment (1990), however, did not address this possibility since a group receiving NAL immediately following LA (limited access) to ethanol was not included. CTAs typically develop when the pairing of the CTA-inducing agent follows the ingestion of a novel fluid. Sinclair (1990) did not observed reductions in ethanol intake in a group of rats that received NAL, 3 hr post-LA drinking. This, however, still does not eliminate the possibility of a CTA, because the unconditioned stimulus-conditioned stimulus interval, may have been too long. The inclusion of a group of animals receiving NAL treatment closer to the period of ethanol drinking would have provided a better test of the CTA hypothesis. Furthermore, a decrease in ethanol intake, if demonstrated in such a group, would make it difficult to argue that the drug was acting by blocking ethanol reinforcement since the drug had been administered after ethanol was already ingested. Any sustained decreases in ethanol intake, observed in the post-injection period would best be explained by the induction of a CTA. It would also suggest that a backward

conditioned CTA may have been induced in the group that was injected 10 min prior to LA, in Sinclair's (1990) experiment.

Experiment 4 was a replication of Sinclair's experiment (1990) with the addition of a group of animals that received NAL immediately following LA drinking. The addition of this group allowed for the appropriate pairing of the pharmacological effects of ethanol with those of NAL which would be required in a traditional CTA paradigm (Hunt & Amit, 1987).

Method

Subjects

Thirty-two naïve, male, Long-Evans rats (Charles River, Canada) weighed 125-150 g at the start of the experiment. Rats were individually housed in standard stainless steel cages and were maintained in a temperature and humidity controlled environment on a 12 hr reverse dark-light cycle (lights on at 11:00 AM). Fluids were presented in two Richter-type tubes mounted on the front of the cages. Food and water were available ad libitum.

Procedure

Baseline: Baseline ethanol recording was the same as those described in Experiment 1. In addition, animals were discarded from the study using the identical exclusion criteria to that described in Experiment 1.

Limited Access: Immediately following baseline measurements, rats began training on a LA schedule. Fluids were delivered to the rats in plastic tubes with steel ball-bearing spouts. They were provided with access to ethanol or water during a daily 4 hr session for a 2 week period. Access to ethanol was then further reduced to a daily 2 hr session for an additional period of 2 weeks. Finally, access was further reduced to a 1 hr

session at which time baseline intake was established. Rats were matched on absolute ethanol intake and then assigned to 1 of 4 groups.

Treatment: Following baseline LA drinking, rats were given daily injections of 10.0 mg/kg NAL or saline in a volume of 1.0 ml, for a period of 4 days. These injections were given either 10 min prior to the start of the LA session (-10MIN), at the end of the LA session (1HR), or 3 hr after the LA session (3HR). The control group received the saline vehicle 10 min prior to the start of the LA session.

Post-injection: On day 5, ethanol intake was measured in all groups for a period of 3 consecutive days, in the absence of NAL or saline injections.

Results

A split-plot analysis of variance with time of injection as the between factor (-10MIN, 1HR, and 3HR) and days (last day of baseline (PRE), 4 days of treatment and the post-injection day (POST) as the within factor was performed. There was a main effect of days, $F(5,90)=15.17, p<.0000$ and days x time interaction, $F(15,90)=2.68, p<.002$ on 1HR absolute ethanol intake. Figure 12 illustrates the gradual decrease in ethanol intake which was apparent in all NAL-treated groups from the second day of treatment, until the post-injection phase.

Post-hoc analysis revealed that the intake of absolute ethanol was significantly reduced in the -10MIN group on all 4 days of NAL treatment and on the post-injection day compared to baseline levels of intake. Intake was also significantly reduced in the 1HR group from the second day of NAL treatment, through to and including the post-injection day compared to baseline levels of intake. Interestingly, ethanol intake was also suppressed in the 3HR group during NAL treatment, however, this suppression was no longer observed in the post-injection phase, compared to baseline levels of intake. Animals continued to gain weight throughout the experiment.

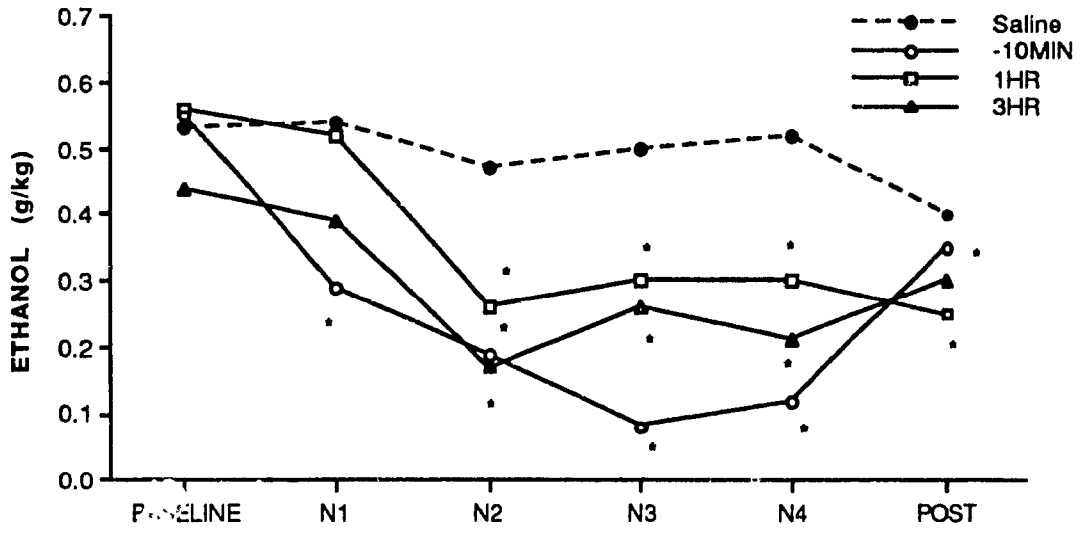

Figure 12. Mean absolute ethanol intake (8% v/v) on the last day of baseline, the 4 days of injections with NAL, and the post-injection day (POST).

Discussion

The purpose of Experiment 4 was to replicate Sinclair's study (1991), and to include an additional group of animals that received NAL treatment following LA drinking. The addition of this group allowed for the pairing of the drug with the pharmacological effects of ethanol. The design of this experiment was essentially a CTA paradigm.

Sinclair's (1990) finding that NAL suppressed ethanol intake in rats in a LA paradigm was confirmed by the data obtained in this experiment. Contrary to Sinclair's study, however, the reduction in ethanol intake was seen following the first day of injections. This suggested that the antidipsogenic actions of NAL on ethanol intake were immediate and therefore, unlikely to have been the consequence of a conditioned process reflecting extinction. Sinclair (1990) argued that in his experiment, NAL was acting by inducing an extinction of the ethanol drinking behavior and because the decrease in ethanol intake was only observed in rats injected with NAL 10 min prior to LA drinking and not in the groups injected 3 hr after limited access drinking. CTAs typically develop when the pairing of the CTA-inducing agent follows the ingestion of a novel fluid (Hunt & Amit, 1987). Unfortunately, injecting a group of animals 3 hr after LA drinking, may not have provided a sufficient test for the presence of a CTA because the USC-CS interval may have been to long. USC-CS intervals which are capable of producing a CTA have been shown to vary, depending on the agent administered to induce the CTA (Hunt & Amit, 1987). The influence of a CTA in this experiment, however, was inferred from the pattern of reduction in ethanol intake observed in the 1 HR group and from the sustained suppression observed during the post-injection phase of the experiment which is considered a critical index of a CTA (Hunt & Amit, 1987). A CTA cannot be observed on the first day, since it occurs when the animal first learns the association between the taste properties of the fluid, and the aversive properties of the CTA-inducing agent. In this experiment, the reduction in ethanol intake in the 1 HR

group, was not observed until the second pairing day. It is possible, therefore, that the decrease observed in the -10MIN group, in both the present experiment, and in Sinclair's experiment (1990) was an example of a backward conditioned CTA. It has been suggested that with shorter UCS-CS intervals, the backward procedure is essentially a forward procedure, since the illness and the flavor may still be experienced continuously (Barker, Smith & Suazrez, 1977).

Sinclair (1990) has also argued that CTAs only develop in response to novel tasting fluids and the rats in his experiment received considerable pre-exposure to ethanol prior to NAL treatment. There are data suggesting, however, that ethanol drinking in rats is unusually sensitive to CTA inducing agents because CTAs have been demonstrated despite long periods of pre-exposure to ethanol, and in both forward and backward conditioning paradigms (Amit, Gill & Ng Cheong Ton, 1991).

Drinking was also suppressed in the 3 HR group during the last 3 days of treatment in the present experiment. This suppression, however, was not seen during the post-injection phase which might suggest that a CTA to ethanol had begun to develop but the time interval was too great for it to be expressed in the post-injection period. The suppression observed on the first day of injections in the -10MIN group suggested that NAL may also be mediating a direct, unconditioned suppressant effect, in addition to its capacity to induce a CTA.

EXPERIMENT 5

The suppression of ethanol intake in the 1HR group observed during the post-injection phase of Experiment 4 was most likely due to a CTA. It is also possible, that a CTA was at least partly responsible for the suppression of ethanol intake measured on the post-injection day in the group injected 10 min prior to LA drinking. This hypothesis was further tested in a pre-exposure paradigm (Ng Cheong Ton & Amit, 1983). Pre-exposure to CTA-inducing agents, were shown to attenuate a drug's capacity to induce a CTA (Ng Cheong Ton & Amit, 1983). Therefore, if the suppression in ethanol intake observed in Experiment 4 was the result of a CTA, the same injection schedule, together with a period of pre-exposure to the drug in the absence of ethanol, should attenuate the development of a CTA.

In Experiment 5, all three groups first received NAL 4 hr after LA drinking when the drug was not paired with the pharmacological effects of ethanol. Following the pre-exposure period, the injectic· schedule used in Experiment 5 was identical to Experiment 4.

Method

Subjects

Twenty-five naïve, male, Long-Evans rats (Charles River, Canada) weighed 125-150 g at the start of the experiment. Rats were individually housed in standard stainless steel cages and were maintained in a temperature and humidity controlled environment on a 12 hr reverse dark-light cycle (Lights on 11:00 AM). Fluids were presented in two Richter-type tubes mounted on the front of the cages. Food and water were available ad libitum.

Procedure

Ethanol Screening: The screening procedure was conducted in the same way described in Experiment 1.

Baseline: Baseline was established by using the same procedure used in experiment 1. Animals were discarded from the study using the same exclusion criteria as those described in Experiment 1.

Limited Access: Limited access training was the same as that described in Experiment 4. Rats were matched on absolute ethanol intake and then assigned to 1 of 4 groups.

Pre-Exposure: Once baseline LA levels of intake were established, the 3 NAL groups were injected with 10.0 mg/kg NAL, in a volume of 1.0 ml/kg, 4 hr following LA drinking for 5 consecutive days. The control group, during this period, received injections of saline in the same volume as that given to the NAL-treated groups. Ethanol intake was recorded during the pre-exposure phase.

Treatment: Following pre-exposure, rats were given daily injections of 10.0 mg/kg NAL or saline for a period of 4 days. These injections were given either 10 min prior to the start of LA session (-10MIN), at the end of the LA session (1HR) or 3 hr after the LA session (3HR). The saline control animals received injections 10 min prior to the start of the LA session.

Post Injection: Following termination of NTX treatment, ethanol intake measures were recorded for an additional period of 3 days.

Results

A split-plot analysis of variance with time of injection (-10MIN, 1HR, and 3HR) as the between factor and days (last day of baseline (PRE), 4 treatment days and the post-injection day (POST)) as the within factor. There was a main effect of days F(5,

105)=9.87, p<.0000 reflecting a significant reduction in ethanol intake in all 4 groups (see Figure 13).

A split-plot measures analysis of variance (days x time of injection) compared the last day of ethanol intake during pre-exposure period to ethanol intake during NAL treatment. There was a significant days by time interaction, F(5,105)=2.04, p<.01. Post hoc comparisons revealed a significant decrease in the -10MIN group on the second and forth days of NAL injections. Ethanol intake was also decreased in the 1HR group, but only on the last day of injections. As mentioned earlier, the critical measure of the presence of a CTA, however, is the post-injection period. In this experiment, all groups returned to the previous levels of ethanol intake during this phase.

Discussion

Although ethanol drinking during the period of pre-exposure to NAL was not reduced, pre-exposure did, however, prevent the induction of a CTA to ethanol. Ethanol intake was not suppressed in the post-injection phase of this experiment, which is the critical test for the presence of a CTA (Hunt & Amit, 1987). It could be argued, therefore, that the decrease in ethanol intake measured during the treatment phase for both the -10MIN and the 1HR group in experiment 4, may have been the result of a CTA. Furthermore, the suppression in ethanol intake observed in the -10MIN group, during NAL treatment, suggested that NAL has a direct, unconditioned effect on ethanol intake that can not be explained by post-ingestion effects such as the induction of CTA, or the capacity of this drug to block ethanol reinforcement. The possibility that the direct effect was mediated through pre-ingestion effects of NAL was therefore tested with another fluid, in the following experiment.

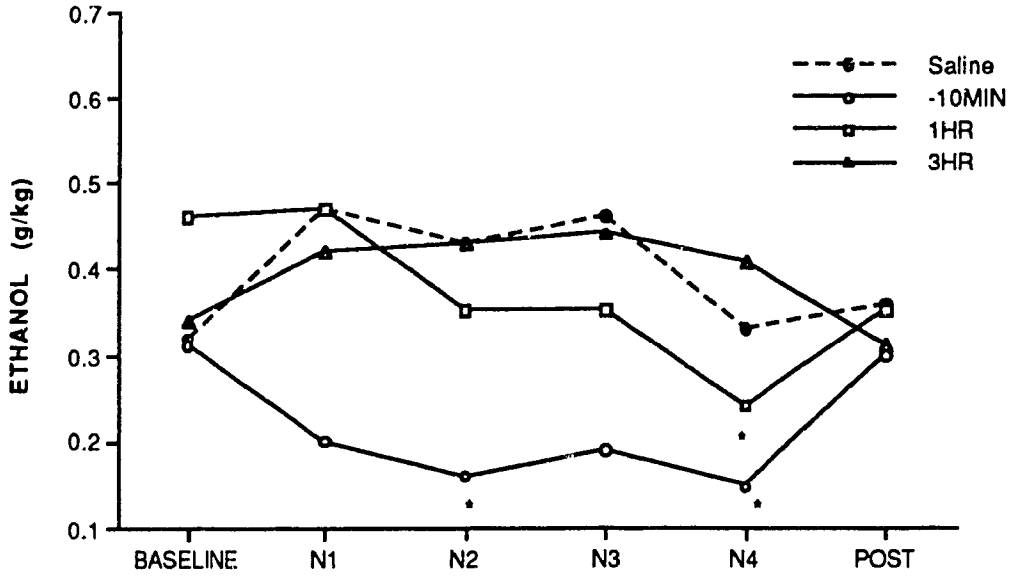

Figure 13. Mean absolute ethanol intake (8%) on the last day of NAL-pre-exposure (BASELINE), the 4 days of injections with NAL, and the post-injection day.

EXPERIMENT 6

Experiment 4 and 5, provided evidence to suggest that one of the mechanisms by which NAL may be mediating its antidipsogenic effect on ethanol intake, was through the induction of a CTA. The reduction in ethanol intake observed on the first day of NAL treatment, measured in the -10MIN group, however, suggested that in addition to a CTA, NAL may be inducing its suppressant effects through an even more direct, unconditioned mechanism. Experiments 2 and 3 provided evidence to suggest that this mechanism might exert its effects through pre-ingestional factors such as a shift in taste sensitivity. If this notion is correct, one would expect a similar pattern of reductions when a different flavored substance is presented instead.

Therefore, Experiment 6 was designed to replicate Experiment 4 but substituting a saccharin solution for ethanol. The results from Experiment 4 would suggest that when saccharin is presented as the conditioned fluid, NAL should also induce a CTA. Furthermore, the direct unconditioned suppressant effect observed in Experiment 4 should also be observed when rats are provided with a saccharin solution, if this decrease is mediated through a capacity of the drug to alter taste sensitivity for flavored substances.

Method

Subjects

48 naïve, male, Long-Evans rats (Charles River, Canada) weighed 125-150 g at the start of the experiment. Rats were individually housed in standard stainless steel cages and were maintained in a temperature and humidity controlled environment on a 12 hr reverse dark-light cycle (Lights on at 11 AM). Fluids were presented in two Richter-type tubes mounted on the front of the cages. Food and water were available ad libitum.

Procedure

Limited Access: Following a period of 2 weeks acclimatization to the reverse dark-light cycle, rats began training on a LA schedule. Fluid was delivered to the rats in plastic tubes with steel ball bearing spouts. They were provided with access to a 0.1 % saccharin solution or to water for 1 hr session per day. Rats were assigned to 1 of 6 groups.

Treatment: Following baseline recording, rats received daily injections of 10.0 mg/kg NAL or a saline vehicle in a volume of 1.0 ml, for a period of 4 days. These injections were given either 10 min prior to the start of the LA session (-10MIN), at the end of the LA session (1HR), or 3 hr after the LA session (3HR). The saline control group was injected either 10 min prior to the start of the LA session (SAL--10MIN), at the end of the LA session (SAL-1HR) or 3 hr after the LA session (SAL-3HR).

Post-injection: On day 5, saccharin intake was measured in all groups for a period of 3 days in the absence of NAL or saline injections.

Results

A split-plot analysis of variance with time of injection (-10MIN, 1HR and 3HR) as the between factor and days (last day of baseline (PRE), 4 days of treatment and the post-injection day (POST) as the within factor. The analysis revealed no significant difference between the 3 saline treated control groups, $F(10,105)=0.03$, $p>0.9$. Therefore, the remaining analysis was carried out on the 3 NAL-treated groups, and the SAL--10MIN control group.

A split-plot analysis of variance (days x time of injection) revealed a main effect of time of injection, $F(3,28)=3.75$, $p<.02$, and of days, $F(5,140)=12.10$, $p<.00001$. An interaction between the time of injection and day of injection, $F(15,140)=4.58$,

$p<.0000$ was also obtained. Post hoc analysis revealed that the -10MIN group drank significantly less of the saccharin solution throughout the injection period and this suppression was maintained in the post-injection phase of the experiment. Saccharin intake was also significantly reduced in the 1HR group and this suppression remained in the post-injection phase of the experiment. There was no effect of NAL on saccharin intake in the 3HR group. These data are illustrated in Figure 14.

Discussion

The -10MIN and 1HR groups showed a significant reduction in their intake of saccharin solution in the post-injection period of this experiment. Unlike Experiment 4, however, no effect of NAL was observed in the 3HR group. The decrease in saccharin intake in the 1HR group was not observed on the first day of NAL injections. This decrease was seen on the second pairing of NAL with the saccharin solution suggesting that the rats had associated the pharmacological actions of NAL with the presentation of the novel fluid and thus had developed a CTA.

Saccharin intake in the -10MIN group was also significantly reduced during the post-injection phase. Similar to the pattern of reduction in the -10MIN group of experiment 4, the decrease was measured on the first day of NAL injections.

Thus, the direct unconditioned suppressant effect of NAL observed with ethanol in experiment 4, was replicated in this experiment when the presented fluid was saccharin. Therefore, the results of experiment 4 and experiment 6 would suggest that the shift in taste sensitivity is similar for both less palatable fluids, such as an 8% solution of ethanol, and preferred sweet substances, such as 0.1% saccharin. These observations are in accordance with other studies that have found similar dipsogenic effects of opiate antagonists on preferred salt and sweet solutions and less palatable bitter solutions (Levin et al, 1982; Touzani, Akarid, & Velly, 1990).

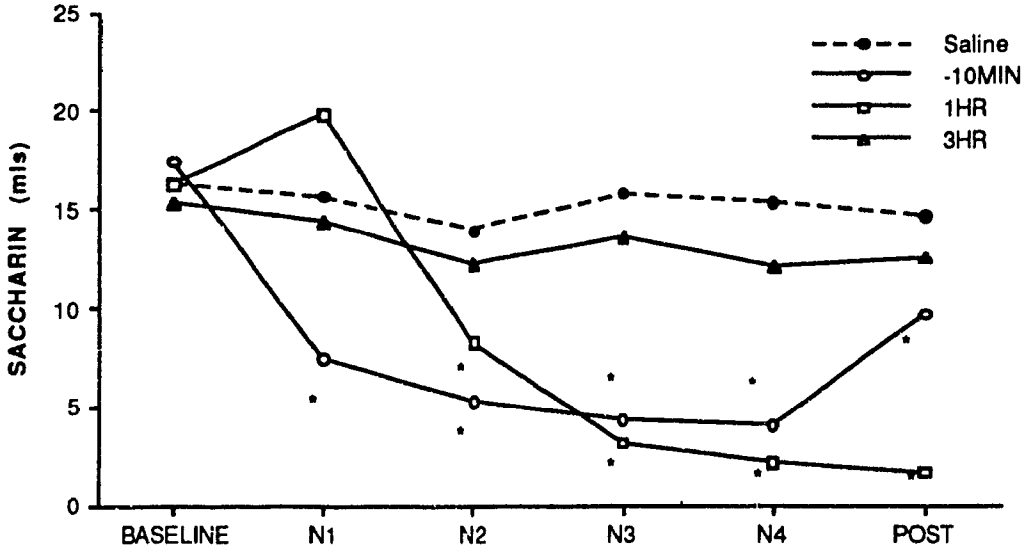

Figure 14. Mean 0.01% saccharin intake on the last day of BASELINE, the 4 days of injections with NAL, and the post-injection day (POST).

GENERAL DISCUSSION

Clinical trials are currently being conducted to examine the effects of the opiate antagonist, Naltrexone (NTX), on ethanol intake in alcoholics (O'Malley et al., 1992; Volpicelli et al., 1992). On the basis of these studies it has been suggested that the motivation to drink ethanol may have been attenuated in NTX-treated patients through the capacity of this drug to block ethanol-mediated reinforcement (Volpicelli et al., 1992). Using an animal model of voluntary oral ethanol self-administration, the experiments contained in this thesis attempted to determine the mechanism by which opiate antagonists were mediating the suppressant effects on ethanol drinking reported in preliminary clinical trials (O'Malley et al., 1992; Volpicelli et al., 1992) and in animal studies (Altshuler et al., 1980, Froehlich et al., 1991; Sinclair, 1990).

NTX Effects on Ethanol-Mediated Reinforcement

Experiments 1 and 2 found no evidence for the capacity of NTX to block ethanol-mediated reinforcement. In experiment 1 a 24-hr drinking paradigm with free-access to ethanol and water was used. NTX-treatment has no effect on 24-hr measurements of voluntary oral ethanol intake in rats at any of the doses administered. In experiment 2 an attempt was made to extinguish ethanol drinking by providing animals with an extended period of ethanol exposure during NTX-treatment. It was assumed that this period of exposure to NTX and ethanol would allow the animals to "learn" that ethanol was no longer reinforcing if NTX had the capacity to block ethanol-mediated reinforcement. Thus, upon termination of NTX-treatment, it was predicted that ethanol drinking behavior would have extinguished. Once free-access to ethanol and water was reinstated and NTX treatment was terminated, however, ethanol intake in NTX-treated groups was not different from ethanol intake prior to NTX-treatment or from ethanol intake measured in the control group.

Measurements taken 4 hr after NTX treatment during forced choice exposure (FCE), however, did reveal a reduction in ethanol intake that was not observed when measurements were taken 24 hr later. This transitory pattern of ethanol suppression suggested that the drug exerted a direct, unconditioned suppression on ethanol intake. It was, therefore, unlikely that this effect could have been mediated through post-ingestional conditioned feedback mechanisms, such as the blockade of ethanol-mediated reinforcement, because so little ethanol was actually ingested during the first 4 hr of FCE. Organisms require a minimum of 5-10 min of drinking a sufficient volume of ethanol, at a pharmacologically-relevant concentration, before blood and brain levels of ethanol may be detected (Gill, 1989). The NTX-treated groups were drinking less than 1 g/kg ethanol before the 4 hr measurement period. The drinking behavior of the rat was not observed between NTX treatment and 4 hr measurement interval. Thus, it is possible that ethanol was consumed in one discrete bout and, therefore, may have produced a pharmacological effect. This possibility is unlikely however, because animals were observed during LA drinking in experiment 4, and it was noted that during treatment with NAL, animals avoided the ethanol tube after only a few licks.

Experiment 1 and 2, found no evidence for the capacity of NTX to block ethanol-mediated reinforcement, therefore, the experiments that followed attempted to explore other mechanisms that might better explain this transient pattern of suppression of ethanol intake. The possibility that pre-ingestional mechanisms, such as alterations in taste sensitivity for ethanol might explain the pattern of decrease in ethanol intake observed in experiment 2, was investigated.

NTX Effects on Taste Sensitivity for Ethanol

In experiment 3, the effects of NTX were tested in an acquisition paradigm where animals were presented with an ascending series of low to high concentrations of ethanol. It was proposed that if NTX-treatment was decreasing ethanol intake through

alterations on the organism's taste sensitivity for ethanol, then the suppression should be observed at the lower, and presumably non-pharmacological concentrations of ethanol offered during acquisition.

At all concentrations of ethanol presented, 2.5 and 10 mg of NTX suppressed ethanol intake compared to intake observed in the saline control group. The pattern of ethanol intake suppression, however, was different in the group receiving the 2.5 mg dose of NAL from that seen in group receiving the 10 mg dose of NAL. Specifically, suppression of ethanol intake in at the high dose of NTX was enhanced with each additional presentation of the same concentration of ethanol. Suppression of ethanol intake at the low dose of NAL was not always enhanced with the second presentation of the same concentration of ethanol. On the basis of these differences it was suggested that the suppression observed at these two doses may have been mediated by two different mechanisms; a possible shift in taste sensitivity at the low dose of NTX, and the possible induction of a CTA at the high dose of NTX. The possibility that the high dose of NTX could induce a CTA to ethanol was investigated in the final 3 experiments of this thesis.

Naloxone and CTAs to Ethanol

Experiment 4 was essentially a conditioned taste aversion (CTA) paradigm. Injections of 10 mg of NAL were administered to rats during 1-hr limited access (LA) to 8% ethanol either 10 min prior to LA (-10MIN), immediately following LA (1HR), or 3 hr following LA (3HR). The design of this experiment was a replication of Sinclair's experiment (1990) but with the addition of the 1HR. The addition of the 1HR group, allowed for the appropriate pairing of post-ingestion effects of ethanol with the pharmacological-effects of NAL.

Ethanol intake during the 1 hr LA was suppressed during NAL-treatment in all three groups. A reduction in ethanol intake was seen on the first day of injections in the

-10MIN group but was not observed in the other 2 groups until the second day of injections. The decrease in intake seen in the -10MIN group following the first day of NAL injections suggested that the suppression of ethanol intake by NAL, was immediate and therefore unlikely to have been the consequence of a conditioning process reflecting extinction.

The measure of a CTA, however, is sustained suppression of fluid intake in the absence of the CTA-inducing agent (Hunt & Amit, 1987). In experiment 4, the presence of a CTA was apparent in the -10MIN and the 1HR NTX-treated groups since the suppression of ethanol intake was sustained in the post-injection phase of the experiment. Although drinking was also suppressed in the 3HR group during the last 3 days of NAL treatment, this suppression was not present during the post-injection phase. In the 3HR group, the time interval between the pairing of the unconditioned stimuli and the conditioned stimuli was likely too long to induce a CTA (Hunt & Amit, 1987).

Pre-exposure to a CTA-inducing agent has been shown to eliminate the capacity of the agent to induce a CTA (Ng Cheong Ton & Amit, 1983). Therefore, it would be reasonable to assume that pre-exposure to NAL should prevent the induction of a CTA, if in fact NAL was decreasing ethanol intake through this mechanism. Indeed, there was no reduction in intake observed in the post-injection phase of this experiment, when NAL-treated groups were pre-exposed to NTX. Therefore, the decrease in ethanol intake, measured during the post-injection period for both the -10MIN and the 1HR group in experiment 4, was most likely due to the induction of a CTA.

If NAL was acting, in part, by inducing a CTA to ethanol, one would assume that the same pattern of effects should also be observed with a flavored non-alcoholic substance. The presentation of a non-alcoholic substance eliminates the possibility that NAL could be decreasing fluid intake by blocking ethanol's pharmacological-effects. Furthermore, the direct, unconditioned suppressant effect observed in experiment 4,

should also be observed with a non-alcoholic substance assuming the decrease in fluid intake was mediated through alterations in taste sensitivity. Experiment 6 therefore, tested the capacity of NAL to induce a CTA to a 0.1% saccharin solution.

NAL-treatment did induce a CTA to saccharin. Furthermore, the direct unconditioned suppressant effect of NAL observed when ethanol was the fluid measured in experiment 4, was also replicated with the saccharin solution. Taken together, experiments 4, 5, and 6 suggest that, at a high dose (10 mg/kg), opiate antagonists may induce their reductions in ethanol intake of rats by the induction of a CTA to ethanol.

In conclusion, the data from this thesis do not support the notion that opiate antagonists are mediating their decreases in ethanol intake via their capacity to block ethanol-mediated reinforcement. Furthermore, two possible mechanisms which may explain the antidipsogenic properties of opiate antagonists on oral ethanol intake, are proposed. It is suggested that opiate antagonists may be mediating their reductions of ethanol intake by modulating pre-ingestional components of oral ethanol self-administration. Specifically, opiate antagonists may alter the taste sensitivity for ethanol. Furthermore, high doses of opiate antagonist (10 mg and upward) may also decrease ethanol intake through post-ingestional mechanisms by inducing a CTA.

Implications for Clinical Application

Clinicians and researchers working in the area of alcohol abuse are interested in developing new and more efficacious methods for the prevention and treatment of alcoholism (Amit & Sutherland, 1975/76; Clair, 1984; Teeling-Smith, 1984). There is evidence to suggest that the self-administration of ethanol is similar to that of any other operant; a behavior whose frequency was controlled by its consequences (Griffiths, Bigelow & Henningfield, 1980; Johanson, 1978; Kelleher & Goldberg, 1975). Thus, clinicians should be interested in the development of pharmacological interventions that

would 1) reduce alcohol craving, 2) moderate alcohol intake, and 3) reduce the reinforcement derived from intoxication (Amit & Sutherland, 1975/76; Clair, 1984; Teeling-Smith, 1984).

Indeed, preliminary clinical trials with NTX have found that when compared to alcoholics treated with placebo, those treated with NTX showed a decrease in the number of drinks consumed on days when ethanol was sampled, a decrease in the rate of relapse following ethanol sampling (O'Malley et al., 1992; Volpicelli et al., 1992), and an increase in the length of abstinence periods (O'Malley et al., 1992). On the basis of the experiments conducted in this thesis, two mechanisms were proposed to explain how opiate antagonists may be inducing their suppression on voluntary oral ethanol intake; a possible effect on pre-ingestional mechanisms, such as a shift in taste sensitivity for ethanol, and the induction of a CTA to ethanol. Although both mechanisms result in the desired decrease in ethanol consumption, the decreases induced by alterations in taste, or by the aversive consequences of a CTA, may be of limited clinical utility. Furthermore, in the case of opiate antagonist's effects on taste sensitivity, experiment 6 of this thesis would suggest that this effect was not specific to ethanol. It seems unlikely that alcoholic patients would be willing to comply with a treatment that may alter their enjoyment of all ingested materials. Similarly, low compliance rates would also be anticipated following medication with an agent that reduced ethanol consumption through the induction of an aversive physiological state.

CONCLUSIONS

In conclusion, the data presented in this thesis provided no evidence for the idea that opiate antagonists have the capacity to block ethanol-mediated reinforcement. At high doses (10 mg), opiate antagonists can mediate decreases in ethanol self-administration through the induction of a CTA to the taste of ethanol. The capacity of NAL to induce a CTA was also observed when animals were given saccharin to drink. A direct suppressant effect of opiate antagonists on ethanol drinking was also observed that appears to be mediated through pre-absorptive mechanisms, possibly through a shift in taste sensitivity.

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