AN INVESTIGATION OF THE MECHANISMS OF ACTION OF 5-HYDROXYTRYPTAMINE UPTAKE BLOCKADE IN THE SUPPRESSION OF VOLUNTARY ETHANOL INTAKE

Gary E. Rockman

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ABSTRACT

AN INVESTIGATION OF THE MECHANISMS OF ACTION OF 5-HYDROXYTRYPTAMINE UPTAKE BLOCKADE IN THE SUPPRESSION OF VOLUNTARY ETHANOL INTAKE

Gary E. Rockman, Ph.D.
Concordia University

The experiments presented in this thesis were designed to elucidate the neuropharmacological mechanisms underlying the attenuating effects of zimelidine, a 5-hydroxytryptamine (5-HT) uptake inhibitor, on ethanol consumption. In the first experiment some specific pharmacological features of zimelidine that might have accounted for the reduction of ethanol intake were explored. When the effects of specific and non-specific inhibitors of 5-HT and norepinephrine (NE) uptake on ethanol consumption were compared, it was found that only those compounds that were specific to 5-HT uptake caused a reduction of ethanol intake. It was also found that norzimelidine, the primary metabolite of zimelidine,

caused an attenuation of ethanol consumption similar to that of zimelidine. These findings suggested that the neuropharmacological action of norzimelidine may account for the reduction in ethanol intake as produced by zimelidine and that specific 5-HT inhibition was the mechanism of action. The possibility existed, however, that a functional depletion of NE and/or an enhancement of 5-HT post-synaptic transmission acted as causal factors in the attenution of ethanol intake. These were examined in additional experiments. The results from Experiments 2 and 3 suggested that zimelidine reduced ethanol intake indirectly by reducing NE activity, whereas, norzimelidine attenuated ethanol intake by some other neurochemical mechanism. In Experiment 4, the role of enhanced 5-HT post-synaptic activity in the attenuation of ethanol intake was examined. Results indicated that the effects of norzimelidine and not zimelidine were mediated by an increased 5-HT post-synaptic activity. In summary, the experiments suggest that zimelidine and norzimelidine reduce ethanol intake by two different neurochemical mechanisms of action and that the neurochemical mechanisms

underlying the control of ethanol consumption involve a complex interaction between 5-HT and NE systems of the brain.

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Introduction

Beverages containing ethyl alcohol (ethanol) have been ingested at social and ritualistic events for thousands of years, in fact, the use of alcoholic beverages dates as far back as 8000 B.C. (Murphree, 1971). Ethanol is presently the most widely consumed mood altering drug in almost every human society (Lieber, 1976) and recent studies indicate that approximately 5-15% of North American and European populations are considered to be problem drinkers; (Cahalan & Room, 1972; Hagnell & Tunving, 1972; Weissman, Myers & Harding, 1980). The resulting impact of ethanol consumption on society has been overwhelming as is evidenced by the prevalence of ethanol-related problems (Grant & Gwinner, 1979). One example of this is that recent estimates indicate that 25 to 40% of severe or fatal injuries resulting from traffic accidents in North America, seem to be related to ethanol consumption (Bacon, 1968; Zylman & Bacon, 1968). Unfortunately, problematic use of ethanol and the incidence of ethanol-related problems are consistently increasing in most societies (Madden, 1979).

Not surprisingly, much work has been done

attempting to understand the social and biological factors involved in ethanol abuse (Madden, 1979). Since the central nervous system is involved in the mediation of many behaviors, it is understandable that researchers have investigated the neuropharmacological basis of ethanol self-administration. To facilitate these investigations, researchers have largely relied on animal models of human alcoholism. To date, evidence accumulated from this avenue of research, although at times equivocal, has indicated that either central noradrenergic or serotonergic systems may be involved in ethanol self-administration (e.g. Myers, 1978a; Myers & Melchior, 1977a).

Recent reports have demonstrated that reductions in central norepinephrine (NE) levels produce attenuations of ethanol self-administration in laboratory animals (Amit, Brown, Levitan & Ogren, 1977; Amit, Levitan & Lindros, 1976; Davis, Smith & Weiner, 1978; Davis, Werner & Smith, 1979). A subsequent study examined the effects of forced-choice ethanol consumption in combination with lowered NE levels (Brown, Amit, Levitan, Ogren & Sutherland, 1977). The results indicated that when ethanol was once again presented in a free-choice with water without drug treatment, ethanol intake was

significantly reduced. These data were interpreted as suggesting that lowered NE levels in some way diminished the positive reinforcing properties of ethanol, thereby, reducing subsequent ethanol consumption (Brown et al., 1977). These results suggest that central NE activity may mediate the reinforcing effects of ethanol.

Another area of research has indicated that increasing the central availability of 5-hydroxytryptamine (5-HT) results in the attenuation of ethanol consumption (Geller, 1973; Hill, 1974; Zabik, Liao, Jeffreys & Maickel, 1978). More recently, it has been demonstrated that increasing 5-HT availability, following treatment with the uptake inhibitor zimelidine (1-4 bromophenyl-1-3-pyridyl-3--dimethylaminopropene; Ross & Renyi, 1977), attenuated voluntary ethanol consumption (Rockman, Amit, Carr, Brown & Ogren, 1979). In this study, zimelidine, a specific neuronal 5-HT uptake inhibitor (Ross & Renyi, 1977), reduced voluntary ethanol consumption without altering body weight or total fluid intake. The results suggested that increasing the availability of central 5-HT may in some way alter the mediation of the positive reinforcing properties of ethanol (Rockman et al., 1979a). Subsequent experimental

evidence, however, has suggested that attenuation of ethanol intake, as produced by increased 5-HT availability, may not be entirely a result of alterations of the serotonergic system. For example, increasing central 5-HT availability has been shown to reduce central NE activity (Everett, 1974; Shaskan & Snyder, 1970; Snyder, Shaskan & Kuhar, 1973). It has been suggested that the reduction in NE activity may be a result of the active transport of available 5-HT into NE neurons via the NE uptake process (Shaskan & Snyder, 1970; Snyder et al., 1973). Therefore, these data suggest that treatment with zimelidine may induce a reduction of NE activity, thereby, reducing ethanol intake. In a recent study, an attempt was made to prevent this proposed invasion of excess 5-HT into NE neurons (Rockman, Amit, Carr & Ogren, 1979). This was accomplished by pretreating ethanol preferring animals with desmethylimipramine (DMI), a selective NE uptake inhibitor (Javaid, Perel & Davis, 1979), prior to treatment with zimelidine. The results indicated that such treatment significantly reduced the magnitude of the zimelidine-induced attenutation of ethanol intake (Rockman et al., 1979b). As a result, the present author suggested that the attenuation of ethanol

intake as produced by zimelidine, may not be directly a result of increased 5-HT availability, but rather, an indirect effect on NE activity (Rockman et al., 1979b).

Due to the contradictory nature of the experimental evidence emanating from studies investigating the role of central 5-HT in ethanol self-administration (e.g. Myers & Melchoir, 1977a), it was necessary to further investigate the mechanism of action of 5-HT uptake blockade with regard to ethanol intake. The present series of experiments were designed to investigate whether the attenuation of ethanol intake as produced by zimelidine and its active metabolite norzimelidine, is solely a result of a direct manipulation of the serotonergic system, an indirect effect on NE activity or some interaction between both systems. We predicted that this research might aid in the understanding of the neurochemical basis of ethanol self-administration.

The following sections of the introduction will review the relevant areas of the literature and outline in more detail the present investigation.

Psychopharmacological Factors in Ethanol Self-Administration

A. Physical dependence and ethanol self-administration. Lester and Freed (1973) have suggested that physical dependence, defined as the occurrence of withdrawal symptoms upon the removal of access to ethanol, is a necessary criterion for an animal model of alcoholism. Furthermore, other investigators have suggested that the experience of withdrawal in animals should lead to an increased voluntary consumption of ethanol (Cicero, Snider, Perez & Swanson, 1971; Ho, Chen & Tsai, 1978). In order to induce physical dependence on ethanol, blood-ethanol levels must exceed the oxidative capacity of the organism for several days without interruption (Goldstein, 1975; Hunter, Riley, Walker & Freund, 1975; Majchrowicz, 1975; Wallgren, 1973). Although, results from some studies have indicated that the development of physical dependence to ethanol increased ethanol consumption, the majority of studies do not support this notion. For example, chronic treatment with intoxicating doses of ethanol presented orally or intragastrically, have been shown to increase subsequent oral consumption of ethanol (Cicero et al., 1971; Deutsch & Koopmans, 1973;

Deutsch & Walton, 1977; Sinclair, Walker & Jordan, 1973; Tang & Falk, 1977). However, other researchers, employing similar procedures, have demonstrated that the development of physical dependence and subsequent manifestations of withdrawal does not induce a preference for ethanol (Begleiter, 1975; Cicero & Smithloff, 1973; Freund, 1969; Goldstein, 1974; Heintzelman, Best & Senter, 1976; Myers, Stoltman & Martin, 1972; Ratcliffe, 1972; Senter & Sinclair, 1967).

Additional evidence suggesting that physical dependence does not induce ethanol self-administration, is derived from studies investigating patterns of ethanol self-administration. Several reports have indicated that both animals and humans will voluntarily and spontaneously abstain temporarily from self-administering ethanol despite the manifestation of withdrawal symptoms (Deneau, Yanagita & Seevers, 1969; Hunter et al., 1975; Mello & Mendelson, 1970, 1972; Winger & Woods, 1973). The above mentioned data from human and animal experiments suggests that although physical dependence may develop from ethanol self-administration, this in itself is not a necessary nor a sufficient condition for the initiation and maintenance of

ethanol self-administration.

On the basis of the literature, therefore, it is suggested that physical dependence may in some way play a supportive role in the maintenance of ethanol self-administration in humans and other animal species. In addition, this implies that other pharmacological properties of ethanol may be the factors primarily responsible for the initiation and maintenance of ethanol self-administration.

B. Ethanol as a reinforcer. Many studies have demonstrated that several species of animals will learn to perform an operant under various schedules of reinforcement in order to receive intravenous (Carney, Llewellyn & Woods, 1976; Karoly, Winger, Ikomi & Woods, 1978; Winger & Woods, 1973) or intragastric (Amit & Stern, 1969; Smith, Werner & Davis, 1976; Yanagita & Takahashi, 1973) infusion of ethanol. These results suggest that ethanol, via either of these two routes of administration, can serve as a positive reinforcer. As humans self-administer ethanol orally, it would therefore seem necessary to demonstrate that animals used as models of human ethanol self-administration, should also consume ethanol orally. Voluntary oral consumption of ethanol in some animal species, has

proved to be difficult to establish. Although rhesus monkeys will readily ingest ethanol (Henningfield & Meisch, 1978, 1976; Meisch, Henningfield & Thompson, 1975), laboratory rats are relunctant to drink ethanol (Richter & Campbell, 1940; Wilson, 1972). The difficulty in establishing and maintaining oral ethanol consumption in rats, seems to stem primarily from the aversive taste of ethanol at higher concentrations (Myers, 1966; Kahn & Stellar, 1960; Richter & Campbell, 1940; Wilson, 1972). As a consequence, several researchers have employed various paradigms which were designed to overcome this problem. The masking of the aversive taste properties of ethanol by sucrose or saccharin has been shown to increase the amount of ethanol consumed (Eriksson, 1969; Lester & Greenberg, 1952). Unfortunately, this increased intake does not continue when unadulterated ethanol is presented (Lester & Greenberg, 1952). Other procedures have been more successful in establishing voluntary oral consumption of ethanol. For example, it has been demonstrated that intermittent presentations of increasing concentrations of ethanol results in an enhanced voluntary oral intake of ethanol (Amit,

Stern & Wise, 1970; Sinclair & Senter, 1968).

It is clear that animals will voluntarily self-administer ethanol by various routes of administration. In addition, since animals will voluntarily ingest ethanol despite its aversive taste, it would then suggest that ethanol has positive reinforcing properties which account for the initiation and maintenance of ethanol self-administration.

In attempting to understand the neurochemical involvement in the mediation of some of the effects of ethanol, researchers have examined the interaction between the biogenic amines and ethanol. The following sections of the introduction will review the relevant literature concerning this relationship.

Neurochemical Factors: Biogenic Amines and Ethanol

A. Effects of ethanol on catecholamine metabolism. Gursey, Verster and Olson (1959) reported that ethanol may alter the metabolism of catecholamines (CA). These authors demonstrated that brain norepinephrine (NE) levels in rabbits were lowered following treatment with ethanol. Since that time, attempts to confirm and expand these findings have resulted in conflicting results. For

example, while it has been demonstrated that acute ethanol treatment does not alter steady state levels of CA (Bacopoulous, Bhatnagar & Van Orden, 1975; Duritz & Truitt, 1966; Hunt & Majchrowicz, 1974a; Pohorecky, 1974), other studies have reported decreases in CA levels as a result of ethanol treatment (Carlsson, Magnusson, Svensson & Waldeck, 1973; Gursey & Olson, 1960). On the other hand, chronic ethanol treatment has been shown to increase the central concentration and levels of both dopamine (DA) and NE (Ortiz, Griffiths & Littleton, 1974).

The results from studies examing the effects of ethanol on turnover of CA have been equally confusing. Turnover of brain NE following acute ethanol treatment has been shown to decrease (Pohorecky, 1974; Thadani, Kulig, Brown & Beard, 1976; Thadani & Truitt, 1973) or increase (Carlsson et al., 1973; Corrodi, Fuxe & Hokfelt, 1966; Hunt & Majchrowicz, 1974a). Similarly, DA turnover has been shown to decrease (Hunt & Majchrowicz, 1974a), increase (Carlsson et al., 1973; Karoum, Wyatt & Majchrowicz, 1976), or remain unchanged (Corrodi et al., 1966).

Needless to say, the relationship between CA

turnover and ethanol treatment is uncertain. When examined in regional areas of the brain, there appears to be a differential effect of ethanol on CA metabolism. For example, following ethanol treatment NE turnover was found to be decreased in the hypothalamus and increased in the pons meddulla (Bacopoulous et al., 1975, 1978). Turnover of DA was reduced in the substantia nigra and caudate nucleus and not in the hypothalamus (Bacopoulous et al., 1975, 1978).

In studies using humans as subjects, acute ethanol administration was shown to induce alteration of peripheral NE metabolism (Davis, Brown, Huff & Cashaw, 1967a). Similarly, Gitlow, Dziedzic, Dziedzic and Wong (1976) measured excretion of the major CA metabolites following chronic ethanol treatment. Their data suggests that ethanol induces an increase in synthesis and turnover of DA and NE. However, a more recent study examining levels of homovanillic acid, a dopamine metabolite, in cerebrospinal fluid (CSF) produced conflicting results (Ballenger, Goodwin, Major & Brown, 1979). It was demonstrated that chronic alcohol consumption did not alter levels of homovanillic acid in CSF, suggesting that DA

metabolism is not altered.

Although there are many conflicting reports regarding the effects of ethanol on CA metabolism, the majority of studies do suggest that ethanol does alter central levels and turnover of CA. Unfortunately, the magnitude, direction and specificity of the ethanol-induced alterations seems to be unclear.

B. Effects of ethanol on 5-hydroxytryptamine (5-HT) metabolism. Although there has been extensive research examining the effects of ethanol on central 5-HT metabolism, the results have proven to be contradictory (e.g. Myers & Melchior, 1977a). For example, 5-HT levels have been reported to be decreased (Gursey et al., 1959) increased (Erickson & Matchett, 1975; Pohorecky, Jaffe & Berkeley, 1974), or unchanged (Carlsson & Lindqvist, 1973; Pohorecky & Newman, 1978; Pohorecky, Newman, Sun & Bailey, 1978; Tabakoff & Boggan, 1974) as a result of acute ethanol treatment. Chronic treatment with ethanol has also been shown not to alter endogenous levels of 5-HT (Pohorecky et al., 1978).

With reference to central 5-HT turnover, several studies have indicated that both acute and chronic ethanol treatment increases turnover of

5-HT (Hunt & Majchrowicz, 1974b; Pohorecky & Newman, 1978; Tabakoff & Boggan, 1974).

In humans, acute ethanol ingestion has been shown to decrease the amount of 5-HT metabolites in urine (Davis, Brown, Huff & Cashaw, 1976b). Similar results have been obtained from a comparison between alcoholic and non-alcoholic patients. Levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in CSF of alcoholics in the abstinence phase were significantly lower than in the non-alcoholic group (Ballenger et al., 1979). In contrast to the animal studies, ethanol seems to lower CSF levels of 5-HT metabolites in humans, suggesting that ethanol may reduce 5-HT synthesis and turnover. At present, the nature of this discrepancy between the effects of ethanol on 5-HT metabolism in animals and humans is unclear.

In summary, the effect of ethanol on CA and 5-HT metabolism has produced interesting but confusing results. Although it is difficult to draw definite conclusions, it does seem clear that ethanol interacts, in some way, with the biogenic amines. Based on this interaction, it is possible that some of the effects of ethanol are mediated by these neurochemical substrates. Not surprisingly,

considerable amount of attention has been given to this fundamental issue of whether the biogenic amines may play a role in the mediation of ethanol-related behaviors.

Biogenic Amines and Ethanol-Related Behaviors

A. Catecholamines. Many studies have implicated catecholaminergic mechanisms in the mediation of some of the pharmacological properties of ethanol. For example, treatment with alpha-methyl-tyrosine (an inhibitor of tyrosine hydroxylase the rate limiting enzyme in the synthesis of CA) has been shown to inhibit the motor excitation produced by ethanol in rats (Carlsson, Engel & Svensson, 1972; Engel, Strombom, Svensson & Waldeck, 1974). In addition, other CA manipulations consisting of pretreatment with CA receptor agonists and antagonists have also been shown to antagonize the ethanol-induced motor excitation (Carlsson, Engel, Strombom, Svensson & Waldeck, 1974; Matchett & Ericksson, 1977; Strombom, Svensson & Carlsson, 1977). Furthermore, reductions of central NE levels via pretreatment with a dopamine-beta--hydroxylase inhibitor, completely antagonized the ethanol-induced motor excitation in rats (Brown, Smith & Sinyor, 1978). These results suggest

that NE may play a primary role in the mediation of the motor excitatory effects of ethanol.

In attempting to investigate whether brain CA are involved in ethanol self-administration, investigators have employed a variety of experimental techniques. For example, correlations between CA content in brains of animals selected for their ethanol intake have been examined. Results have indicated that DA concentrations were significantly higher in brains of ethanol-preferring rats (AA strain) as compared to the concentrations in brains of water-preferring rats (ANA strain, Ahtee, Attila & Kiianmaa, 1980; Ahtee & Eriksson, 1975). Brain NE concentrations were found either not to differ (Ahtee & Eriksson, 1975) or slightly lower in the AA strain (Ahtee et al., 1980). A similar study with two strains of mice found no difference in brain CA levels (Ho, Tsai & Kissin, 1975). In this latter study central CA concentrations were measured in mice with either high (C57 Bl/6J strain) or low (DBA/2J strain) preference for ethanol. No difference in brain concentrations of either DA or NE were found.

Other studies which have employed procedures designed to manipulate central CA system have produced more consistent findings. Electrical

stimulation of the lateral hypothalamus, an area rich in catecholamines (Ungerstedt, 1971), has been shown to produce a permanent increase in ethanol consumption in rats (Amit & Stern, 1971; Amit, Stern & Wise, 1970). Conversely, electrolytic lesions of the ventral hypothalamus has been shown to attenuate ethanol self-administration (Amit, Meade, Levitan & Singer, 1976). Additional evidence suggesting a relationship between central CA and ethanol self-administration is derived from studies employing alpha-methyl-tyrosine (AMPT) in both animals and humans. Reduction in central levels of DA and NE as a result of AMPT treatment has been shown to slightly reduce the preference for ethanol in rats (Myers & Veale, 1968) and to suppress the euphoric effects of alcohol in humans (Ahlenius, Carlsson, Engel, Svensson & Sodersten, 1973). Similarly, pharmacological destruction of CA neurons by treatment with the neurotoxin 6-hydroxydopamine (6-OHDA) has also been shown to alter ethanol consumption. Treatment with 6-OHDA was shown to reduce ethanol consumption (Brown & Amit, 1977; Melchior & Myers, 1976; Myers & Melchior, 1975a). These results suggest that CA may be involved in the mediation of ethanol

self-administration. However, since procedures used in these studies reduced both DA and NE levels, the relative importance of either DA or NE is unclear. Consequently, subsequent studies have examined the effects of selective depletions of either DA or NE on ethanol consumption. Brown and Amit (1977) pretreated ethanol preferring animals with desmethylimipramine, a NE uptake inhibitor, prior to treatment with 6-OHDA. This resulted in depletions of DA while leaving NE neurons intact. Ethanol drinking animals treated in this way did not alter their consumption of ethanol, suggesting that NE rather than DA is involved in the mediation of ethanol drinking. Similar results were obtained following the selective depletion of forebrain NE without affecting brain DA (Mason, Corcoran & Fibiger, 1979). Such treatment reduced oral consumption of ethanol thereby supporting the notion that NE and not DA is primarily involved in the mediation of ethanol self-administration. This notion was supported further by the results from a study investigating the effect of 6-OHDA-induced lesions of ascending DA systems (Kiianmaa, Andersson & Fuxe, 1979). Such treatment was shown not to

alter ethanol intake. It is important to note, however, that 6-OHDA-induced lesions of ascending NE pathways in the brain, resulting in partial depletion of NE, has been shown to produce a transient increase in ethanol consumption (Kiianmaa, 1980; Kiianmaa, Fuxe, Jonsson & Ahtee, 1975). While the data support the notion that NE and not DA is involved in the mediation of ethanol consumption, the nature of the discrepancy in relation to the direction of these results is unclear. It is possible that the magnitude of NE depletions plays an important role in determining the direction of the effect on ethanol intake.

The role of NE in the mediation of ethanol self-administration has been further supported by the results of studies employing inhibitors of dopamine-beta-hydroxylase (DBH). Inhibition of DBH, the enzyme necessary for the conversion of DA to NE, results in a specific depletion of central NE levels. It has been demonstrated that DBH inhibition attenuates both intragastric (Davis et al., 1978, 1979) and oral (Amit et al., 1976, 1977) ethanol self-administration. In addition, forced-choice ethanol presentations in combination with FLA-57, a DBH inhibitor,

resulted in a marked decrease in ethanol consumption when ethanol was subsequently presented in a free-choice situation without injections (Brown et al., 1977). These results were interpreted as suggesting that the combination of ethanol consumption and depletion of NE resulted in a reduction of the positive reinforcing properties of ethanol, thereby, reducing subsequent ethanol consumption (Brown et al., 1977).

These data strongly suggest that central CA and specifically NE play a major role in the mediation of the positive reinforcing properties of ethanol.

Further evidence implicating biogenic amines in the mediation of ethanol self-administration is obtained from the area of research investigating the role of tetrahydroisoquinolines (TIQ) and tetrahydro-β-carbolines in ethanol intake. Although this area of research is not directly related to the present investigation, a brief review is included since this area has received a considerable amount of attention in the alcohol intake literature.

B. Tetrahydroisoquinoline (TIQ), β-carbolines and ethanol self-administration. It has been

demonstrated that acetaldehyde, the primary metabolite of ingested ethanol, can condense with CA to form TIQ alkaloids (Cohen & Collins, 1970; Davis & Walsh, 1970). In addition, it has been suggested that TIQ alkaloids can be transported, via the CA uptake process, into CA neurons (Cohen, 1976, 1978, 1979). Once located and stored in CA neurons, it has been suggested that TIQ alkaloids may act as false neurotransmitters which ultimately may be involved in the regulation of ethanol consumption (Cohen, 1978, 1979; Davis, 1973).

Although there seems to be an agreement in the literature regarding a possible role for TIQ alkaloids in ethanol consumption (e.g. Brown, Amit & Smith, 1980a, 1980b; Myers, 1978b), there exists sharp contradictions in the behavioral data emanating from investigations of this relationship. For example, Myers (1978), Myers and Melchior (1977b, 1977c) and Myers and Oblinger (1977) have reported that following intraventricular infusions of several TIQ alkaloids, rats would consume large quantities of ethanol. These authors argue that the formation of TIQ alkaloids during the course of normal ethanol consumption, may be involved in the

continued preference for ethanol. In contrast, other investigators using similar procedures have failed to produce any effect on ethanol consumption following treatment with the same TIQ alkaloids (Brown et al., 1980a, 1980b; Smith, Brown & Amit, 1980). In light of these discrepancies, the role of condensation products of acetaldehyde and CA in ethanol self-administration remains to be elucidated.

Similarly, it has been demonstrated that 5-HT may condense with acetaldehyde to form tetrahydro-β-carbolines (Cohen, 1976; McIsaac, 1961). Consequently, it has been suggested that β-carbolines may somehow be involved in the maintenance of ethanol intake (Myers, 1978b; Myers & Melchior, 1977b). For example, central infusions of a β-carboline has been shown to significantly increase the preference for ethanol in rats (Myers, 1978b; Myers & Melchior, 1977b; Myers & Oblinger, 1977). However, intraperitoneal injections of other β-carboline compounds reduced ethanol intake (Geller & Purdy, 1975). Therefore, although β-carbolines seem to alter ethanol intake, the role of condensation products of 5-HT and acetaldehyde in ethanol consumption is unclear.

C. 5-hydroxytryptamine (5-HT) and ethanol self-administration. Various approaches have been used in an attempt to determine whether 5-HT may play a role in the mediation of ethanol self-administration (e.g. Myers & Melchior, 1977a). For example, investigators have examined the relationship between central levels and rate of turnover of 5-HT and ethanol consumption in different rat strains. In a comparison of two strains of rats selected as either ethanol preferring (AA strain) or water preferring (ANA strain), it was determined that central 5-HT levels were significantly higher in the ethanol preferring rats (Ahtee, 1972; Ahtee & Eriksson, 1972). Results of a subsequent study indicated that the 5-HT levels were elevated primarily in the hypothalamic and midbrain regions (Ahtee & Eriksson, 1973). A more recent study, however, has yielded contradictory findings. Murphy, McBride, Lumeng and Li (1980) have demonstrated that a strain of rats selectively bred to prefer ethanol (P) had a much lower level of 5-HT in hypothalamic and midbrain regions when compared to rats bred net to prefer ethanol (NP).

In attempting to further investigate the possible relationship between 5-HT and ethanol

consumption, researchers have employed pharmacological agents capable of reducing central 5-HT content and have monitored subsequent effects on ethanol consumption. The results emanating from from these studies have served to further confuse an already unclear relationship. Originally, it was reported that reductions of central 5-HT, as produced by p-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, caused an attenuation of the preference for ethanol (Myers & Veale, 1968; Veale & Myers, 1970). Subsequent studies, however, have demonstrated that the effects of PCPA were not specific to ethanol intake. It was demonstrated that ingestion of a saccharin solution and total fluid intake were also decreased following PCPA treatment (Nachman, Lester & Le Magnen, 1970; Stein, Wayner & Tilson, 1977). Furthermore, reductions in central 5-HT levels by treatment with either PCPA or the neurotoxin 5,6-dihydroxytryptamine have been shown to either increase (Geller, 1973; Ho, Tsai, Chen, Begleiter & Kissin, 1974) or not alter (Kiianmaa, 1975) ethanol intake. As a result of these contradictory findings, there does not appear to be any consistent relationship between lowered levels of central 5-HT

and ethanol consumption.

Numerous studies have examined the effects of increased levels of 5-HT on ethanol intake. One approach has involved the addition of tryptophan in the diet of animals in an attempt to increase 5-HT both peripherally and centrally (Fernstrom & Wurtman, 1971). Animals treated in such a manner were shown to significantly increase their ethanol consumption (Sprince, Parker, Smith & Gonzales, 1972; Myers & Melchior, 1975). Whether this observed increase in ethanol intake is solely a result of increased 5-HT levels or is a result of activity in other monoaminergic systems is uncertain. This uncertainty is based 1) on the observation that only certain rat strains are susceptable to tryptophan treatment (Myers & Melchior, 1975b) and 2) that other amino acids such as L-DOPA, which increases DA levels, also produces increased ethanol intake (Sprince et al., 1972).

In contrast, more consistent results have been obtained from studies examining the effects of other procedures designed to increase levels and availability of central 5-HT on ethanol intake. Several studies have demonstrated that

intraperitoneal or intraventricular treatment with either 5-hydroxytryptophan, the precursor of 5-HT, or 5-HT itself, produces significant reductions in ethanol consumption (Geller, 1973; Geller, Purdy & Merritt, 1973; Myers, Evans & Yaksh, 1972; Myers & Martin, 1973; Zabik et al., 1978). Based on these studies, it has been suggested that increasing the central availability and levels of 5-HT may somehow interfere with the mediation of ethanol consumption. These results have recently been confirmed and expanded as a result of data emanating from studies investigating the effect of 5-HT uptake blockade on ethanol consumption (Rockman et al., 1979a, 1979b). The mechanism of uptake blockade and its relation to ethanol intake will be reviewed in detail in a subsequent section. Since 5-HT uptake blockade and the resultant effects on neurotransmission form the basis of the present investigation, a discussion of these effects and interactions will precede the review of the literature concerning uptake blockade and ethanol consumption.

Uptake Blockade - Effects Within the Central Nervous System

A. General properties. Following the

release of a neurotransmitter into the synaptic gap, a specific uptake mechanism accounts for the primary process of inactivation by transporting the neurotransmitter back into the pre-synaptic neuron. This active transport mechanism has been shown to be operating within both CA and 5-HT systems (Agranoff, 1975; Fuller & Wong, 1977; Iversen, 1975; Snyder, Kuhar, Green, Coyle & Shaskan, 1970; Snyder et al., 1973). As a consequence, inhibiting the uptake process, via pharmacological uptake inhibitors, produces various changes within the neurotransmitter system. Acute or short-term inhibition of 5-HT uptake, has been shown to produce an increased availability of 5-HT in the synaptic gap (Anderson, 1972; Carlsson, Jonason, Lindqvist & Fuxe, 1969; Kantak, Wayner, Tilson, Dwoskin & Stein, 1978; Meek, Fuxe & Anden, 1970). The increased availability of 5-HT, as a result of uptake blockade, produces enhanced synaptic transmission (Svensson, Dahlof, Engberg & Hallberg, 1981) and activity at post-synaptic receptor sites (Fuller, 1980; Sangdee & Franz, 1979). Due to a postulated negative feedback system, the increased availability in the synaptic gap may result in a reduction in neuronal firing

rate and turnover (Aghajanian, 1972; Schubert, Nyback & Sedvall, 1970; Sheard, Zolovick & Aghajanian, 1972). As a result, chronic treatment (14 daily treatments) with 5-HT uptake inhibitors, seems to produce an overall reduction in synthesis, turnover and transmission (Fuxe, Ogren & Agnati, 1979; Fuxe, Ogren, Agnati, Eneroth, Holm & Andersson, 1981; Hwang, Magnussen & Van Woert, 1980; Sugrue, 1980).

B. 5-HT uptake blockade - interactions with brain NE. The literature reviewed in previous sections of the introduction strongly suggests that central NE plays a major role in the mediation of ethanol consumption. Studies examining the role of 5-HT in ethanol intake have been inconclusive with the exception of those studies investigating the effects of increased 5-HT availability on ethanol consumption. It is therefore conceivable that some interaction between increased 5-HT availability and NE activity may explain the role of biogenic amines in the mediation of ethanol intake.

An examination of the anatomical, biochemical and pharmacological data suggest an interaction between 5-HT and NE systems in the brain (Baraban

& Aghajanian, 1981; Pickel, Joh & Reis, 1977; Pujol, Stein, Blondaux, PetitJean, Froment & Jouvet, 1973; Samanin & Garattini, 1975). More specifically, it has been demonstrated that increasing the central availability of 5-HT induces a decrease in brain NE levels (Everett, 1974). Based on these results it was suggested that increasing the availability of 5-HT may in some way displace NE from the storage vessicles (Everett, 1974). A mechanism for this proposed displacement of NE by surplus 5-HT has been suggested which involves the NE uptake system. Due to the pharmacological similarities of the 5-HT and NE uptake processes (Iversen, 1975), it has been indicated that the NE uptake process may not be specific for NE alone, but may also accumulate 5-HT (Shaskan & Snyder, 1970; Snyder et al., 1973; Thoa, Eccleston & Axelrod, 1969). These investigators have demonstrated that increasing the availability of 5-HT may result in an invasion by 5-HT of NE neurons via the NE uptake mechanism. It was further suggested that once located and stored in the NE neuron, the 5-HT could act as a false neurotransmitter within the NE system and subsequently cause a functional depletion of NE

(Shaskan & Snyder, 1970; Snyder et al., 1970; Thoenen & Tranzer, 1971; Tranzer, Thoenen, Snipes & Richards, 1969).

5-HT Uptake Blockade and Ethanol Consumption

In a recent study, zimelidine, a selective neuronal 5-HT uptake inhibitor (Ogren, Ross, Hall, Holm & Renyi, 1981; Ross, Ogren & Renyi, 1976; Ross & Renyi, 1977) was used to produce an increased availability of central 5-HT. Results indicated that short-term treatment with zimelidine produced an attenuation of voluntary ethanol consumption in rats (Rockman et al., 1979a). Treatment with zimelidine was shown not to effect body weight or total fluid intake. Since ethanol has aversive tasting properties (Wilson, 1972) the possibility that zimelidine somehow induced an increased sensitivity to the taste of ethanol, thereby, reducing ethanol intake was examined. Additional animals ingesting an aversive tasting quinine-sucrose solution were treated with zimelidine in a similar manner as the ethanol drinking animals (Rockman et al., 1979a). The results indicate that zimelidine did not alter the consumption of the quinine-sucrose solution, suggesting that the zimelidine-induced

attenuation of ethanol intake was not a result of an increased sensitivity to an aversive tasting liquid. It was also considered that zimelidine may somehow interfere with the metabolism of ethanol which may then account for the alteration of ethanol intake. However, since other investigators have determined that zimelidine does not alter the rates of metabolism of ethanol and acetaldehyde (Rydeberg, 1979; Lindros, 1978; personal communications), alterations of ethanol metabolism does not seem to be a causal factor. Based on these data it was suggested that zimelidine attenuated ethanol consumption as a consequence of a manipulation of the neurochemical process which may mediate the positive reinforcing properties of ethanol (Rockman et al., 1979a). Therefore, these data served to implicate 5-HT in the mediation of ethanol self-administration.

A subsequent study was designed to further investigate the notion, that zimelidine, as a result of 5-HT uptake blockade, may diminish the positive reinforcing properties of ethanol (Rockman et al., 1979a). This experiment was based on the view that when a behavior is maintained by positive reinforcement and that

reinforcing factor is removed, while responding continues, the result would be the elimination of the behavior (extinction) followed by the reacquisition of the particular response when reinforcement is reinstated (Mackintosh, 1974; Thompson & Pickens, 1975). In order to fulfill the requirements of an extinction paradigm, ethanol was presented to the animals in a forced-choice situation in combination with zimelidine treatment (Rockman et al., 1979a). The results indicated that such treatment significantly reduced ethanol intake when ethanol was once again available in a free-choice with water without drug treatment. The reduction in ethanol intake and subsequent reacquisition of ethanol drinking behavior suggests that zimelidine treatment can effectively extinguish the ethanol drinking response. The data serve to suggest that zimelidine, via 5-HT uptake blockade, may alter the neurochemical mediation of ethanol self-administration (Rockman et al., 1979a).

Based on the literature review in the previous section, it seems possible that zimelidine-induced attenuation of ethanol intake may be a function of an interaction between central 5-HT and NE neurons.

Since zimelidine causes an increased availability of 5-HT in synaptic gaps via uptake blockade (De Montigny, Blier, Caille & Kouassi, 1981; Ogren et al., 1981), the resulting surplus of 5-HT could invade NE neurons (Shaskan & Snyder, 1970; Snyder et al., 1973). Therefore, it was proposed that if the invasion of surplus 5-HT into NE neurons was somehow prevented, the effectiveness of zimelidine in reducing ethanol intake may be diminished (Rockman et al., 1979b). In an attempt to prevent this proposed invasion, ethanol preferring animals were pretreated with desmethylimipramine (DMI), a selective neuronal NE uptake blocker (Carlsson et al., 1969; Javaid et al., 1979), prior to treatment with zimelidine. The results suggested that pretreatment with DMI prior to administration of zimelidine, partially attenuated the zimelidine-induced suppression of ethanol intake (Rockman et al., 1979b). These preliminary results indicated that the attenuation of ethanol intake produced by zimelidine may be partially due to a 5-HT-induced functional depletion of NE.

The Present Investigation

As mentioned previously, the data emanating from studies designed to investigate the possible role of 5-HT in the mediation of ethanol self-administration are at times contradictory. In addition, due to the preliminary nature of the data concerning 5-HT uptake blockade and ethanol intake, the mechanism by which 5-HT uptake blockade reduces ethanol consumption is largely unclear. Therefore, it seems necessary to further investigate the role of 5-HT uptake blockade in ethanol intake. The present series of experiments was undertaken to investigate two major issues. The first goal was to identify some specific attributes of zimelidine which may account for the reduction of ethanol intake. Secondly, this thesis was designed to examine the neuropharmacological mechanism of action of zimelidine with regard to the suppression of ethanol intake. Since norzimelidine, the primary active metabolite of zimelidine (Ross & Renyi, 1977; Ogren et al., 1981), has been shown to be a potent inhibitor of 5-HT uptake, it is possible that the effects of zimelidine may actually be

a result of the action of norzimelidine. Therefore, the first experiment was designed to evaluate the effect of norzimelidine on voluntary ethanol consumption. In addition, this experiment was also designed to investigate the relative importance of uptake blockade specificity in the suppression of ethanol intake. This was accomplished by comparing the effects of specific and non-specific inhibitors of 5-HT and NE uptake on ethanol consumption. Subsequent experiments of this thesis were designed to investigate the neuropharmacological mechanism of action of zimelidine and norzimelidine with regard to ethanol intake. The possibility of both a functional depletion of NE and/or enhanced 5-HT post-synaptic transmission as causal factors in the attenuation of ethanol intake were evaluated. Experiment 2 was designed to examine whether norzimelidine, as a consequence of 5-HT uptake blockade, induces a functional depletion of NE, thereby, reducing ethanol intake. In an attempt to block this proposed reduction in NE activity, ethanol preferring animals were pretreated with DMI, a specific NE uptake inhibitor (Javaid et al., 1979) prior to treatment with norzimelidine. The third

experiment was designed to further investigate the notion that both zimelidine and norzimelidine reduce ethanol intake as a result of an indirect reduction of NE activity. In an attempt to counteract this proposed reduction of NE activity, ethanol preferring animals were pretreated with clonidine, an adrenergic receptor agonist (Anden, Corrodi, Fuxe, Hokfelt, Hokfelt, Rydin & Svensson, 1970) prior to treatment with zimelidine and norzimelidine. In the final experiment, the role of enhanced 5-HT post-synaptic transmission as a causal factor in the attenuation of ethanol intake by zimelidine and norzimelidine was examined. If 5-HT uptake blockade reduces ethanol intake as a result of increasing 5-HT post-synaptic activity, then artificially reducing 5-HT post-synaptic activity in combination with 5-HT uptake inhibition should alter the reduction in ethanol intake. Therefore, in Experiment 4, pretreatment with methergoline, a 5-HT post-synaptic receptor blocker (Fuxe, Ogren, Agnati & Jonsson, 1978) was used in an attempt to reduce 5-HT post-synaptic activity prior to treatment with zimelidine and norzimelidine.

The present investigation was designed to

examine the neuropharmacological mechanism of action of 5-HT uptake blockade in the suppression of voluntary ethanol intake. It was our view that an understanding of the neuropharmacological mechanism of 5-HT uptake blockade would contribute to our knowledge of the neurochemical processes which underly and mediate ethanol self-administration.

Experiment 1

In an attempt to investigate the role of 5-HT uptake blockade in ethanol consumption, the present study was designed to determine whether other 5-HT uptake inhibitors would attenuate voluntary ethanol consumption in a manner similar to that demonstrated with zimelidine. Furthermore, the relative importance of uptake blockade specificity was evaluated through comparisons of specific 5-HT uptake inhibitors, a relatively non-specific inhibitor of both 5-HT and NE uptake and specific NE uptake inhibition on ethanol consumption. Included in the group of selective 5-HT uptake inhibitors, norzimelidine, the primary active metabolite of zimelidine (Ogren et al., 1981; Ross & Renyi, 1977), was evaluated as to its effect on voluntary ethanol intake. Since norzimelidine is also a potent inhibitor of 5-HT uptake (Ogren et al., 1981), the possibility that norzimelidine accounts for the reduction of ethanol intake observed following treatment with zimelidine was examined.

Method

Subjects

Subjects were male Wistar rats (Canadian Breeding Farm Laboratories Ltd.), weighing between 200-250 g at the beginning of the experiment. All subjects were individually housed in stainless steel cages, in a room regulated for constant temperature and humidity and a 12 hour lights-on schedule. Drinking solutions were presented in calibrated Richter tubes, mounted on the front of the cage. Purina Rat chow was available ad libitum.

Procedure

Ethanol screening. The ethanol used in this study was 95% ethyl alcohol (ETOH) diluted with tap water to form concentrations ranging from 3 to 11% (v/v).

Animals were presented with a free choice between ETOH and water every alternate day, commencing with a 3% ETOH solution. The position of the Richter tubes was alternated with each ethanol presentation. The ETOH concentrations were increased by 2% when 50% or more of the total daily fluid was consumed as ethanol. Once drinking of an 11% (v/v) ethanol solution was established

the animals were then switched to a schedule consisting of an everyday free choice between water and ethanol. All animals were maintained on this schedule throughout the remaining phases of the experiment. Only those animals whose ETOH consumption was 50% or more of their total daily intake were included in the experiment.

Baseline Period

This period consisted of an everyday free choice between ethanol and water. After a 5-day baseline measure was attained, animals were randomly divided into drug treatment and control groups.

Injection Period

During the injection period, the groups received 1 intraperitoneal injection per day for 5 consecutive days at approximately 15:00 hr. The drugs and dosages used in this study are shown in Table 1. The 5-HT uptake inhibitors were the following. Fluoxetine hydrochloride (Lilly, Lemberger, Rowe, Carmichael, Crabtree, Horng, Bymaster & Wong, 1978), norzimelidine dihydrochloride (Astra LaKemedel AB, Ross & Renyi, 1977), Lu-10-171 (Lundbeck & Co., Hyttel, 1977) and alaproclate (Lindberg, Thorberg, Bentsson, Renyi,

Table 1

Dosage and neuropharmacological actions of uptake inhibitors injected I.P. in Experiment 1

Drug	Dose (mg/kg)	Action
fluoxetine	10 + 15	5-HT uptake blockade
norzimelidine	10 + 20	5-HT uptake blockade
lu-10-171	10 + 15	5-HT uptake blockade
alaproclate	25 + 30	5-HT uptake blockade
amitriptyline	5 + 10	5-HT + NE uptake blockade
desmethylimipramine (DMI)	2.5 + 5	NE uptake blockade
doxepin	5 + 15	NE uptake blockade

Ross & Ogren, 1978, Astra LaKemedel AB). Inhibitor of both 5-HT and NE uptake: amitriptyline hydrochloride (Schering Corp., Fielding & Lal, 1975; Tuomisto, Tukiainen, Voutilainen & Tuomainen, 1980). Selective inhibitors of NE uptake: desmethylimipramine (DMI, CIBA, Javaid et al., 1979) and doxepin hydrochloride (Pfizer; Fielding & Lal, 1975). The drug doses used in this experiment were previously determined in this laboratory to be the highest effective dose causing no effect on body weight and water consumption. All drugs were dissolved in Ringer's solution and were injected in volumes of 1 or 2 ml/kg. Ringer's control animals received equivalent volumes of the vehicles. Free choice ethanol consumption was monitored during the injection period and for an additional 5 days thereafter.

Results

Ethanol consumption was calculated both in terms of daily ethanol preference (mean per cent of total daily fluid intake) and in terms of daily absolute amount of ethanol ingested (mean grams per kilogram). The data were analysed using a two-way analysis of variance (group x period) and

post hoc Tukey tests.

Figure 1 illustrates the ethanol consumption for the groups treated with fluoxetine. The analysis revealed a significant group x period interaction ((Ethanol preference: $F(4,30) = 9.33$, $p<.001$; Absolute ethanol intake: $F(4,30) = 8.25$, $p<.001$)). Post hoc Tukey tests revealed that during the injection period fluoxetine (10 and 15 mg/kg) produced an attenuation of ethanol consumption both in terms of ethanol preference ($p<.01$) and absolute ethanol intake ($p<.01$) when compared to baseline and control levels. During the post-injection period, ethanol intake for both experimental groups remained below control and baseline levels ($p<.01$).

Ethanol consumption following treatment with norzimelidine is shown in Figure 2. The analysis yielded a significant group x period interaction ((Ethanol preference: $F(4,30) = 8.79$, $p<.001$; Absolute ethanol intake: $F(4,30) = 8.35$, $p<.001$)). Treatment with norzimelidine (10 mg/kg) produced a significant reduction in ethanol preference ($p<.05$) and absolute ethanol intake ($p<.01$) when compared to baseline and control levels. Norzimelidine (20 mg/kg) treatment attenuated ethanol preference to

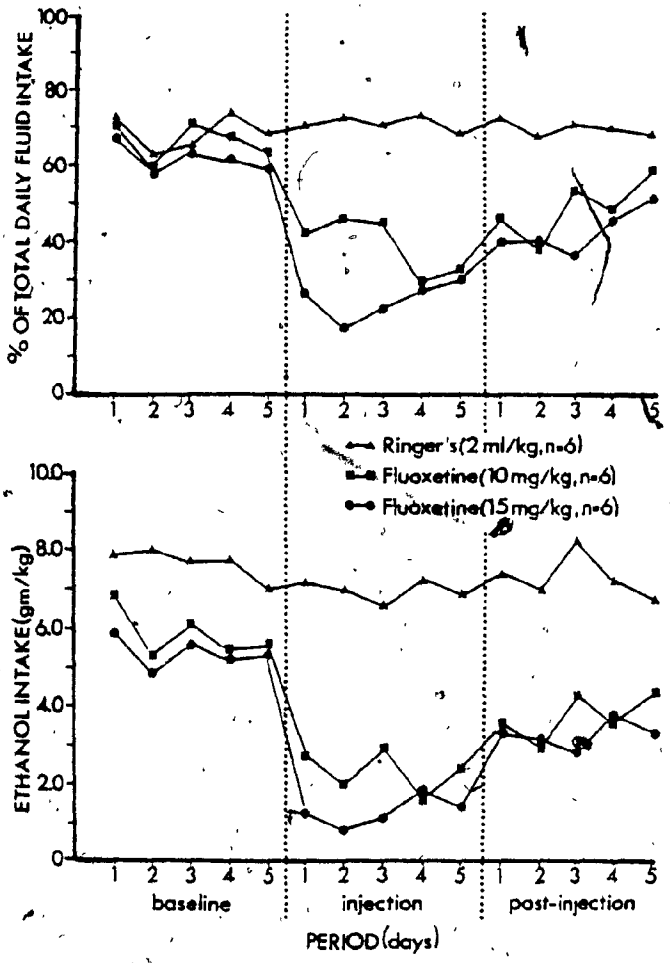

Figure 1 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats treated with Fluoxetine (10 + 15 mg/kg, i.p.) and Ringer's solution (2 ml/kg, i.p.).

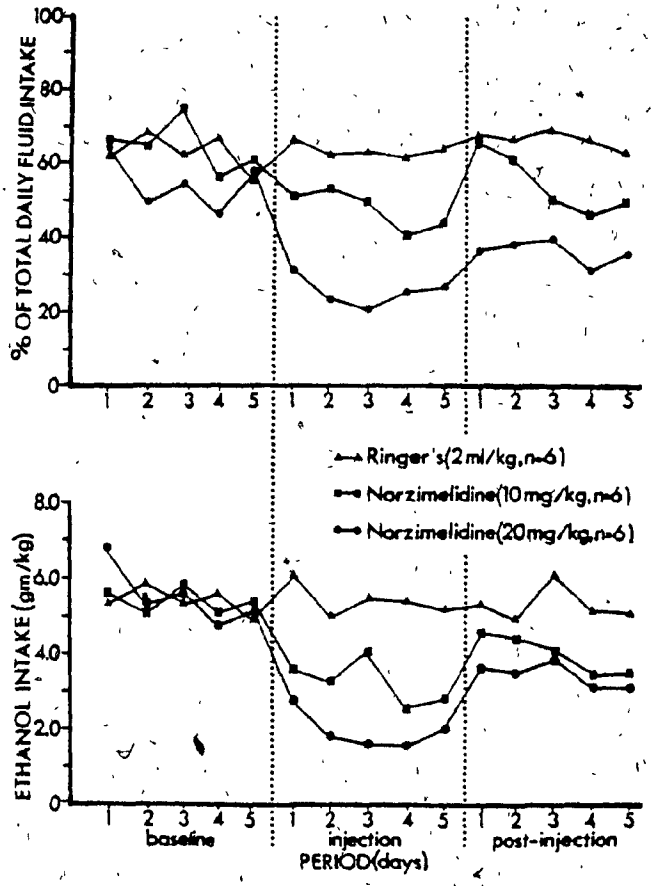

Figure 2 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats treated with Norzimelidine (10 + 20 mg/kg, i.p.) and Ringer's solution (2 ml/kg, i.p.).

a great extent ($p<.01$) than 10 mg/kg of norzimelidine. In terms of absolute ethanol intake, differences between the effectiveness of these doses of norzimelidine approached significance ($p>.05$). In the post-injection period, animals that were treated with 20 mg/kg of norzimelidine increased their ethanol consumption compared to injection period levels ($p<.05$).

Similarly, treatment with lu-10-171 produced an attenuation of ethanol consumption as illustrated in Figure 3. A group x period interaction was evident ((Ethanol preference: $F(4,36) = 5.65$, $p<.002$; Absolute ethanol intake: $F(4,36) = 6.42$, $p<.001$)). Post hoc Tukey tests revealed that treatment with lu-101-171 (10 + 15 mg/kg) similarly attenuated ethanol preference and absolute ethanol intake when compared to baseline and control levels ($p<.01$). During the post-injection period ethanol consumption remained below baseline and control levels ($p<.05$).

As illustrated in Figure 4, treatment with alaproclate produced a decrease in ethanol consumption (group x period interaction; (Ethanol preference: $F(4,34) = 2.60$, $p<.05$; Absolute

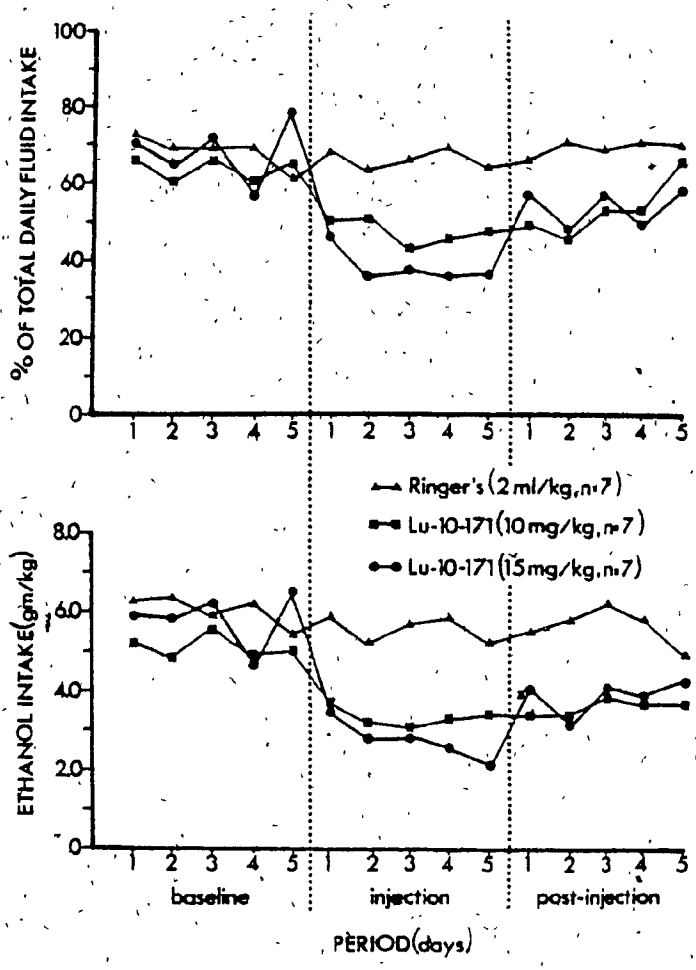

Figure 3 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats treated with Lu-10-171 (10 + 15 mg/kg, i.p.) and Ringer's solution (2 ml/kg, i.p.).

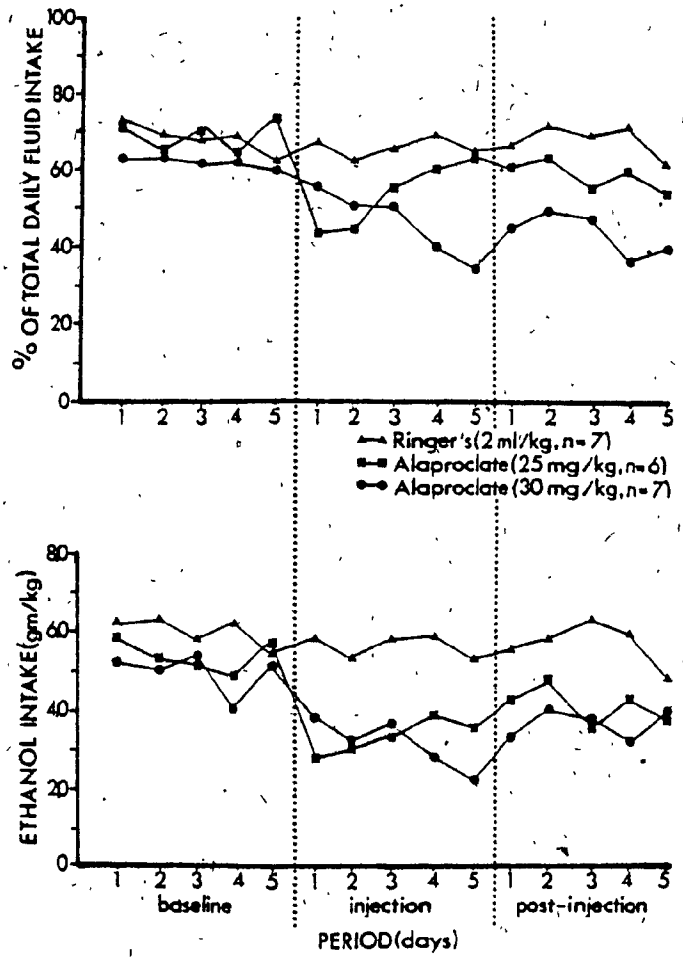

Figure 4 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats treated with Alaproclate (25 + 30 mg/kg, i.p.) and Ringer's solution (2 ml/kg, i.p.).

ethanol intake: $F(4,34) = 3.64$, $p<.02$)).
During the injection period alaproclate (25 mg/kg) attenuated ethanol consumption only in terms of absolute ethanol intake ($p<.01$) in relation to control and baseline levels. Alaproclate (30 mg/kg) caused a decrease in ethanol consumption both in terms of ethanol preference ($p<.05$) and absolute ethanol intake ($p<.01$). Ethanol consumption for the group treated with 30 mg/kg of alaproclate remained significantly below control and baseline levels during the post-injection period ($p<.05$).

Treatment with amitriptyline, DMI and doxepin as shown in Figures 5, 6 and 7 respectively, did not affect ethanol consumption. The analysis revealed no group x period interaction. (Amitriptyline: (Ethanol preference: $F(4,34) = 1.38$, $p>.05$; Absolute ethanol intake: $F(4,34) = 1.49$, $p>.05$); DMI (Ethanol preference: $F(4,28) = 1.16$, $p>.05$; Absolute ethanol intake: $F(4,28) = 1.84$, $p>.05$); Doxepin (Ethanol preference: $F(4,32) = 2.16$, $p>.05$; Absolute ethanol intake: $F(4,32) = .526$, $p>.05$)).

In general, it should be noted that in each of the above experiments control animals maintained a consistent intake of ethanol drinking in all phases

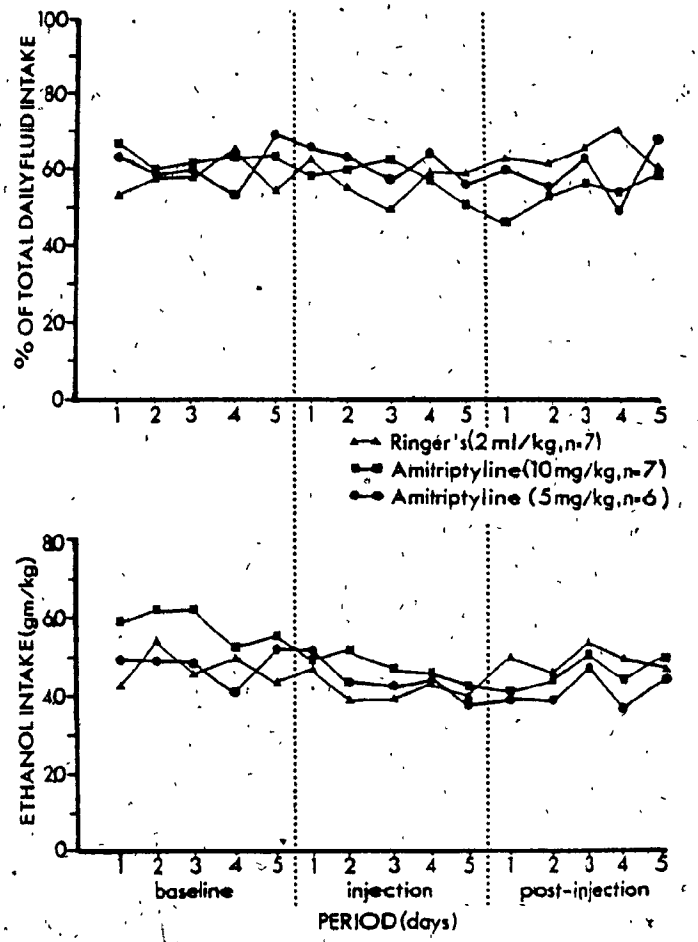

Figure 5 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats treated with Amitripytline (5 + 10 mg/kg, i.p.) and Ringer's solution (2 ml/kg, i.p.).

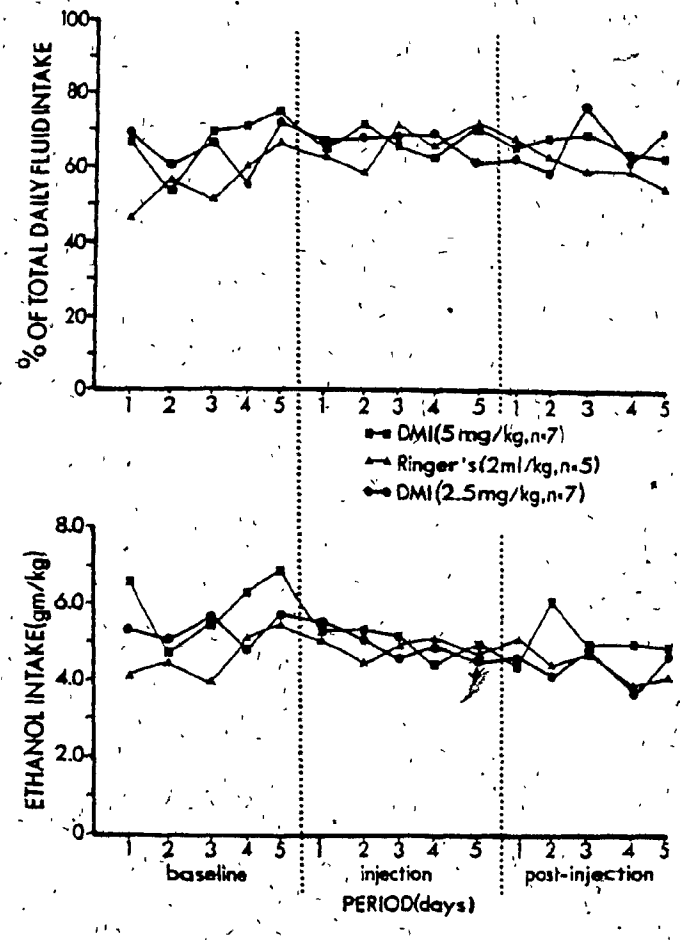

Figure 6 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats treated with DMI (2.5 + 5 mg/kg, i.p.) and Ringer's solution (2 ml/kg, i.p.).

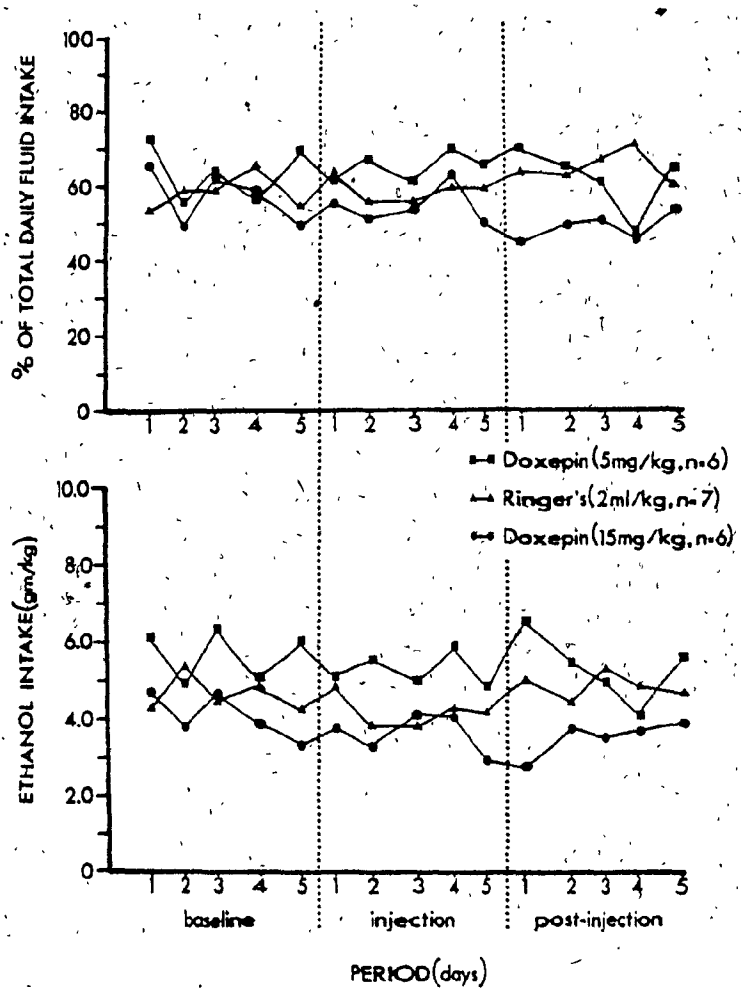

Figure 7 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats treated with Doxepin (5 + 15 mg/kg, i.p.) and Ringer's solution (2 ml/kg, i.p.).

of the experiment. Furthermore, none of the drug treatments administered in the experiment produced reductions in body weight or total fluid intake.

Discussion

The present study revealed that those drugs which have been shown to specifically inhibit 5-HT uptake (fluoxetine, norzimelidine, lu-10-171 and alaproclate) produced a significant attenuation of ethanol consumption. Conversely, those drugs that do not specifically inhibit 5-HT uptake, did not affect ethanol consumption. Amitriptyline, which moderately inhibits both 5-HT and NE uptake had no affect on voluntary ethanol consumption. Similarly, DMI and doxepin, both selective inhibitors of NE uptake, did not alter ethanol consumption.

These results are consistent with reports indicating that increasing the availability of central 5-HT, via treatment with either 5-hydroxytryptophan, 5-HT or 5-HT uptake inhibitors, produced reductions of ethanol intake (Geller, 1973; Hill, 1974; Rockman et al., 1979a; Zabik et al., 1978). These results considered together suggest that increasing the central availability

of 5-HT at the level of the synapse may alter the mediation of the positive reinforcing properties of ethanol.

One of the most interesting findings of the present experiment involves the norzimelidine--induced attenuation of ethanol intake. Norzimelidine, the primary active metabolite of zimelidine, has been shown to be a potent selective inhibitor of 5-HT uptake. In fact, norzimelidine appears to be considerably more potent than zimelidine (Ogren et al., 1981; Ross & Renyi, 1977; Siwers, Ringberger, Tuck & Sjoqvist, 1977). However, both zimelidine and norzimelidine appear to be equally effective in reducing ethanol intake. Therefore, the data suggests that although specificity of 5-HT uptake blockade seems to be an important factor in the mechanism underlying the reduction of ethanol intake, increased potency does not result in an increased attenuation of ethanol intake. In other words, norzimelidine may be creating a "floor effect" in the attenuation of ethanol intake, in that the increased potency does not further suppress ethanol consumption.

Finally, since norzimelidine is the primary active metabolite of zimelidine, the data suggest

that the effect of zimelidine on ethanol intake may in fact be due to the action of norzimelidine.

The possibility that norzimelidine like zimelidine (Rockman et al., 1979b) produces indirectly a functional depletion of NE, thereby reducing ethanol intake, is examined in Experiment 2.

Experiment 2

As previously mentioned, the data concerning the relationship between central 5-HT and ethanol self-administration is largely contradictory (e.g. Myers & Melchior, 1977a). The exception to this confused relationship is the observation that increasing central 5-HT availability results in a reduction of ethanol intake. On the other hand, experimental evidence strongly suggests that central NE plays a major role in the mediation of ethanol self-administration (e.g. Amit et al., 1977). These data lead one to speculate as to whether some interaction between increased 5-HT availability and NE activity may account for the effects of 5-HT uptake blockade on ethanol intake. This relationship between 5-HT and NE activity is supported by the anatomical, biochemical and pharmacological findings which suggest an interaction between 5-HT and NE neurons in the brain (Baraban & Aghajanian, 1981; Pickel et al., 1977; Samanin & Garattini, 1975). More specifically, it has been demonstrated, that due to the similarities of 5-HT and NE uptake processes, 5-HT may be taken up into NE neurons (Iversen,

1975; Shaskan & Snyder, 1970; Snyder et al., 1973; Thoa et al., 1969). Thus, it has been reported that an increase in the availability of central 5-HT may result in the invasion of NE neurons by surplus 5-HT via the NE uptake mechanism. It was further suggested that once located in NE neurons, the accumulated 5-HT could act as a false neurotransmitter within the NE system and subsequently cause a functional depletion of NE (Shaskan & Snyder, 1970; Snyder et al., 1973; Thoenen & Tranzer, 1971; Tranzer et al., 1969).

The possibility that zimelidine attenuated ethanol intake as a result of an interaction between surplus 5-HT and NE neurons was examined in a recent study (Rockman et al., 1979b). In an attempt to prevent the proposed invasion of NE neurons, ethanol preferring animals were pretreated with desmethylimipramine (DMI; 5 mg/kg), an NE uptake inhibitor, prior to treatment with zimelidine. The results revealed that pretreatment with DMI prior to the administration of zimelidine, partially attenuated the zimelidine-induced suppression of ethanol intake. Therefore, it was suggested that the observed attenuation of ethanol drinking following

treatment with zimelidine may be partially due to a functional depletion of NE (Rockman et al., 1979b).

In light of the data from Experiment 1, it seems quite possible that the effect of zimelidine on ethanol intake may in fact be due to the action of norzimelidine. Therefore, this experiment explored the possibility that norzimelidine induced attenuation of ethanol intake is a result of an interaction between surplus 5-HT and NE neurons. In the present experiment ethanol preferring rats were pretreated with DMI (5 and 7.5 mg/kg) prior to treatment with norzimelidine.

Method

Subjects

Subjects were naive male Wistar rats (Canadian Breeding Farm Laboratories Ltd.) weighing between 200-250 g at the beginning of the experiment. All subjects were housed under the same conditions as those described in Experiment 1.

Procedure

Naive male Wistar rats were presented with a free choice between water and increasing

concentrations of ethanol according to procedures described in Experiment 1. The design of this experiment was similar to Experiment 1 with the exception of the injection period.

Injection Period

During the injection period, animals received one intraperitoneal injection per day for 5 consecutive days at approximately 15:00 hr. Group 1 (DMI-norzimelidine 5 mg/kg; 20 mg/kg, n = 5) received pretreatment with desmethylimipramine (DMI, 5 mg/kg, Ciba Co. Ltd.) followed 30 minutes later by injections of norzimelidine (20 mg/kg). Similarly, Group 2 (DMI-norzimelidine 7.5 mg/kg; 20 mg/kg, n = 5) was treated with 7.5 mg/kg of DMI 30 minutes prior to norzimelidine (20 mg/kg) treatment. DMI and norzimelidine were dissolved in Ringer's solution and injected in volumes of 2 ml/kg. The doses of DMI chosen were previously determined in this laboratory, to be the highest effective dose shown not to produce effects on body weight and fluid intake. Group 3 (DMI, 5 mg/kg, n = 5) and Group 4 (DMI, 7.5 mg/kg, n = 5) received injections of DMI alone in doses of 5 and 7.5 mg/kg respectively. Group 5 (norzimelidine, 20 mg/kg, n = 5) was injected with Ringer's

solution (2 ml/kg) prior to norzimelidine (20 mg/kg). Group 6 (Ringer's, 2 ml/kg, n = 5) was injected with Ringer's solution in a volume of 2 ml/kg. Approximately 4 hr following the injections, fluid consumption of the previous 24 hr was measured and tubes were refilled and replaced. For 5 days following the injection period, the consumption of ethanol in a free-choice with water was measured without drug treatments.

Results

Ethanol consumption was calculated both in terms of daily ethanol preference (mean percent of total daily intake) and in terms of daily absolute ethanol ingested (mean grams per kilogram). The data were analysed using a two-way analysis of variance (group x period) and post hoc Tukey tests. In the benefit of clarity, the data are represented in two figures. Figure 8 illustrates the ethanol consumption for the animals pretreated with DMI (5 mg/kg) followed by injections of norzimelidine (20 mg/kg). The analysis yielded a significant group x period interaction ((Ethanol preference: $F(6,32) = 14.15$, $p<.001$; Absolute ethanol intake: $F(6,32) = 8.02$, $p<.001$)). Post hoc tests (Tukey)

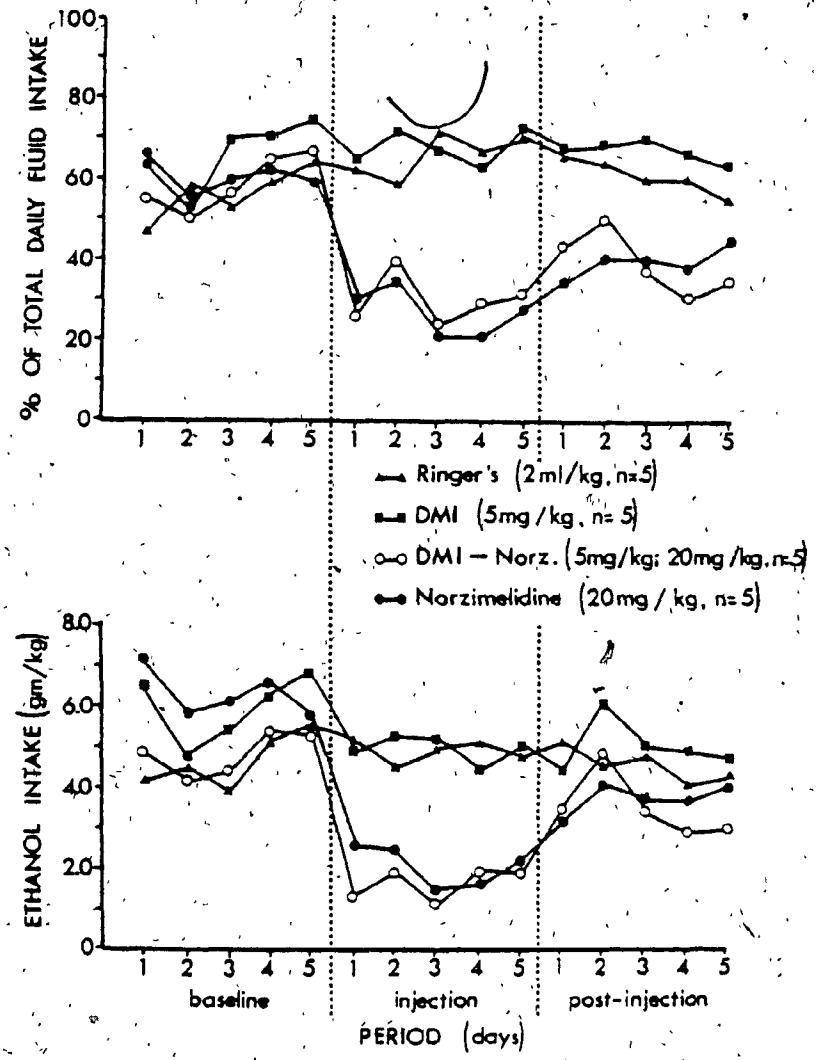

Figure 8 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats pretreated with DMI (5 mg/kg, i.p.) prior to treatment with norzimelidine (20 mg/kg, i.p.).

revealed that during the injection period norzimelidine (20 mg/kg) significantly attenuated ethanol consumption both in terms of ethanol preference ($p<.01$) and absolute ethanol intake ($p<.01$) when compared to baseline and control levels. More importantly, the group treated with DMI prior to norzimelidine treatment also reduced their ethanol intake ($p<.01$) in relation to control and baseline levels. Ethanol consumption of this group did not differ from the group treated with norzimelidine alone ($p>.05$). During the post-injection period both these groups significantly increased their preference for ethanol ($p<.01$) and absolute ethanol intake ($p<.05$) in relation to consumption during the injection period. Control animals treated with either DMI (5 mg/kg) or Ringer's solution did not alter their ethanol intake throughout the experiment ($p>.05$).

Figure 9 illustrates ethanol consumption for the group pretreated with DMI (7.5 mg/kg) prior to norzimelidine (20 mg/kg) treatment. A two-way analysis of variance yielded a significant group x period interaction ((Ethanol preference: $F(6,32) = 35.15$, $p<.001$; Absolute ethanol intake: $F(6,32) = 17.78$, $p<.001$). Post hoc Tukey tests

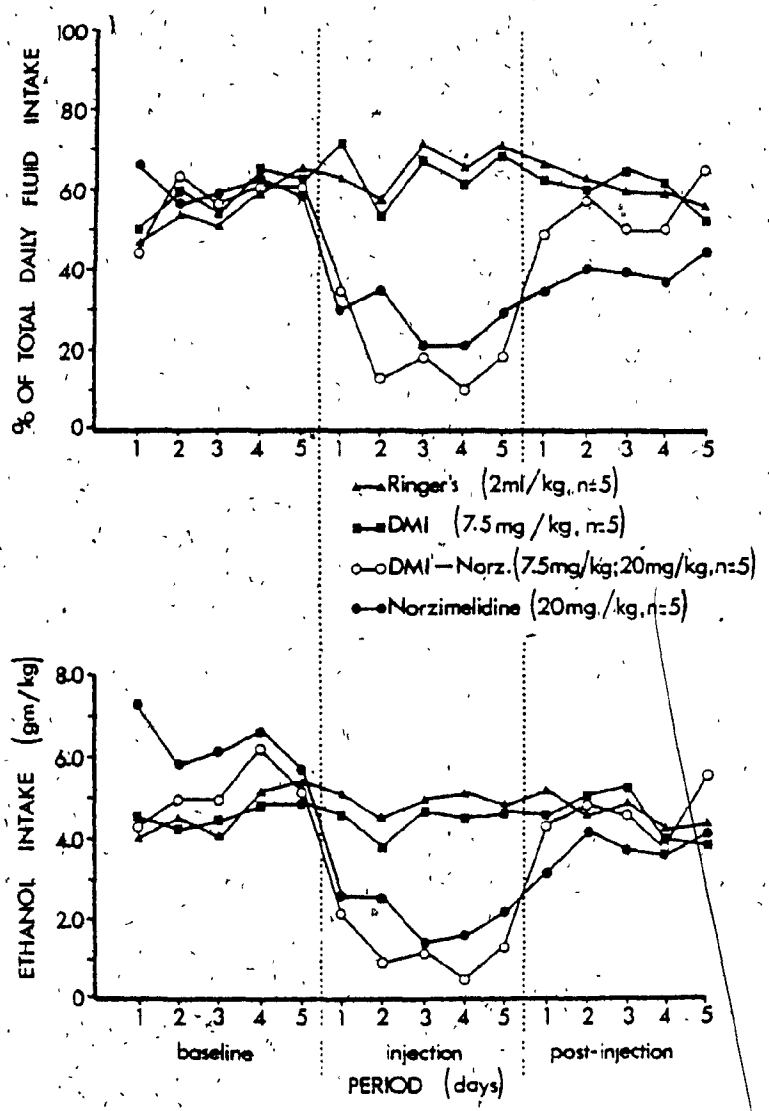

Figure 9 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats pretreated with DMI (7.5 mg/kg, i.p.) prior to treatment with norzimelidine (20 mg/kg, i.p.).

reveal that although groups treated with DMI (7.5 mg/kg) prior to norzimelidine and norzimelidine alone reduced their ethanol intake ($p<.01$), pretreatment with DMI did not alter the norzimelidine induced attenuation of ethanol intake ($p>.05$). Both these groups significantly increased their ethanol intake during the post-injection period ($p<.01$). Control animals treated with DMI (7.5 mg/kg) or Ringer's solution (2 ml/kg) did not alter their ethanol intake throughout the experiment ($p>.05$). In addition, none of the drug treatments administered in the experiment produced reductions in body weight or total fluid intake.

Discussion

The results of this study indicated that pretreatment with DMI (5 and 7.5 mg/kg) did not alter norzimelidine induced attenuation of ethanol intake. As previously mentioned, it has been suggested that a surplus of central 5-HT may invade central NE neurons via the NE uptake processes, causing a functional depletion of NE (e.g. Shaskan & Snyder, 1970; Snyder et al., 1973). Since, as mentioned earlier, norzimelidine is a

potent central 5-HT uptake inhibitor, the resulting increased availability of 5-HT in the synaptic gap could subsequently be taken up into NE neurons. Since it has been proposed that the NE uptake processes may be responsible for the accumulation of surplus 5-HT, blocking NE uptake via DMI pretreatment, should theoretically eliminate the invasion of 5-HT into NE neurons. However, the results of the present study do not support this view. Since pretreatment with DMI did not alter norzimelidine-induced attenuation of ethanol intake, it is suggested that the observed attenuation of ethanol drinking following norzimelidine treatment does not seem to be a result of a 5-HT induced functional depletion of NE. It is important to note that these results are in sharp contrast to those obtained with zimelidine (Rockman et al., 1979b). The discrepancy between these results may lead to some speculations. In an attempt to understand the results of the present study, it seems possible that norzimelidine may reduce ethanol intake by some other mechanism of action rather than by an interaction with brain NE. However, it is not clear as to why the mechanism of action of norzimelidine as effecting ethanol

intake should differ from that of zimelidine. Since both drugs are inhibitors of 5-HT uptake, one would expect similar interactions with brain NE, if in fact, such an interaction occurs. Hence, a re-examination of the notion of a functional depletion of NE as a result of zimelidine and norzimelidine treatment is presented in the following study.

Experiment 3

Based on the results from the previous experiment, it was necessary to determine whether increasing 5-HT availability, via 5-HT uptake blockade, may result in an invasion by surplus 5-HT into NE neurons, thereby attenuating ethanol intake. Since it has been suggested that increasing 5-HT availability results in a reduction of NE activity (Everett, 1974; Snyder et al., 1973; Thoa et al., 1969), a procedure which could counteract this reduced activity is desirable. Pharmacological and behavioral data indicate that clonidine, a noradrenergic receptor agonist (Anden et al., 1970) can effectively reverse some behavioral consequences of reduced NE activity as produced by inhibition of dopamine-beta-hydroxylase, the enzyme necessary for the conversion of dopamine to NE (Freedman, Backman & Quartermain, 1979). Therefore, clonidine, by virtue of its noradrenergic stimulating properties seems capable of counteracting a reduction of NE activity. The present study was designed to examine the effect of clonidine pretreatment on the zimelidine and norzimelidine-induced attenuation of ethanol intake.

Method

Subjects

Subjects were naive male Wistar rats (Canadian Breeding Farm Laboratories Ltd.) weighing between 200-250 g at the beginning of the experiment. All subjects were housed under the same conditions as those described in Experiment 1.

Procedure

The rats participating in this experiment were presented with a free choice between water and increasing concentrations of ethanol according to procedures described in Experiment 1. The design of this experiment was similar to Experiment 1 with the exception of the injection period.

Injection Period

During the 5-day injection period, animals were pretreated with daily intraperitoneal injections (at approximately 17:00 hr) of clonidine hydrochloride (.25 mg/kg; n = 5, Boehringer Ltd.) dissolved in Ringer's solution (.25 mg/ml) 30 minutes prior to treatment with zimelidine (20 mg/kg; n = 5) and norzimelidine (20 mg/kg; n = 5). Dose of clonidine chosen was the highest effective dose previously determined

not to alter fluid intake or body weight. Control animals received injections of either Ringer's (2 mg/kg; n = 5) prior to norzimelidine (20 mg/kg; n = 5) and zimelidine (20 mg/kg; n = 5), clonidine (.25 mg/kg; n = 5) or Ringer's solution (2 ml/kg; n = 5). For 5 days following the injection period free choice ethanol intake was monitored.

Results

As in the previous studies ethanol consumption was calculated both in terms of daily ethanol preference and absolute amount of ethanol ingested. Figure 10 illustrates ethanol consumption for those animals pretreated with clonidine (.25 mg/kg) prior to zimelidine (20 mg/kg) treatment. A two-way analysis of variance revealed a significant group x period interaction ((Ethanol preference: $F(6,32) = 14.26$, $p<.001$; Absolute ethanol intake: $F(6,32) = 20.93$, $p<.001$)). Post hoc Tukey tests indicated that groups treated with clonidine prior to zimelidine and zimelidine alone both reduced their ethanol intake when compared to control and baseline levels ($p<.01$). However, during the injection period the clonidine-zimelidine group consumed ethanol to a greater degree than those

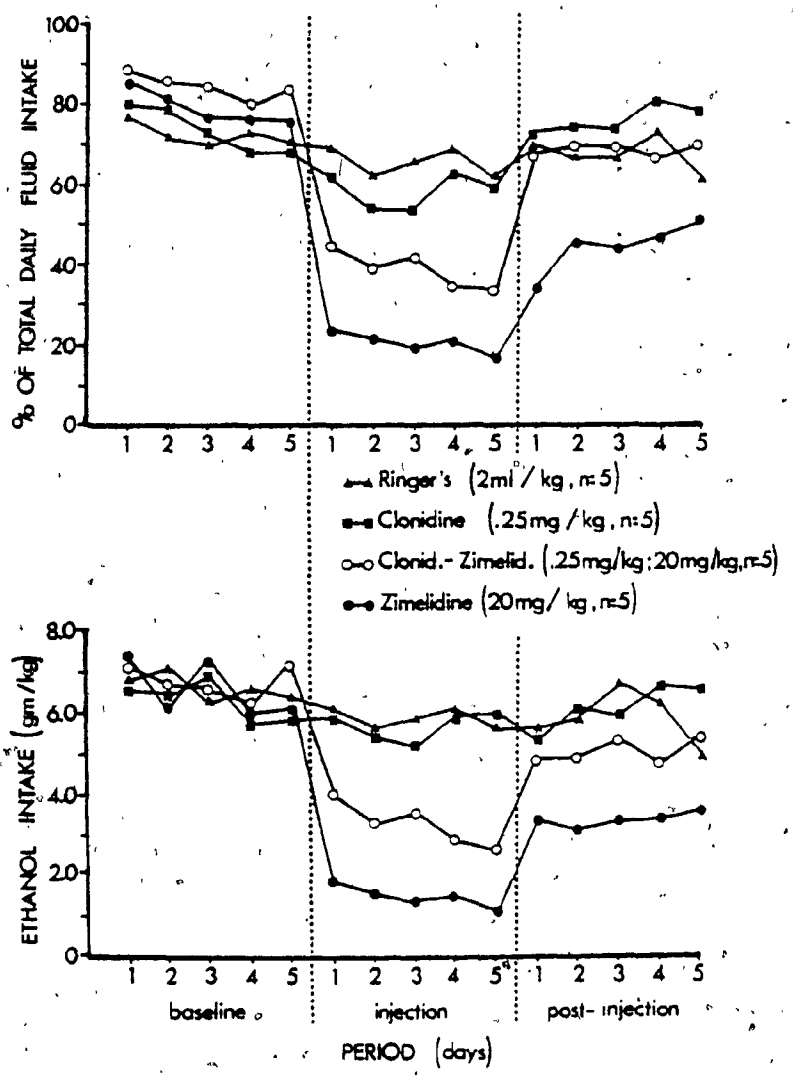

Figure 10 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats pretreated with clonidine (.25 mg/kg, i.p.) prior to zimelidine (20 mg/kg, i.p.) treatment.

animals treated with zimelidine alone (Ethanol preference, $p<.05$; Absolute ethanol intake, $p<.01$). In addition, during the post-injection period ethanol consumption of the clonidine-zimelidine group returned to baseline and control levels ($p>.05$), whereas ethanol consumption for the zimelidine treated group increased ($p<.01$) but remained below baseline and control levels ($p<.01$). Treatment with clonidine alone or injection of Ringer's solution did not alter ethanol intake ($p>.05$).

Figure 11 shows ethanol consumption for those animals pretreated with clonidine (.25 mg/kg) prior to norzimelidine (20 mg/kg) treatment. A two-way analysis of variance yielded a significant group x period interaction ((Ethanol preference: $F(6,32) = 11.86$, $p<.001$; Absolute ethanol intake: $F(6,32) = 10.74$, $p<.001$)). Post hoc Tukey tests revealed that the groups treated with clonidine prior to norzimelidine and norzimelidine alone both reduced their ethanol intake when compared to control and baseline levels ($p<.01$). More importantly, during the injection period no difference in ethanol intake existed between the clonidine-norzimelidine and norzimelidine alone

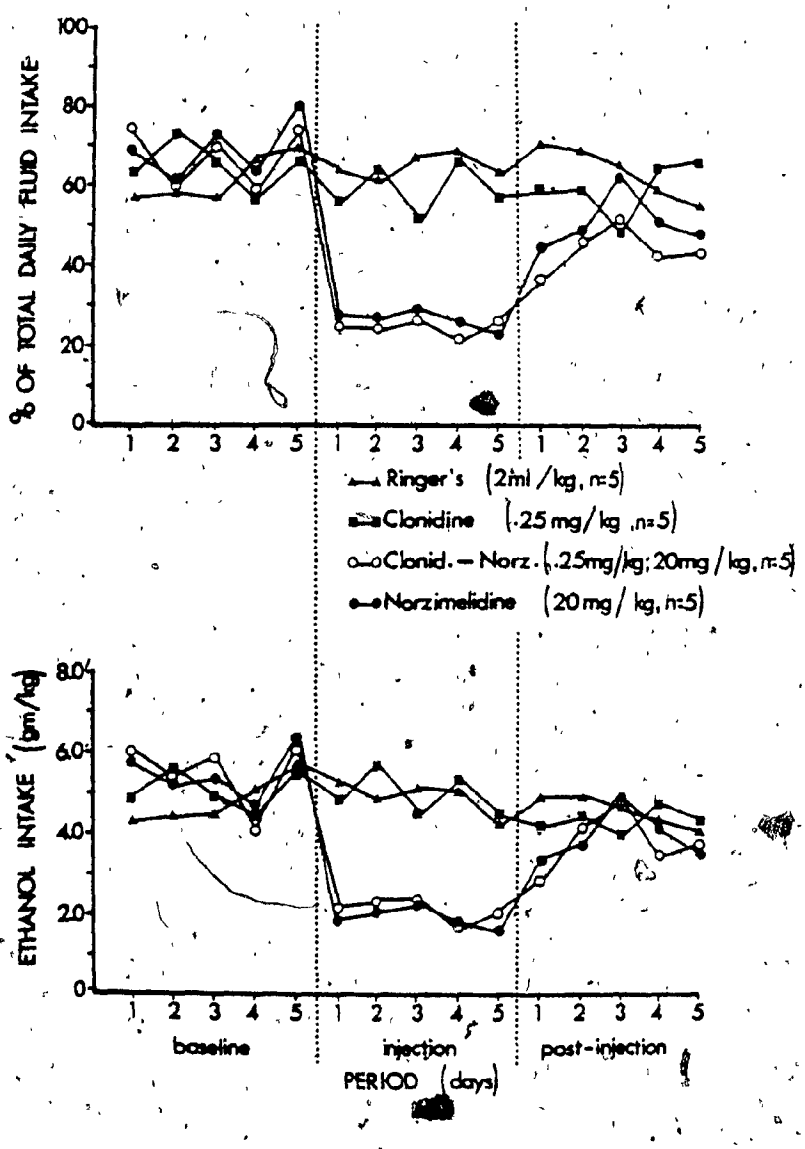

Figure 11 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats pretreated with clonidine (.25 mg/kg, i.p.) prior to norzimelidine (20 mg/kg, i.p.) treatment.

groups ($p>.05$). Following the termination of the injections, ethanol consumption for these two groups during the post-injection period returned to control levels ($p>.05$). Treatment with clonidine alone or injections of Ringer's solution did not alter ethanol consumption. In addition, it should be noted that all treatments during the injection periods did not alter body weight or total fluid intake.

Discussion

The results of this experiment demonstrated that pretreatment with clonidine, a noradrenergic agonist, significantly reduced the attenuation of ethanol intake produced by zimelidine. These data confirm the earlier report that treatment with a 5-HT uptake inhibitor may result in a 5-HT induced reduction of NE activity, producing the observed reductions in ethanol intake (Rockman et al., 1979b). These results support the notion that NE mediates ethanol self-administration (Amit et al., 1977; Davis et al., 1978, 1979). In contrast, pretreatment with clonidine prior to norzimelidine treatment does not alter the

norzimelidine-induced attenuation of ethanol intake. This result supports the findings of Experiment 2 in which it was demonstrated that attempts to either prevent or as in the present experiment, counteract the proposed 5-HT induced funcational depletion of NE, does not seem to alter the effectiveness of norzimelidine in reducing ethanol intake. Therefore, while zimelidine seems to reduce ethanol intake via an indirect reduction of NE activity, norzimelidine, however, seems to attenuate ethanol intake by some other yet unidentified mechanism. These data may lead one to some important speculations concerning the neuropharmacological mechanism of action of zimelidine and norzimelidine. It is of course possible, that the reduction of ethanol intake produced by zimelidine and norzimelidine are a result of effects on two separate systems. The possibility seems at first somewhat unlikely since norzimelidine is the active metabolite of zimelidine, and both compounds have been shown to inhibit 5-HT uptake (Ogren et al., 1981). Alternatively, it is possible that the degree of 5-HT uptake blockade may play an important role in the mechanism underlying the

norzimelidine-induced reduction of ethanol intake. Biochemical and pharmacological data demonstrate that norzimelidine is considerably more powerful an inhibitor of 5-HT uptake than zimelidine (Bertilsson, Tuck & Siwers, 1980; Ogren et al., 1981; Siwers et al., 1977). As has been previously mentioned, the direct effect of 5-HT uptake blockade is an increase in the availability of 5-HT in the synaptic gaps (Carlsson et al., 1969; Corrodi & Fuxe, 1968; Meek et al., 1970). As a consequence, an increased activity at 5-HT post-synaptic receptor sites in know to occur (Sangdee & Franz, 1979; Svensson et al., 1981). It is therefore possible that norzimelidine significantly enhances 5-HT transmission, and through this mechanism reduce ethanol intake.

The following experiment was designed to investigate the role of enhanced 5-HT post-synaptic transmission as a causal factor in the norzimelidine and zimelidine-induced attenuations of ethanol intake.

Experiment 4

In has been established that treatment with drugs that inhibit uptake of either 5-HT or NE cause an increased availability of the neurotransmitter in the synaptic gap (Anderson, 1972; Carlsson et al., 1969; Meek et al., 1970). As a result of 5-HT uptake blockade, an enhanced synaptic transmission and activity at post-synaptic receptor sites is known to occur (De Montigny et al., 1981; Fuller, 1980; Sangdee & Franz, 1979; Svensson et al., 1981). It is therefore possible, that the attenuation of ethanol intake produced by norzimelidine and zimelidine may be a result of an enhanced post-synaptic activity within the serotonergic system. The present study was designed in an attempt to counteract the proposed enhanced post-synaptic activity. This was accomplished by reducing 5-HT post-synaptic activity by pretreatment with methergoline, a 5-HT post-synaptic receptor blocker (Fuxe, Agnati & Everitt, 1975; Fuxe, Ogren, Agnati & Jonsson, 1978) prior to the administration of norzimelidine and zimelidine.

Method

Subjects

Subjects were male Wistar rats (Canadian Breeding Farm Laboratories Ltd.) weighing between 200-250 g at the beginning of the experiment. All subjects were housed under the same conditions as those described in Experiment 1.

Procedure

The rats participating in this experiment were presented with a free-choice between water and increasing concentrations of ethanol according to procedures described in Experiment 1. The design of this experiment was similar to Experiment 1 with the exception of the injection period.

Injection Period

During the 5-day injection period, animals were pretreated (approximately 15:00 hr) with daily intraperitoneal injections of methergoline (.5 or 1 mg/kg; n = 5, supplied by Astra LaKemedel AB) dissolved in a .01% ascorbic acid Ringer's solution 30 minutes prior to treatment with zimelidine (20 mg/kg, n = 5). Doses of methergoline were previously determined not to alter fluid intake or body weight. Similarly,

additional animals were pretreated with daily injections of methergoline (1 mg/kg, n = 5) prior to norzimelidine (20 mg/kg, n = 5). Control animals received either methergoline (.5 or 1 mg/kg), vehicle solution (2 ml/kg) prior to zimelidine (20 mg/kg, n = 5) and norzimelidine (20 mg/kg, n = 5) or injections of the vehicle solution (2 ml/kg, n = 5). Following the injection period, animals in the post-injection period were presented with a free-choice between ethanol and water for 5 consecutive days without drug treatments.

Results

As in the previous study ethanol consumption was calculated both in terms of daily ethanol preference and daily absolute amount of ethanol ingested.

Figure 12 illustrates the ethanol consumption for those animals pretreated with .5 mg/kg of methergoline prior to zimelidine (20 mg/kg) treatment. The analysis revealed a significant group x period interaction ((Ethanol preference: $F(6,32) = 7.68$, $p<.001$; Absolute ethanol intake: $F(6,32) = 6.27$, $p<.001$)). Post hoc Tukey tests indicated that zimelidine alone produced an

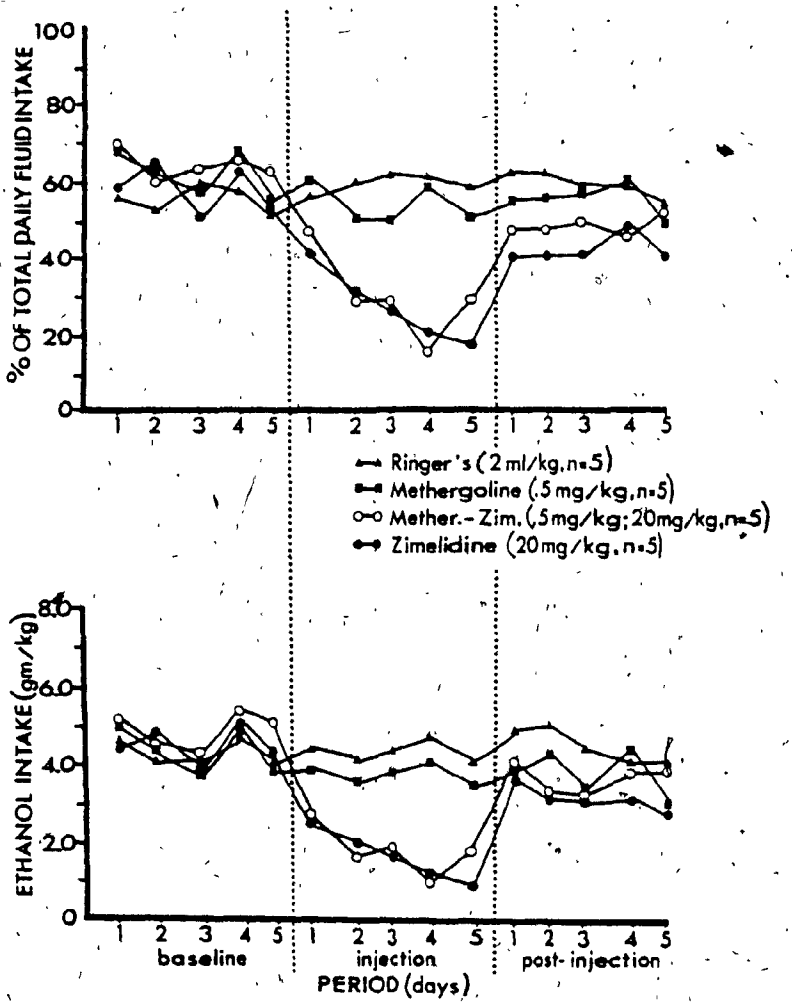

Figure 12 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats pretreated with Methergoline (.5 mg/kg, i.p.) prior to zimelidine (20 mg/kg, i.p.) treatment.

attenuation in both the preference for ethanol and absolute ethanol intake as compared to baseline and control levels ($p<.01$). Pretreatment with methergoline followed by injections of zimelidine also produced a decrease in ethanol consumption ($p<.01$). No difference existed between the above mentioned treatment groups during the injection period ($p>.05$). Treatment with methergoline alone or injections of the vehicle solution did not alter ethanol consumption ($p>.05$).

As shown in Figure 13, pretreatment with 1 mg/kg of methergoline followed by zimelidine (20 mg/kg) yielded similar results ((Ethanol preference: $F(6,32) = 5.65$, $p<.001$; Absolute ethanol intake: $F(6,32) = 9.573$, $p<.001$)). As was evident following pretreatment with .5 mg/kg of methergoline, post hoc Tukey test revealed that 1 mg/kg of methergoline prior to treatment with zimelidine (20 mg/kg) did not effect the zimelidine induced attenuation of ethanol intake ($p>.05$). In addition both methergoline (1 mg/kg) and the vehicle solution did not affect ethanol consumption.

Figure 14 illustrates ethanol consumption for those animals pretreated with 1 mg/kg of

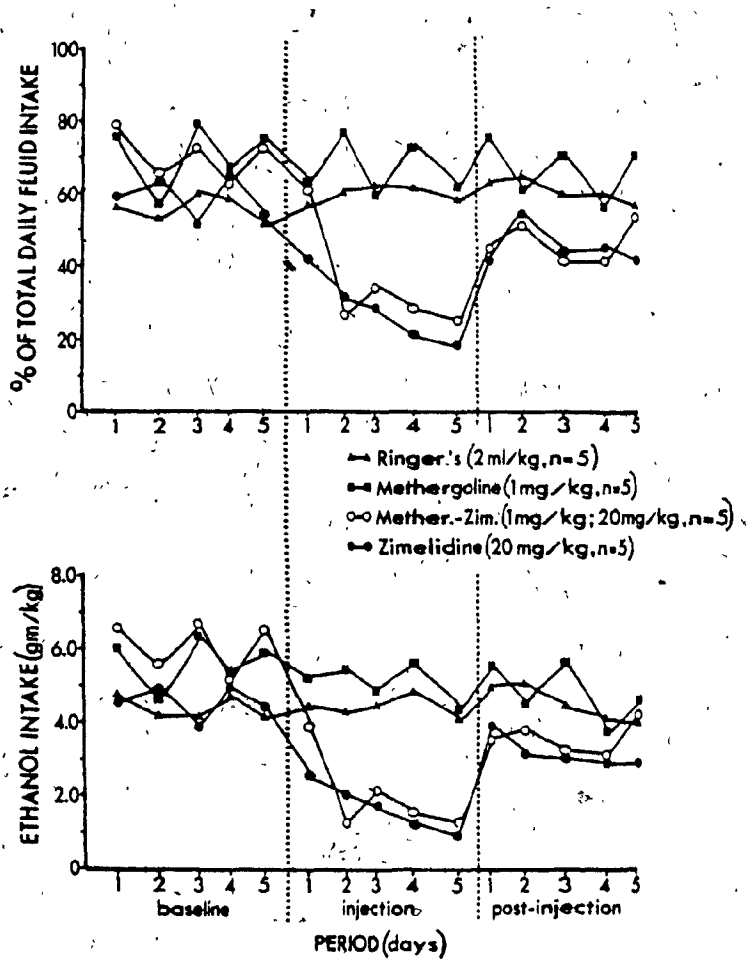

Figure 13 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats pretreated with methergoline (1 mg/kg, i.p.) prior to zimelidine (20 mg/kg, i.p.) treatment.

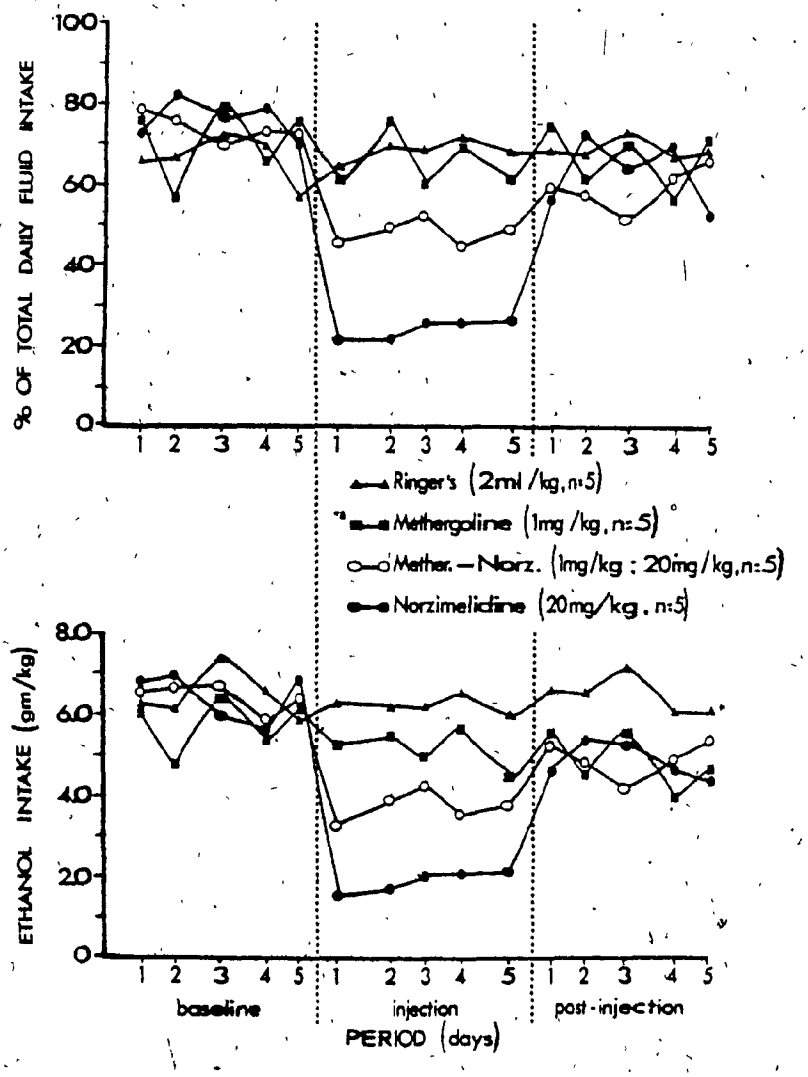

Figure 14 Ethanol consumption in terms of mean per cent of total daily fluid intake and mean absolute ethanol intake in rats pretreated with methergoline (1 mg/kg, i.p.) prior to norzimelidine (20 mg/kg, i.p.) treatment.

methergoline prior to norzimelidine (20 mg/kg) treatment. A two-way analysis of variance revealed a significant group x period interaction ((Ethanol preference: $F(6,32) = 9.32$, $p<.001$; Absolute ethanol intake: $F(6,32) = 9.85$, $p<.001$)). Post hoc Tukey tests indicated that the groups treated with methergoline prior to norzimelidine and norzimelidine alone both reduced their ethanol intake when compared to control and baseline levels ($p<.01$). More importantly, during the injection period the methergoline-norzimelidine group consumed ethanol to a greater degree than those animals treated with norzimelidine alone ($p<.05$). During the post-injection period ethanol consumption for these two groups increased and did not differ from control levels ($p>.05$). Treatment with methergoline (1 mg/kg) alone or injections of the vehicle solution did not alter ethanol consumption ($p>.05$). In addition, it should be noted that all treatments during the injection periods did not alter body weight or total fluid intake.

Discussion

In an attempt to determine the neurochemical mechanism of action of zimelidine and norzimelidine and its effect on ethanol intake, the possibility of enhanced 5-HT post-synaptic transmission as a causal factor was investigated. This experiment demonstrated that inhibition of 5-HT post-synaptic activity prior to zimelidine treatment, does not alter the zimelidine-induced attenuation of ethanol intake. Therefore, zimelidine does not seem to reduce ethanol consumption as a result of enhanced 5-HT post-synaptic activity. On the other hand, pretreatment with methergoline prior to administration of norzimelidine, diminished the effectiveness of norzimelidine in reducing ethanol intake. The data indicate that the suppression of ethanol consumption produced by norzimelidine seems to be primarily due to an increase in 5-HT post-synaptic receptor activation. The results if not directly supportive, are in line with the notion that the potency of norzimelidine in inhibiting 5-HT uptake may play important role in the mechanism underlying the reduction of ethanol intake. In addition, the data serve to

indicate that 5-HT may play an important role in the mediation of ethanol self-administration.

General Discussion

The present series of experiments were undertaken to investigate two major issues. The first goal was to identify some specific attributes of zimelidine which may account for the reduction of ethanol intake. Secondly, this thesis was designed to examine the neuropharmacological mechanism of action of zimelidine and norzimelidine with regard to the suppression of ethanol consumption.

Following a comparison of the effects of several 5-HT and NE uptake inhibitors on ethanol intake, it was demonstrated in Experiment 1 that only those compounds which specifically inhibit 5-HT uptake altered ethanol consumption. This suggested that one pharmacological property of zimelidine which may be responsible for zimelidine-induced reduction of ethanol intake, is its ability to specifically inhibit 5-HT uptake. These results were in agreement with data indicating that increasing the availability of central 5-HT produces an attenuation of ethanol intake (Geller, 1973; Hill, 1974; Rockman et al., 1979a; Zabik et al., 1978). In addition, results from Experiment 1 indicated that norzimelidine, the primary active

metabolite of zimelidine, also reduced ethanol consumption. Therefore, based on the results of the first experiment, it was suggested that the neuropharmacological mechanism of action of norzimelidine may account for the reduction of ethanol intake as produced by zimelidine.

Consequently, Experiments 2, 3 and 4 were designed to investigate the mechanism of action of zimelidine and norzimelidine and its effects on ethanol intake. Based on previous data suggesting that zimelidine may produce a 5-HT-induced functional depletion of NE (Rockman et al., 1979b), it was necessary to determine whether norzimelidine attenuated ethanol intake through a similar process. Experiments 2 and 3 demonstrated that attempts to prevent or counteract the proposed reduction of NE activity had differential effects on zimelidine and norzimelidine induced reductions of ethanol intake. The data support the earlier report (Rockman et al., 1979b) that zimelidine reduces ethanol consumption by indirectly inducing a reduction of NE activity. In contrast, both procedures employed to block and/or counteract the reduction of NE activity had no effect on norzimelidine-induced attenuation of ethanol intake. Therefore,

paradoxically, norzimelidine which selectively inhibits 5-HT uptake, seems to affect ethanol intake primarily as a result of effects on another system rather than indirectly reducing NE activity.

Experiment 4 was conducted to evaluate the direct involvement of the serotonergic system in norzimelidine and zimelidine-induced attenuation of ethanol consumption. Since increasing the availability of central 5-HT increases 5-HT post-synaptic activity (Sangdee & Franz, 1979; Svensson, 1981), the possibility that both zimelidine and norzimelidine reduce ethanol intake by increasing 5-HT post-synaptic activity was investigated. In addition, the relative potency of norzimelidine as compared to zimelidine was hypothesized to be an important factor in the mechanism underlying the attenuation of ethanol intake. Recent biochemical data have indicated that norzimelidine is approximately 10 times as potent as zimelidine in inhibiting 5-HT uptake (Ogren et al., 1981). Therefore, it seemed possible that the difference in potency between these two compounds may play an important role in the process leading to the reduction of ethanol

consumption. In an attempt to counteract the proposed enhanced 5-HT post-synaptic activity, animals in Experiment 4 were pretreated with methergoline, a 5-HT post-synaptic receptor blocker, prior to administrations of zimelidine and norzimelidine. It was demonstrated that such treatment reduced the effectiveness of norzimelidine but not zimelidine. The results obtained in these latter studies indicated that norzimelidine-induced attenuation of ethanol intake may be primarily due to an enhanced 5-HT post-synaptic activity. Since methergoline did not affect the zimelidine-induced reduction of ethanol intake, it is suggested that an enhanced 5-HT post-synaptic activity may not be a primary cause of the reduction of ethanol intake produced by zimelidine.

Following an examination of the data from the present series of experiments, one seems obliged to postulate that zimelidine and norzimelidine may exert their effects on ethanol intake via different neuropharmacological mechanisms. As would seem somewhat unlikely, nevertheless, the data suggest that 5-HT uptake blockade produced by zimelidine, reduces ethanol intake as a result of a 5-HT-induced reduction

of NE activity. On the other hand, norzimelidine, the primary metabolite of zimelidine, seems to reduce ethanol intake as a function of an enhanced 5-HT post-synaptic activity. In an attempt to explain the notion that zimelidine and norzimelidine reduce ethanol intake by different neuropharmacological mechanisms of action, one must consider the pharmacological properties of these two compounds. As mentioned earlier, the only apparent significant pharmacological difference between these two compounds appears, to be potency of 5-HT uptake blockade (Ogren et al., 1981). Thus, this difference in potency between zimelidine and norzimelidine appears to be an important factor underlying two neuropharmacological mechanisms capable of influencing ethanol intake. The present author is suggesting that due to the putative higher degree of 5-HT uptake blockade produced by norzimelidine, treatment with this compound may alter 5-HT activity sufficiently, resulting in a reduction of ethanol intake. Zimelidine, on the other hand seems not to alter ethanol intake via increasing 5-HT post-synaptic activity. Rather, this compound increases availability of 5-HT

which seems to result in a reduction of NE activity, thereby, reducing ethanol intake.

While considering the data from the present series of experiments, one is left to ponder why the norzimelidine induced attenuation of ethanol intake is resistant to pretreatment with DMI and clonidine. Since norzimelidine increases 5-HT availability, one would expect norzimelidine also to induce a functional depletion of NE. Yet the data from Experiments 2 and 3 suggest that norzimelidine does not reduce ethanol intake via a reduction of NE activity. In an attempt to reconcile these data, the author suggests that norzimelidine induces a large immediate and direct impact on 5-HT activity. In this way norzimelidine may produce a maximal effect on ethanol intake. Such an increase in 5-HT synaptic transmission may then gain dominance over the putative indirect reduction of NE activity. Furthermore, it is suggested that this would possibly result in the contribution of NE activity to the variance accounting for this phenomenum neglible.

One of the most important implications of this investigation is related to the neurochemical basis of ethanol intake. Considering the data

from the present series of studies, one must arrive at a tentative conclusion that both 5-HT and NE systems are somehow involved in the mediation of ethanol consumption. It is interesting to note that reductions of ethanol intake occurs both as a result of an indirect reduction of NE activity (produced by zimelidine) and an increase in 5-HT post-synaptic activity (produced by norzimelidine). The observation that both a reduction of NE activity and increase in 5-HT post-synaptic transmission reduce ethanol intake is certainly interesting. If true, it seems to suggest that some relationship between 5-HT and NE activity plays an important role in the mediation of ethanol intake. This type of relationship between 5-HT and NE and its effects on ethanol intake seems to be supported by the pharmacological and biochemical literature. Recent biochemical data demonstrated that there exists an inverse relationship between central 5-HT and NE levels, synthesis and turnover. For example, treatments which reduce central 5-HT levels have been shown to produce concurrent increases in synthesis and turnover of NE (Degueurce, Wiklund, Leger & Pujol, 1979; Keane, Degueurce, Renaud, Crespi & Pujol, 1978;

McRae-Degueurce & Pujol, 1979). Conversely, reductions of NE activity have been demonstrated to induce increases in 5-HT synthesis and activity (Blondaux, Juge, Sordet, Chouvet, Jouvet & Pujol, 1973; Johnson, Kim & Boukma, 1972). This inverse relationship between 5-HT and NE activity is also evident following procedures which increase 5-HT availability. As mentioned previously, an increase in central 5-HT availability seems to induce a decrease in NE activity (Everett, 1974; Shaskan & Snyder, 1970; Snyder et al., 1973; Thoenen & Tranzer, 1971).

This notion of an inverse relationship between 5-HT and NE also seems to be evident within the ethanol intake literature. For example, reduction in NE activity and increases in 5-HT availability both have been shown to reduce ethanol intake (Amit et al., 1977; Brown et al., 1977; Davis et al., 1978; Geller, 1973; Hill, 1974; Rockman et al., 1979a, 1979b). Conversely, increasing NE synthesis and turnover by electrical stimulation induces large increases in ethanol intake (Amit & Stern, 1971; Amit, Wise & Stern, 1970). The only data not fitting this model is derived from studies examining the effects of

5-HT depletions on ethanol intake. Nevertheless, the data from the present investigation in conjunction with the literature serve to indicate that a certain "relationship" between 5-HT and NE activity may be important in the mediation of the positive reinforcing properties of ethanol. However, the properties of this "relationship" between 5-HT and NE and their influence on ethanol intake remain to be elucidated.

The present investigation also raises a number of issues for further investigation. It is important to note that 5-HT uptake blockade also alters pre-synaptic activity within the serotonergic system (De Montigny et al., 1981; Svensson et al., 1981). Therefore, it is possible that zimelidine and norzimelidine alter 5-HT pre-synaptic activity, which may also contribute to the reduction of ethanol intake. At present, the extent to which alterations in 5-HT pre-synaptic activity may affect ethanol intake is unknown. However, since increasing 5-HT availability, via 5-HT uptake blockade, seems to increase 5-HT pre-synaptic activity, thereby, altering 5-HT synthesis and turnover through a negative feedback system (Aghajanian, 1973; Sheard et al., 1972), it

suggests that 5-HT pre-synaptic activity may also play an important role in the mediation of ethanol intake. The involvement of 5-HT pre-synaptic activity in the effects of 5-HT uptake blockade on ethanol intake has not been investigated to date and clearly warrants investigation.

An additional issue emanating from this thesis concerns the potency of norzimelidine. As mentioned previously, it has been suggested that the degree to which norzimelidine alters 5-HT synaptic activity may play a role in rendering the norzimelidine-induced attenuation of ethanol intake resistant to pretreatment with DMI and clonidine. If true, one would expect that the effectiveness of lower doses of norzimelidine in reducing ethanol intake may be altered by pretreatment with DMI and clonidine. Therefore, experiments are being designed to examine the effects of a dose response relationship of norzimelidine in combination with DMI and clonidine on ethanol intake.

Finally, it should be noted that the present series of experiments represent an indirect demonstration of the neuropharmacological

mechanism of action of zimelidine and norzimelidine. In an attempt to shed additional light on the notions emanating from this investigation, it is suggested that some direct measurement techniques be employed. Toward this goal, studies are presently being designed employing the "single cell recording" technology in order to directly measure activity within the 5-HT and NE systems following norzimelidine and zimelidine treatment.

In conclusion, the data from this thesis seems to suggest that zimelidine and norzimelidine reduce ethanol intake via two different neuropharmacological mechanisms of action. In addition, these data indicate that both 5-HT and NE may be involved in the mediation of the positive reinforcing properties of ethanol. Considered in this context, the data from this investigation may contribute to our understanding and subsequently to the development of novel approaches to the treatment of alcohol oriented behavior.

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