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LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L’AVONS RÉCU
ATTENUATION OF ETHANOL INTAKE BY 5-HT REUPTAKE BLOCKADE IN RATS: POSSIBLE INTERACTION WITH BRAIN NOREPINEPHRINE

Gary E. Rockman

A Thesis
in
The Department
of
Psychology

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Arts at Concordia University, Sir George Williams Campus Montreal, Quebec, Canada

September 1978
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ABSTRACT

ATTENUATION OF ETHANOL INTAKE BY 5-HT REUPTAKE BLOCKADE IN RATS: POSSIBLE INTERACTION WITH BRAIN NOREPINEPHRINE

Gary E. Rockman

The role of serotonin reuptake blockade in the mediation of the positive reinforcing properties of ethanol in laboratory rats was investigated. In the first experiment it was demonstrated that treatment with H102/09, a selective serotonin reuptake inhibitor, specifically attenuated ethanol consumption. This result was attributed to an increased availability of central serotonin, suggesting that serotonin may in some way mediate the positive reinforcing properties of ethanol. In an attempt to extinguish the ethanol drinking response, ethanol preferring animals in the second experiment, were provided with ethanol as the only source of fluid in combination with H102/09 treatment. Animals treated in such a manner subsequently reduced their ethanol consumption when presented with a free-choice between ethanol and water. The final experiment was undertaken to examine whether the attenuation of ethanol consumption following treatment with H102/09 could be due to an invasion of surplus central serotonin into norepinephrine.
neurons. In an attempt to prevent this proposed invasion by serotonin, ethanol preferring animals were pre-treated with desmethylimipramine (DMI), a norepinephrine reuptake inhibitor, prior to treatment with H102/09. The results demonstrated that those animals treated in such a manner consumed significantly more ethanol than those animals treated with H102/09 alone. Based on these results it was suggested that the observed attenuation of ethanol consumption following H102/09 treatment, could be partially due to a serotonin induced functional depletion of norepinephrine.
Acknowledgements

My deepest gratitude is extended to Dr. Zalman Amit for his constant encouragement and guidance provided throughout the course of these studies.

I wish to thank Drs. Jane Stewart, Peter Seraganian, Zavie Brown and Shimon Amir for their criticisms during the writing of this thesis.

I would also like to thank Franc Rogan and Geoff Carr for their technical assistance.

Partial support and generous supplies of the compound M102/09 received from Astra Chemical Company, Sweden are gratefully acknowledged.
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Beverages containing ethyl alcohol (ethanol) have been ingested at social and ritualistic events for thousands of years. Excessive use of ethanol has proved to be a problem to some persons in every society introduced to it. Ethanol is now the most widely consumed mood altering drug in North America; "alcoholism" constitutes the most prevalent form of drug dependence in our society (Sardesai, 1969; Roach, 1971; Lieber, 1976). The nature and causes of ethanol dependence in both human and animal subjects have been the subject of intensive experimental investigation. In order to study the development of ethanol dependence researchers have attempted to establish criteria for an animal model of human alcoholism (Mello, 1973).

A common view of alcoholism in our society is that when ethanol is ingested in excess it is because the individual concerned must have ethanol to stave off the unpleasant physical symptoms of withdrawal from the drug. It is not surprising therefore in light of this commonly held view that Lester and Freed (1973) have suggested that one criterion for an animal model of alcoholism is physical dependence, a state which is said to be inferred from the occurrence of withdrawal symptoms upon the removal of access to ethanol. Furthermore, other investigators have suggested that the experience of withdrawal
in animals should lead to voluntary consumption of ethanol (Ho, Chen & Tsai, 1978; Cicero, Snider, Perez & Swanson, 1971). This negative reinforcement model of ethanol self-administration is however, not supported by data emanating from both human and animal investigations. It is true that animals made physically dependent through forced exposure to either oral or intragastric administration of large quantities of ethanol demonstrate withdrawal reactions upon the termination of treatment. In spite of this however, animals given ethanol under such conditions do not increase their preference for ethanol or consume sufficient quantities of it to avoid or terminate the withdrawal symptoms (Ratcliffe, 1972; Cicero & Smithloff, 1973; Myers, Stöltman & Martin, 1972; Begleiter, 1975). In addition, both animals and humans have been shown to voluntarily and spontaneously abstain from self-administering ethanol, despite the occurrence of withdrawal symptoms (Hunter, Riley, Walker & Freund, 1975; Deneau, Yanagita, Seavers, 1969; Winger & Woods, 1973; Mello & Mendelson, 1970; 1972).

The above mentioned data from human and animal experiments demonstrates quite convincingly that although physical dependence may develop from the ingestion of ethanol, this in itself is not a sufficient condition for the initiation and maintenance of ethanol self-ad-
ministration (Amit, Sutherland & White, 1975/76; Spealman & Goldberg, 1978). An alternative explanation for ethanol self-administration is that ethanol is consumed for its positive reinforcing properties. Ethanol has been shown to serve as a positive reinforcer in non-dependent animals under various schedules of reinforcement whether presented orally (Henningfield & Meisch, 1978; Meisch & Beardsley, 1975; Henningfield & Meisch, 1976), intragastrically (Smith, Werner & Davis, 1976; Amit & Stern, 1969), or intravenously (Carney, Llewellyn & Woods, 1976; Smith, Werner & Davis, 1976).

It would appear from these studies that the initiation and maintenance of ethanol self-administration is primarily due to ethanol's positive reinforcing properties rather than the avoidance of the negative withdrawal symptoms. This premise is to be the working hypothesis upon which the following studies are based.

**Involvement of Catecholamines**

During the past fifteen years the possible relationship between the pharmacological actions of ethanol and the biogenic amines has been experimentally investigated (e.g. Myers, 1978).

With regards to the effects of ethanol on catecholamines, acute ethanol treatment has been shown to sig-
nificantly increase the turnover of central catecholamines (Karoum, Wyatt & Majchrowicz, 1976), having a greater effect on noradrenergic neurons than dopaminergic neurons (Pohorecky, 1974; Carlsson, Magnusson, Svensson & Waldeck, 1973; Corrodi, Fuxe & Hökfelt, 1966; Pohorecky & Jaffe, 1975). Additional evidence suggesting a relationship between central catecholamines and the pharmacological properties of ethanol is derived from studies employing alpha-methyl-tyrosine, an inhibitor of tyrosine hydroxylase the rate limiting enzyme in the synthesis of catecholamines. Pretreatment with alpha-methyl-tyrosine has been reported both to inhibit the motor excitation produced by ethanol in rats (Carlsson, Engel & Svensson, 1972; Engel, Strömbom, Svensson & Waldeck, 1974) and suppress the euphoric effects of ethanol in humans (Ahlenius, Carlsson, Engel, Svensson & Sodersten, 1973). Electrical stimulation of the lateral hypothalamus, an area rich in catecholamines, has been shown to produce a permanent increase in ethanol consumption in laboratory rats (Amit, Stern & Wise, 1970; Amit & Stern, 1971), while electrolytic lesions of the ventral portion of this area has been shown to attenuate the preference for ethanol (Amit, Meade, Levitan & Singer, 1976). Indirect evidence further implicating catecholamines in the pharmacological actions of ethanol, is based on the observation that
acetaldehyde, the primary metabolite of ingested ethanol, can react with catecholamines to produce tetrahydroisoquinoline alkaloids (TIQ). It has been suggested that once formed, these TIQ alkaloids may play a role in ethanol ingestion (Cohen, 1973; Cohen & Collins, 1970; Davis & Walsh, 1970). This hypothesis has some support since it has been reported that the intraventricular infusion of several TIQ alkaloids produce a substantial increase in the preference for ethanol (Myers & Melchior, 1977 a; Myers & Melchior, 1977 b; Melchior & Myers, 1977). However, other investigators have reported that the intraventricular infusion of the same TIQ alkaloids produces no change in the preference for ethanol (Amit, Brown & Smith, 1978).

Overall, these experiments suggest that catecholamines may somehow play a role in the mediation of the pharmacological properties of ethanol.

Additional evidence implicating the involvement of catecholamines and specifically norepinephrine in the mediation of some of the pharmacological actions of ethanol, is derived from studies examining the pharmacological properties of drugs employed in the treatment of human alcoholism. One of the most commonly employed agents in the treatment of human alcoholism is disulfiram (Antabuse; Hald & Jacobsen, 1948), which is known to inhibit aldehyde dehydrogenase, the enzyme necessary for the
oxidation of acetaldehyde. Consequently, the consumption of ethanol results in a toxic accumulation of acetaldehyde in the blood (Ericksson & Sippel, 1977; Sauter, Boss & Von Wartburg, 1977). Therefore, because of this toxic effect it has been suggested that the alcoholic is deterred from drinking (Kitson, 1977). In addition disulfiram has also been shown to inhibit dopamine-beta-hydroxylase (DBH), the enzyme necessary for the conversion of dopamine (DA) to norepinephrine (NE) (Goldstein, Anagnoste & Lauber, 1964; Catignani & Neal, 1975). This inhibition of DBH according to Collier (1972) may cause a depletion of NE levels in the brain, sufficient to block the "ethanol-induced-euphoria". This viewpoint has recently been supported by Brown & Amit (1977), demonstrating the involvement of NE rather than DA in the mediation of the positive reinforcing properties of ethanol. Furthermore, Amit, Levitan, and Lindros (1976) compared the effects of disulfiram (both an aldehyde dehydrogenase and DBH inhibitor), Temposil, (an aldehyde dehydrogenase inhibitor), and FLA-63 (a DBH inhibitor) on ethanol ingestion in rats. It was shown that Temposil had virtually no effect, whereas disulfiram moderately suppressed ethanol intake and FLA-63 had the largest attenuating effect on ethanol ingestion. However, due to the toxicity of FLA-63, the effect of DBH inhibition alone could not be adequately eval-
uated. More recently, it was reported that the administration of a non-toxic DBH inhibitor FLA-57, decreasing central NE levels, produced a pronounced attenuation of ethanol drinking (Amit, Brown, Levitan & Ögren, 1977). It was further demonstrated that extinction of the ethanol-drinking response could be obtained by combining forced ethanol presentations with lowered NE levels (Brown, Amit, Levitan, Ögren & Sutherland, 1977). These results have further implicated the role of NE in the mediation of the positive reinforcing properties of ethanol.

Involvement of Serotonin

Since the late 1950's considerable experimental data have been reported, suggesting a relationship between serotonin (5-HT) and the pharmacological effects of ethanol (e.g. Myers & Melchior, 1977c). At present, however, the experimental evidence concerning the relationship between 5-HT and the pharmacological properties of ethanol is contradictory. For example, Goldstein (1973) has suggested that serotonin is not directly involved in ethanol induced withdrawal reactions. It has been demonstrated subsequently however, that 5-HT receptor blockade produced by methysergide significantly enhanced the ethanol-induced withdrawal convulsions (Blum, Wallace, Schwertner & Embanks, 1976). The data from studies examining the ef-
ffect of ethanol on central levels of serotonin are equally confusing. Following acute treatment with ethanol, serotonin levels have been reported to be increased (Pohorecky, Jaffe, Berkeley, 1974; Erickson & Matchett, 1975), decreased (Gursey, Vester & Olson, 1959) or unchanged (Pohorecky & Newman, 1978; Tabakoff & Boggan, 1974). With regards to the relationship between lowered levels of central serotonin and ethanol consumption, reduction of central 5-HT levels by p-chlorophenylalanine (PCPA) was originally reported to cause an attenuation of the preference for ethanol (Veale & Myers, 1970; Myers & Veale, 1968). Subsequent studies have demonstrated that the effects of PCPA were not specific to ethanol, in that ingestion of a saccharin solution was also affected (Stein, Wayner & Tilson, 1977; Nachman, Lester & LeMagnen, 1970). Moreover, reduction in central levels of 5-HT have also been shown to increase (Geller, 1973) or have no effect on ethanol self-administration (Kiiianmaa, 1975). It would therefore seem quite obvious that it would be impossible to draw any conclusions regarding the relationship between lowered levels of 5-HT and ethanol self-administration. A more consistent finding is observed following the treatment with compounds that subsequently increase the central availability of serotonin. Animals treated with intra-peritoneal or intraventricular administrations of 5-
hydroxytryptophan (5-HTP), the precursor of 5-HT, or 5-HT itself, significantly reduced their ethanol consumption (Myers & Martin, 1973; Geller, 1973; Hill, 1974; Geller, Purdy & Merritt, 1973; Myers, Evans & Yaksh, 1972). This suggests that an increase in the central availability of serotonin may be involved in the self-administration of ethanol.

It is well documented that pharmacological agents, designed to block the reuptake of central neurotransmitters produces an increase in the availability of that neurotransmitter in the synaptic gap (Anderson, 1972; Corrodi & Fuxe, 1968; Meek, Fuxe & Andén, 1970; Carlsson, Jonason, Lindqvist & Fuxe, 1969). Therefore, in the following series of experiments, H102/09 (Zimelidine) a selective central serotonin reuptake inhibitor (Ross, Ögren & Renyi, 1976; Ross & Renyi, 1977), was employed in an attempt to produce an increased availability of central serotonin. The effect of treatment with H102/09 on ethanol consumption in laboratory rats was investigated in Experiments 1 & 2. The final experiment was designed to investigate whether an interaction between the increased availability of serotonin and the norepinephrine neurons could be responsible for the observed effect.
EXPERIMENT 1

Increases in the central availability of serotonin have been shown to produce a substantial decrease in ethanol consumption (e.g. Hill, 1974). In addition it has been demonstrated that drugs blocking the reuptake process of serotonin cause an increased availability of serotonin in the synaptic gap (e.g. Meek, Fuxe & Andén, 1970). The first experiment was designed to investigate whether increasing the availability of serotonin by employing a selective 5-HT reuptake blocker (H102/09), would specifically affect voluntary ethanol consumption in laboratory rats.

Method

Subjects

Subjects were male Wistar rats (Canadian Breeding Farm Laboratories Ltd.), weighing between 200 – 250 grams 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 cycle. 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 15% (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 were alternated with each ethanol presentation. The ETOH concentrations were increased by 2% increments when 50% or more of the total daily fluid was consumed as ethanol. Once drinking of an 11-15% (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 and on their specific ETOH concentration throughout the remaining phases of the experiment. Only those animals whose ETOH consumption was 50% or more of their total daily fluid intake were included in the experiment.

Baseline period: This period consisted of an everyday free choice between ethanol and water. Once a 5-day baseline measure was attained, animals were randomly divided into 4 groups.

Injection period: During the injection period, the four groups received 1 intraperitoneal injection per day for 5 consecutive days, at approximately 15:00 hours.
Groups 1 (n=9), 2 (n=11) and 3 (n=9) received H102/09 (Astra Chemical Co.) in a dose of 10, 20 and 30 mg/kg respectively, prepared in a concentration of 10 mg/ml dissolved in Ringer's solution. Group 4 (n=9) received daily injections of the vehicle (2 ml/kg). Approximately 4 hours following the injections, fluid consumption of the previous 24 hours was measured and tubes were refilled and replaced.

Post-injection period: For 5 days following the injection period, the consumption of ethanol in a free choice with water, was measured.

Taste and activity controls: To determine whether the effects of H102/09 were specific to ethanol consumption, H102/09 was administered to rats ingesting an aversive tasting Quinine-Sucrose solution. Animals were presented with a free choice between water and 0.002% (w/v) Quinine Sulfate in a 10% (w/v) Sucrose solution (QS) on alternate days. The concentration of Quinine was raised by increments of 0.002% (w/v) when 50% or more of the total daily fluid consumed was the QS solution. When drinking of a 0.008% - 0.016% (w/v) QS solution was established, the animals were switched to a schedule consisting of an everyday free choice between water and the QS solution. These animals were then treated in a similar manner to the ethanol drinking animals. Group 1 (n=8) received H102/09 (20 mg/
kg, I.P.) and group 2 (n=6) received Ringer's solution
(2 ml/kg, I.P.) for 5 consecutive days.

To determine the effect of H102/09 on the motor ac-
tivity in rats, additional animals were injected with
either H102/09 (20 mg/kg, I.P., n=7) or Ringer's solution
(2 ml/kg, I.P., n=7) for 5 consecutive days. Four hours
following the injections, on the first, third and fifth
days of the injection period, animals were placed in an
open field (black wooden boxes, 45.7 cm sq x 39.4 cm high)
for 1 hour. Motor activity was automatically recorded
via counters connected to 4 intersecting photocell beams
placed 15.2 cm apart and 3.8 cm above the floor.

Biochemical assays: Assays were done to determine
the effect of treatment with H102/09 on the whole brain
levels of serotonin, norepinephrine and dopamine. Addi-
tional animals (n=24) of equivalent weight were injected
with H102/09 (20 mg/kg I.P.) or Ringer's solution (2 ml/
kg I.P.) for 5 consecutive days. Four hours following
the injections, on the first, third and fifth days of the
injection period, animals were decapitated and the brains
were removed and immediately frozen on dry ice. Whole
brain levels of serotonin, norepinephrine and dopamine
were determined, using spectrophotofluorometric techniques
(Barchas, Erdelyi & Angwin, 1972; Shellenberger & Gordon,
1971).
Results

Ethanol consumption for the present and subsequent experiments was calculated both in terms of daily ethanol preference (mean percent of total daily fluid intake) and in terms of daily absolute amount of ethanol ingested (mean grams per kilogram). Daily ethanol consumption expressed in terms of percent of total fluid intake for each period of the experiment is shown in Figure 1. A two-way analysis of variance (groups x periods) revealed both a significant group effect ($F(3, 34) = 4.45, p < .01$) and period effect ($F(2, 68) = 58.65, p < .001$). More importantly the analysis yielded a significant group x period interaction ($F(6, 68) = 5.95, p < .001$). Post hoc tests (Tukey) revealed the following. During the injection period animals treated with H102/09 (20 and 30 mg/kg) reduced their ethanol preference to a greater degree ($p < .01$) than the group treated with 10 mg/kg of H102/09 ($p < .05$) as compared to baseline levels. In comparison to control levels, only the groups treated with 20 and 30 mg/kg of H102/09 reduced their ethanol intake ($p < .01$). During the post-injection period, all groups treated with H102/09 were significantly lower in intake than control levels ($p < .05$). The control group did not change their preference for ethanol throughout the entire ex-
periment (p > .05).

Daily absolute ethanol intake for each period of the experiment is illustrated in Figure 2. A two-way analysis of variance (groups x periods) revealed both a significant group effect (F(3, 34) = 3.46, p < .05) and period effect (F(2, 68) = 104.55, p < .001). More importantly, the analysis yielded a significant group x period interaction (F(6, 68) = 7.71, p < .001). Post hoc Tukey tests revealed that during the injection period, all groups treated with H102/09 significantly reduced their ethanol intake when compared to baseline levels (p < .01). In relation to control levels doses of 20 and 30 mg/kg produced a greater effect (p < .01) than did 10 mg/kg of H102/09 (p < .05). During the post-injection period, the groups treated with 20 and 30 mg/kg significantly increased their absolute ethanol intake as compared to their intake during the injection period (p < .01). Control animals were shown not to differ in absolute ethanol intake across periods (p > .05). In addition, a two-way analysis of variance revealed that treatment with H102/09 (20 mg/kg) as shown in Figure 3, had no effect either on body weight (F(2, 36) = .552, p > .05) or total fluid intake (F(2, 36) = 1.96, p > .05).

Taste and activity controls: As illustrated in Figure 4, H102/09 (20 mg/kg) did not affect the ingestion
of the Quinine-Sucrose solution. A two-way analysis of variance yielded no significant differences ($F(2, 24) = 1.26, p > .05$).

Figure 5 illustrates the motor activity for both vehicle and H102/09 (20 mg/kg) treated animals. Although following the first injection the animals treated with H102/09 were significantly lower in motor activity than control animals ($t(12) = 3.139, p < .01$), no significant differences were obtained on the third ($t(12) = .868, p > .05$) and fifth ($t(12) = .96, p > .05$) days of treatment.

**Biochemical assays:** Whole brain levels of serotonin, norepinephrine and dopamine following treatment with H102/09 for one, three and five days are shown in Table 1. No significant differences were obtained (Student's t tests, $p > .05$).

**Discussion**

The results of this experiment demonstrates that treatment with H102/09, a serotonin reuptake inhibitor, produces an attenuation in ethanol drinking during the injection period. Following the termination of treatment, ethanol intake was observed to increase back to near baseline levels. A dose response relationship was evident since 10 mg/kg of H102/09 produced only a moder-
Figure 1. Ethanol consumption in terms of mean percent of total daily fluid intake in rats treated with H102/09.
Figure 2. Ethanol consumption in terms of mean absolute ethanol intake in rats treated with H102/09.
Figure 3. Mean total fluid intake (ethanol and water consumption combined) and body weight in rats treated with H102/09.
Figure 4. Quinine-Sucrose consumption in terms of mean percent of total daily fluid intake in rats treated with H102/09.
Figure 5. Motor activity in terms of mean activity counts in rats treated with H102/09.
### Table 1
Effects of Treatments with H102/09 on the Levels of Serotonin and Catecholamines in Whole Brain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SEROTONIN</th>
<th></th>
<th>NOREPINEPHRINE</th>
<th></th>
<th>DOPAMINE</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Levels (ng/g)</td>
<td>% Control</td>
<td>Levels (ng/g)</td>
<td>% Control</td>
<td>Levels (ng/g)</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>422.09</td>
<td>-</td>
<td>444.71</td>
<td>-</td>
<td>539.86</td>
</tr>
<tr>
<td>1 x (2ml/kg Ringer's)</td>
<td>4</td>
<td>468.21</td>
<td>110.92</td>
<td>425.35</td>
<td>95.6</td>
<td>619.84</td>
</tr>
<tr>
<td>H102/09</td>
<td>4</td>
<td>393.71</td>
<td>-</td>
<td>432.73</td>
<td>-</td>
<td>613.99</td>
</tr>
<tr>
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<td>104.72</td>
<td>430.00</td>
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<td>-</td>
<td>446.05</td>
<td>-</td>
<td>693.78</td>
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<td>5 x (2 ml/kg Ringer's)</td>
<td>4</td>
<td>387.02</td>
<td>88.0</td>
<td>461.38</td>
<td>103.4</td>
<td>654.16</td>
</tr>
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</table>
ate attenuation of ethanol consumption, whereas, doses of 20 and 30 mg/kg were shown to be significantly and equally more effective. Treatment with H102/09 was shown not to affect body weight, total fluid consumption, or the ingestion of a Quinine Sucrose solution. The effect of H102/09 would appear to be specific to ethanol consumption. Although H102/09 was shown to produce a decrease in motor activity (Fig. 5) on the first day of treatment, this effect was absent by the third injection. Because the attenuation of ethanol intake continued throughout the injection period, it would appear that a decrease in motor activity cannot be assumed to be responsible. Since treatment with H102/09 seems to be specific to ethanol consumption while not producing motor deficits, it is suggested that the attenuation of ethanol consumption was a consequence of the manipulation of the positive reinforcing properties of ethanol.

Assay results revealed no significant changes in whole brain levels of NE, DA and 5-HT, although there was a slight increase in 5-HT levels on the first and third days of injection. These results are consistent with other reports, indicating that blocking the reuptake process of biogenic amines results in either no significant changes (Hyttel, 1977a) or extremely small increases in whole brain levels followed by decreases
of a similar magnitude after continued treatment (Hyttel, 1977b; Roslof & Davis, 1978). It has been suggested that the lack of large increases in endogenous levels of the neurotransmitter following the initial treatment with a reuptake blocker may be due to a reduction in the neuronal firing rate and hence a decreased turnover, which results as a consequence of the increased availability in the synaptic cleft (Sheard, Zolovick & Aghajanian, 1972; Aghajanian, 1972; Schubert, Nybäck & Sedvall, 1970).

As previously mentioned, several studies have indicated that increasing the availability of serotonin in the brain produces a decrease in ethanol consumption (e.g. Geller, 1973). Present results confirm these findings that increasing the availability of 5-HT via reuptake blockade, produces a profound attenuation in ethanol consumption. In addition, these results suggest that serotonin may in some way mediate the positive reinforcing properties of ethanol. The following experiment was designed to further examine this suggestion and to determine whether a longer lasting effect could be produced following H102/09 treatment.
EXPERIMENT 2

A variety of treatment procedures that have been designed to suppress ethanol self-administration in both humans and infra-human subjects have, by and large, been minimally effective (Amit & Sutherland, 1975/76). Recently, extinction of the ethanol drinking response was accomplished by combining the attenuation of the positive reinforcing properties of ethanol with the previously reinforced response (Brown et al., 1977). This is consistent with the view that when a response is maintained by positive reinforcement and when that response is performed in the absence of reinforcement, extinction should occur. Upon reinstatement of the reinforcer, reacquisition of the behavior should occur (Thompson & Pickens, 1975; Mackintosh, 1974).

Immediately following the termination of treatment in Experiment 1 a sharp increase in ethanol drinking was observed, indicating that extinction of the response had not occurred. This is not surprising, since both necessary conditions of an extinction paradigm were not met. During the injection period in Experiment 1 the reinforcement derived from ethanol consumption may have been diminished, however the drinking response was also absent. Therefore, the second experiment was conducted
to investigate whether H102/09 would effectively extinguish the ethanol drinking response. In order to ensure the performance of the response in absence of reinforcement, ethanol was presented as the only source of fluid combined with injections of H102/09.

**Method**

**Subjects**

Subjects were male Wistar rats (Canadian Breeding Farm Laboratories Ltd.), weighing between 200 - 250 grams 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 cycle. Drinking solutions were presented in calibrated Richter tubes mounted on the front of the cage. Purina Rat chow was available ad libitum.

**Procedure**

Animals were presented with a free choice between water and increasing concentrations of ethanol on alternate days according to procedures described in Experiment 1. Only those animals whose ETOH consumption was 50% or more of their total daily fluid intake were
included in the experiment. The ethanol concentrations used in this experiment were 15 or 17% (v/v). The first phase of the experiment consisted of a schedule of alternate day free-choice presentations between water and ethanol. After a 5-day baseline measure was attained, animals were randomly divided into 3 groups for the remaining phases of the experiment. The second phase consisted of the first treatment session (Tr-1) in which animals were presented with ethanol as the only source of fluid for 10 consecutive alternate days. Therefore, on these days both Richter tubes available to each animal contained only the appropriate ethanol concentration. On the 10 intervening days, only water was available. During this 20 day treatment period, 4 hours prior to the presentation of either ethanol or water, the two experimental groups received daily intraperitoneal injections of H102/09 in a dose of 20 mg/kg (Group 1, n=4) or 30 mg/kg (Group 2, n=4). The drug was dissolved in an identical manner to that described in Experiment 1. The control group (n=4) were similarly injected with Ringer's solution (2 ml/kg). Therefore, during this period, all animals received 20 injections, 10 of which were combined with forced ethanol ingestion. Following completion of the second phase animals were given in the third phase (reacquisition) a free choice between
water and ETOH for 15 consecutive alternate days without injections. The fourth phase (Tr-2), which followed was similar to the first treatment period except it consisted of 5 consecutive alternate days of forced ethanol consumption and 10 daily injections. Animals were injected in an identical manner to that described in the first treatment period. The final phase consisted of a free choice between ETOH and water for 5 consecutive alternate days without injections for all animals.

Results

Daily ethanol consumption expressed in terms of percent of total daily fluid intake for the baseline and reacquisition periods is shown in Figure 6. A two-way analysis of variance (groups x periods) revealed both a significant period effect \(F(2,18) = 19.69, \ p < .001\) and a group x period interaction \(F(4,18) = 5.85, \ p < .01\). Post hoc Tukey tests revealed the following. During the first 10 days following the treatment period (reacquisition), both the experimental groups significantly reduced their ethanol intake in relation to baseline \(p < .01\) and control levels \(p < .01\). During days 10-15 of reacquisition all groups preferred ethanol at baseline levels \(p > .05\). Following the second treatment period, all
groups did not differ in ethanol intake from baseline (p > .05) or the previous five days of reacquisition (p > .05). The control group did not change their preference for ethanol throughout the experiment (p > .05).

Daily absolute ethanol intake for the baseline and reacquisition periods is shown in Figure 7. A two-way analysis of variance (groups x periods) revealed both a significant period effect (F (2, 18) = 35.39, p < .001) and a group x period interaction (F (4, 18) = 3.5, p < .02). Post hoc Tukey tests revealed that during the first 10 days of reacquisition both the experimental groups significantly reduced their intake as compared to baseline (p < .01) and control levels (p < .01). Intake during days 10-15 of reacquisition returned to control levels (p > .05) but was still significantly below baseline levels (p < .01). Following the second treatment period, no difference in ethanol intake was observed between groups (p > .05) or as compared to intake during days 10-15 of the reacquisition period (p > .05). Absolute ethanol intake for the control group throughout the experiment did not change (p > .05). During the treatment periods when ethanol was the only source of fluid, absolute ethanol intake for all groups was not significantly different from baseline levels (p > .05).
Figure 6. Ethanol consumption in terms of mean percent of total daily fluid intake in rats subjected to extinction treatments (Tr) with H102/09.
Figure 7. Ethanol consumption in terms of mean grams per kilogram in rats subjected to extinction treatments (Tr) with H102/09.
Discussion

The results of this experiment indicate that forced ethanol intake in combination with H102/09 treatment can effectively extinguish the ethanol drinking response. As previously mentioned, in order to extinguish a response, that response must be performed in absence of reinforcement. When reinforcement is once again available, a characteristic reacquisition pattern reliably occurs (Thompson & Pickens, 1975; Mackintosh, 1974). In the present experiment, when ethanol was again available in a free choice without drug treatment, a slow reacquisition of the response occurred. This behavior during the reacquisition period would indicate that the ethanol consumed was once again reinforcing. These results would therefore further implicate serotonin in the mediation of the positive reinforcing properties of ethanol.

Following the second treatment period, animals' ethanol consumption was not altered. This is consistent with the report that following a second extinction treatment period no additional attenuating effect was observed (Brown et al., 1977).

In relation to possible treatment procedures for
human alcoholics, the application of such a treatment procedure is promising. Wikler (1973) has suggested that the high relapse rate with human alcoholics following detoxification in a hospital or clinical setting may be due to the environmental stimuli (secondary reinforcers) which confront the addict following treatment. The treatment procedure which may emanate from this experiment would involve the alcoholic performing the response (drinking) in absence of reinforcement in his natural environment, thereby eliminating not only the primary reinforcing properties of alcohol, but also the environmental conditioned reinforcers.
EXPERIMENT 3

Several investigators have demonstrated and suggested an interaction between central serotonin and norepinephrine (Ho, Singer & Gershon, 1971; Pickel, Joh & Reis, 1977; Pujol, Stein, Blondaux, Petitjean, Froment & Jouvet, 1973). Shaskañ and Snyder (1970) have indicated that an increase in the availability of central 5-HT results in an invasion by 5-HT of the NE neurons via the NE reuptake mechanism. Once located in the NE neurons, the 5-HT could act as a false neurotransmitter. They further suggested that small amounts of surplus serotonin could subsequently cause a functional depletion of NE. As previously mentioned, considerable data is available suggesting the involvement of NE in the mediation of the positive reinforcing properties of ethanol (e.g. Brown et al., 1977). It is, therefore, conceivable that the decrease in ethanol consumption, produced by the increased availability of serotonin, could be due to an interaction between the surplus serotonin and the norepinephrine neurons. The following experiment was designed to determine whether an attempt to prevent the proposed invasion of 5-HT into the norepinephrine neurons by blocking the NE reuptake process, would alter the effectiveness of H102/09 treatment on ethanol con-
sumption. This was accomplished by treating ethanol preferring animals with desmethyliimipramine (DMI), a selective neuronal NE reuptake blocker (Carlsson, Corrodi, Fuxe & Hökfelt, 1969; Carlsson, Fuxe, Hamberger & Lindqvist, 1966) prior to treatment with H102/09.

Method

Subjects

Subjects were male Wistar rats (Canadian Breeding Farm Laboratories Ltd.), weighing between 200 - 250 grams 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

Animals were presented with a free choice between water and increasing concentrations of ethanol on alternate days in a similar manner to that described in Experiment 1. When drinking of an 11-15% (v/v) ethanol solution was established, the animals were then switched to a schedule consisting of an everyday free choice be-
between water and ethanol. All animals were maintained on this schedule throughout the remaining phases of the experiment. Only those animals whose ethanol consumption was 50% or more of their total daily fluid intake were included in the experiment. Following a 5-day baseline measure period animals were divided into 4 groups:

**Injection period**

This period consisted of 5 consecutive injections for all groups in a similar paradigm as described in Experiment 1. Group 1 (H102, n=11) received 5 consecutive injections of H102/09 (20 mg/kg) prepared and injected in a similar manner to that described in Experiment 1. Group 2 (DMI-H102, n=11) received pretreatment with desmethylimipramine (DMI, 5 mg/kg, I.P., CIBA Company Ltd.) followed 30 minutes later by injections of H102/09 (20 mg/kg, I.P.). DMI was dissolved in Ringer's solution in a volume of 2.5 mg/ml. The dose of DMI chosen was previously determined to be the highest dose shown to produce no effect on body weight and fluid intake. Group 3 (DMI, n=11) received injections of DMI (5 mg/kg, I.P.) alone, whereas Group 4 (n=9) received injections of the Ringer's solution (2 ml/kg). Approximately 4 hours following the injections, fluid consumption of the previous 24 hours 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.

**Results**

Daily ethanol intake in terms of mean percent of total fluid intake for each period of the experiment is shown in Figure 8. A two-way analysis of variance (groups x periods) yielded both a significant group effect ($F(3, 38) = 9.38, p < .001$) and period effect ($F(2, 76) = 76.7, p < .001$). More importantly, the analysis revealed a significant group x period interaction ($F(6, 76) = 12.30, p < .001$). Post hoc Tukey tests revealed the following. Treatment with H102/09 significantly reduced the preference for ethanol when compared to baseline and control levels ($p < .01$). Ethanol preference for this group significantly increased during the post-injection period as compared to the injection period ($p < .01$). Animals pretreated with DMI prior to H102/09 treatment significantly reduced their ethanol preference in relation to baseline and control levels ($p < .01$). More importantly, during the injection period the DMI-H102 group preferred ethanol to a greater degree than those animals treated with H102/09 alone ($p < .05$).
The control groups did not alter their preference for ethanol throughout the experiment ($p > .05$).

Daily absolute ethanol consumption in terms of mean grams per kilogram was calculated. A two-way analysis of variance (groups x periods) and subsequent post hoc tests revealed a significant difference in ethanol intake between the groups during the baseline period ($F(6, 76) = 10.88, p < .001$; Tukey test, $p < .01$). For this reason the data as shown in Figure 9 was calculated in terms of the difference from baseline in grams per kilogram for each day of the injection and post-injection periods. A two-way analysis of variance (groups x periods) revealed both a significant group effect ($F(3, 38) = 13.28, p < .001$) and period effect ($F(1, 38) = 87.02, p < .001$). More importantly, the analysis yielded a significant group x period interaction ($F(3, 38) = 3.84, p < .01$). Post hoc Tukey revealed that during the injection period, both the DMI-H102 and H102 groups significantly reduced their ethanol intake as compared to the control groups ($p < .01$). The DMI-H102 group consumed significantly more ethanol than did the H102 group during both the injection and post-injection periods ($p < .01$). The DMI and Ringer's control groups did not significantly differ in ethanol intake throughout the experiment ($p > .05$).
Figure 8. Ethanol consumption in terms of mean percent of total daily fluid intake in rats given DMI prior to H102/09 treatment.
Figure 9. Ethanol consumption in terms of difference from baseline (gm/kg) in rats given DMI prior to H102/09 treatment.
Discussion

The results of Experiment 3 demonstrate that animals pretreated with DMI prior to treatment with H102/09, reduced their ethanol intake to a significantly lesser extent than those animals treated with H102/09 alone. As previously mentioned, it has been suggested that a surplus of central serotonin may invade central norepinephrine neurons via the NE reuptake process, causing a functional depletion of norepinephrine (Shaskan & Snyder, 1970). Since H102/09 is a potent and selective central serotonin reuptake blocker, the resulting surplus of 5-HT in the synaptic gap could subsequently be taken up into the NE neurons. Therefore, it is possible that the observed effect on ethanol drinking following H102/09 treatment is actually a result of a functional depletion of norepinephrine. This view is supported by the findings of the present experiment. Blocking norepinephrine reuptake would theoretically eliminate the invasion of the surplus serotonin into the NE neurons. Animals treated in such a manner reduced their ethanol intake to a much lesser extent than animals treated with H102/09 alone. Based on this evidence, it is suggested that the observed attenuation of ethanol drinking following treatment with H102/09 may be partially due to a functional
depletion of norepinephrine. Since the dose of DMI used was relatively low, it is possible that the blockade of the NE reuptake process was not complete. Therefore, a more pronounced attenuation of the H102/09 effect could be observed by raising the DMI pretreatment dose. However, due to toxicity problems with higher doses of DMI, this at the present time cannot be done.
GENERAL DISCUSSION

Several investigators have demonstrated that increasing the central availability of serotonin in the synaptic gap produces an attenuation of ethanol consumption in laboratory rats (e.g. Geller, 1973). Consistent with this observation as described in Experiment 1, H102/09, a selective central 5-HT reuptake inhibitor, produced an attenuation of ethanol self-administration. Because of the lack of effect on body weight, on total fluid consumption and on the ingestion of a Quinine-Sucrose solution, it is suggested that the effect of H102/09 is specific to ethanol consumption. These results implicate the involvement of serotonin in the mediation of the positive reinforcing properties of ethanol. Since ethanol consumption significantly increased upon the termination of treatment, a treatment procedure producing longer lasting effects was desirable. The results from Experiment 2, in which extinction of the ethanol drinking response was accomplished, served to highlight two major issues. First, the extinction of the drinking response, during the treatment period, indicates that the reinforcing property of ethanol was diminished. This finding implicates serotonin in the mediation of the positive reinforcing properties of
ethanol. Secondly, with regards to treatment procedures for human alcoholics, this type of procedure, as described in Experiment 2, may have possible clinical applications. The value of such a program would be that during the time when alcohol reinforcement is diminished, new alternate behavior patterns can be introduced, thus resulting in the extinction of both primary and secondary reinforcing properties of the drug-taking behavior.

Although, as previously mentioned, there is some experimental evidence implicating serotonin in the pharmacological properties of ethanol, there is an abundance of evidence suggesting the involvement of catecholamines as mediators of some of the pharmacological properties of ethanol. More recently, it appears that primarily NE may mediate ethanol self-administration (e.g. Brown et al., 1977). Therefore, both increasing the availability of 5-HT and decreasing brain NE levels, results in an attenuation of ethanol consumption. It is, of course, possible that both central serotonin and norepinephrine could be involved in ethanol self-administration. However, Shaskan and Snyder (1970) have suggested an interaction between surplus central 5-HT and the norepinephrine neurons. This possibility was examined in Experiment 3, where it was found that by blocking the NE reuptake process prior to the treatment with
H102/09, resulted in a significant moderation of the effect produced by H102/09 alone. It is therefore suggested that the observed attenuation of ethanol consumption may be partially due to a functional depletion of NE. This is consistent with the notion that NE plays a role in the mediation of the positive reinforcing properties of ethanol. Whether other mechanisms can account for the observed attenuation of ethanol consumption in the preceding experiments, remains to be investigated further.
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