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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
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Montreal, Québec, Canada

September 1978

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 experi-
ment it was demonstrated that treatment with H102/09, a
selective serotonin reuptake inhibitor, specifically at-
tenuated ethanol consumption. This result was attrib-
uted to an increased availability of central serotonin,
suggesting that serotonin may in some way mediate the
positive reinforcing properties of ethanol. In an at-
tempt to extinguish the ethanol drinking response, eth-
anol 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 con-
sumption when presented with a free-choice between eth-
anol 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 in-
vasion 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.

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Table of Contents

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 eth-
anol (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 adminis-
tration of large quantities of ethanol demonstrate with-
drawal 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 ter-
minate 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-administrating ethanol, despite the occurrence
of withdrawal symptoms (Hunter, Riley, Walker & Freund,
1975; Deneau, Yanagita, Seevers, 1969; Winger & Woods,
1973; Mello & Mendelson, 1970; 1972).

The above mentioned data from human and animal ex-
periments 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 eth-
anol 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), intra-
gastrically (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 initia-
tion and maintenance of ethanol self-administration is
primarily due to ethanol's positive reinforcing proper-
ties 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 relation-
ship 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 catechol-
amines, acute ethanol treatment has been shown to sig-

nificantly increase the turnover of central catecholamines
(Karoum, Wyatt & Majchrowicz, 1976), having a greater ef-
fect 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 be-
tween central catecholamines and the pharmacological pro-
perties 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,
Strombom, Svensson & Waldeck, 1974) and suppress the
euphoric effects of ethanol in humans (Ahlenius, Carlsson,
Engel, Svensson & Sodersten, 1973). Electrical stimula-
tion of the lateral hypothalamus, an area rich in cate-
cholamines, has been shown to produce a permanent in-
crease in ethanol consumption in laboratory rats (Amit,
Stern & Wise, 1970; Amit & Stern, 1971), while electro-
lytic lesions of the ventral portion of this area has
been shown to attenuate the preference for ethanol (Amit,
Meade, Levitan & Singer, 1976). Indirect evidence fur-
ther 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 tetrahydroiso-
quinoline alkaloids (TIQ). It has been suggested that
once formed, these TIQ alkaloids may play a role in eth-
anol 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 intraventricu-
lar infusion of the same TIQ alkaloids produces no change
in the preference for ethanol (Amit, Brown & Smith, 1978).

Overall, these experiments suggest that catechol-
amines 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 eth-
anol, is derived from studies examining the pharmacolog-
ical 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 in-
hibit 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 adminis-
tration 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 eth-
anol-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 me-
diation 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 se-
rotonin (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 con-
tradictory. For example, Goldstein (1973) has suggested
that serotonin is not directly involved in ethanol induced
withdrawal reactions. It has been demonstrated subse-
quently however, that 5-HT receptor blockade produced by
methysergide significantly enhanced the ethanol-induced
withdrawal convulsions (Blum, Wallace, Schwertner &
Eubanks, 1976). The data from studies examining the ef-

fect of ethanol on central levels of serotonin are equal-
ly confusing. Following acute treatment with ethanol, se-
rotonin levels have been reported to be increased (Poho-
recky, 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 cen-
tral serotonin and ethanol consumption, reduction of cen-
tral 5-HT levels by p-chlorophenylalanine (PCPA) was orig-
inally 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). More-
over, reduction in central levels of 5-HT have also been
shown to increase (Geller, 1973) or have no effect on eth-
anol self-administration (Kiianmaa, 1975). It would there-
fore 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). Approximate-
ly 4 hours following the injections, fluid consumption
of the previous 24 hours was measured and tubes were re-
filled and replaced.

Post-injection period: For 5 days following the in-
jection 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 incre-
ments 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 ani-
mals were switched to a schedule consisting of an everyday
free choice between water and the QS solution. These ani-
mals 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 activity 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 photocel 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. Additional 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 spectrophotoflurometric 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 ($\underline{F}$ (3, 34)= 4.45, $\underline{p} < .01$) and period effect ($\underline{F}$ (2, 68)= 58.65, $\underline{p} < .001$). More importantly the analysis yielded a significant group x period interaction ($\underline{F}$ (6, 68)= 5.95, $\underline{p} < .001$). 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 ($\underline{p} < .01$) than the group treated with 10 mg/kg of H102/09 ($\underline{p} < .05$) 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 ($\underline{p} < .01$). During the post-injection period, all groups treated with H102/09 were significantly lower in intake than control levels ($\underline{p} < .05$). 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-
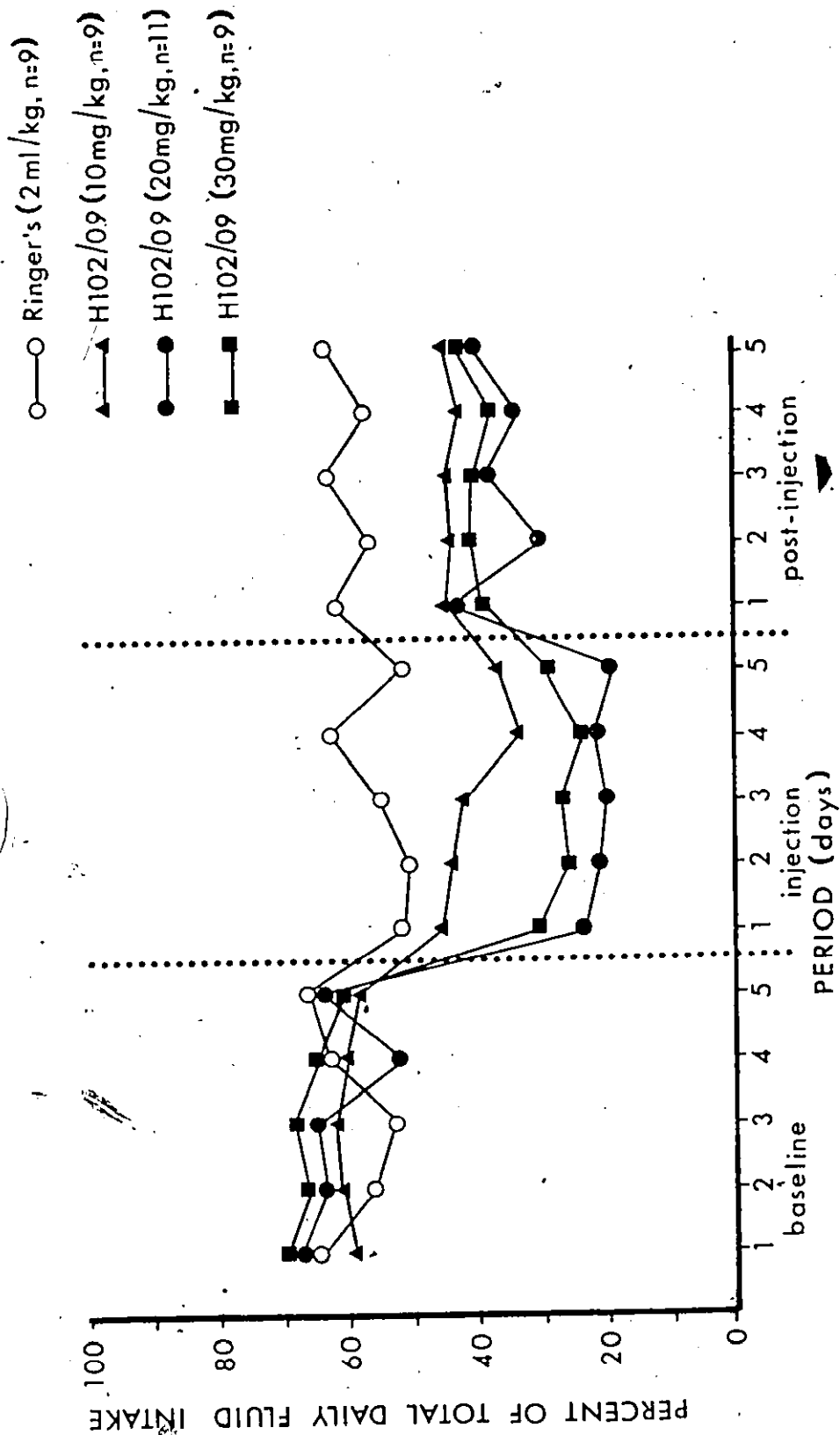
Figure 1. Ethanol consumption in terms of mean percent of
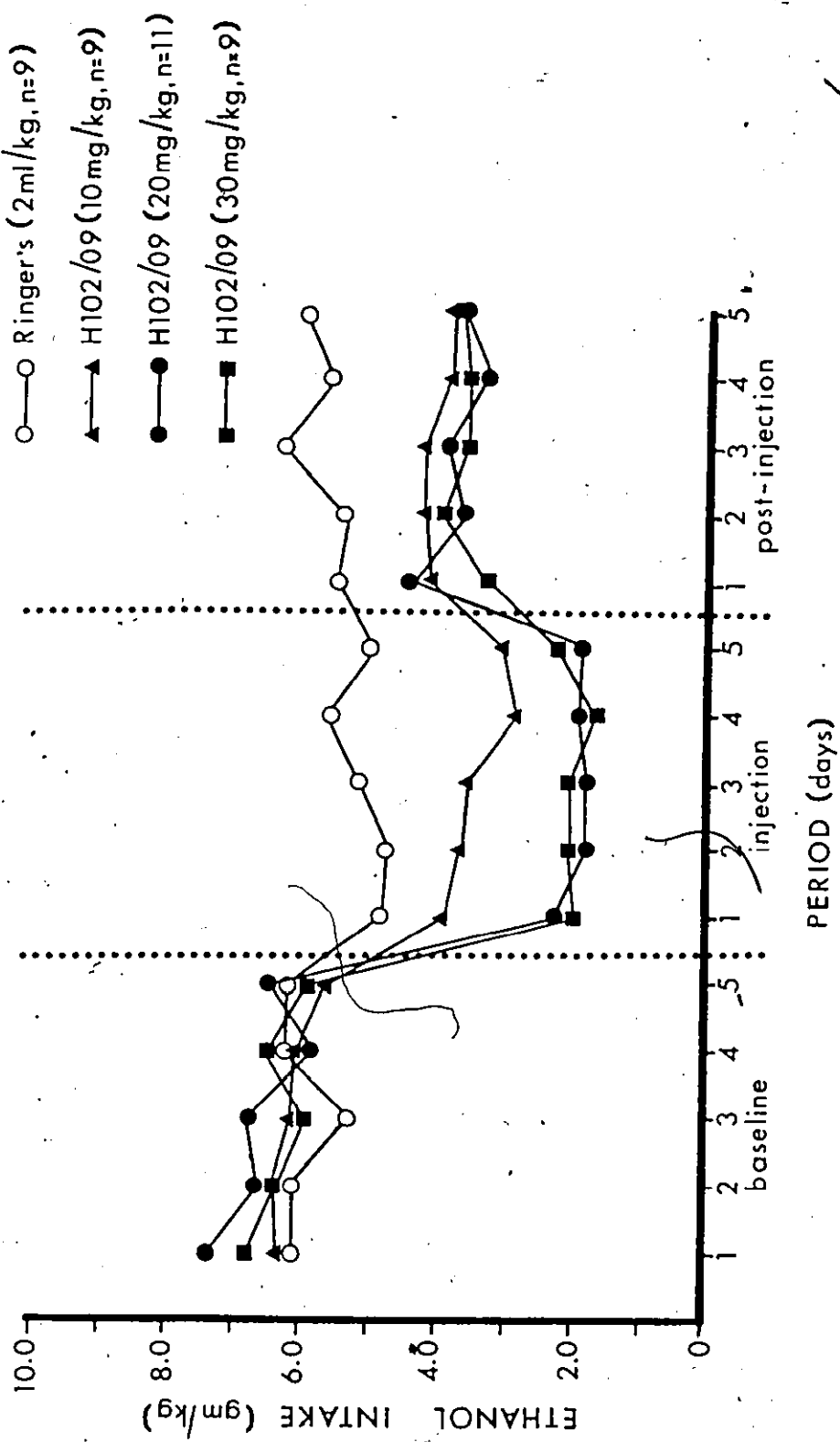total daily fluid intake in rats treated with
H102/09.

Figure 2. Ethanol consumption in terms of mean absolute ethanol intake in rats treated with H102/09.
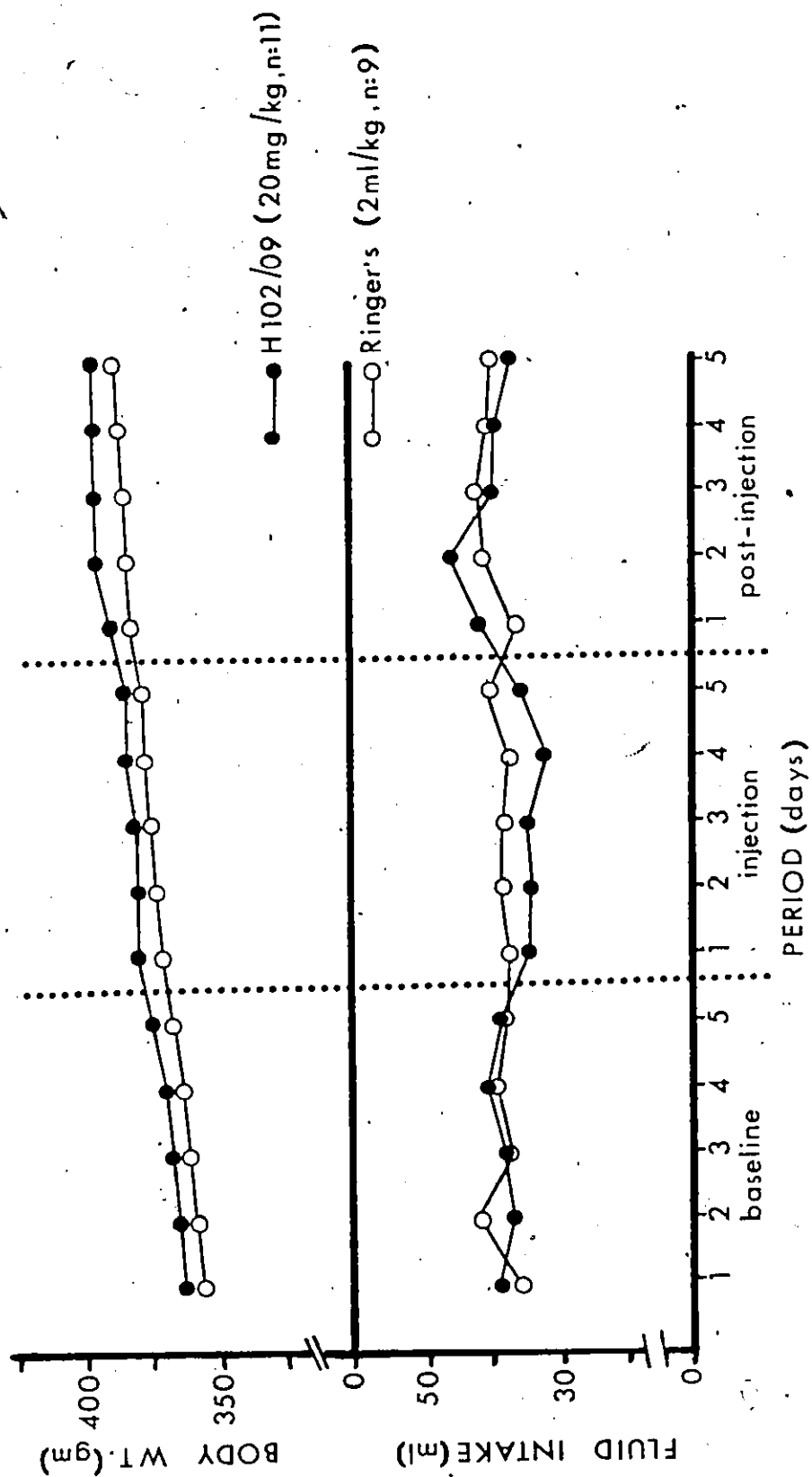
19

Figure 3.   Mean total fluid intake (ethanol and water consumption combined) and body weight in rats treated with H102/09.

20
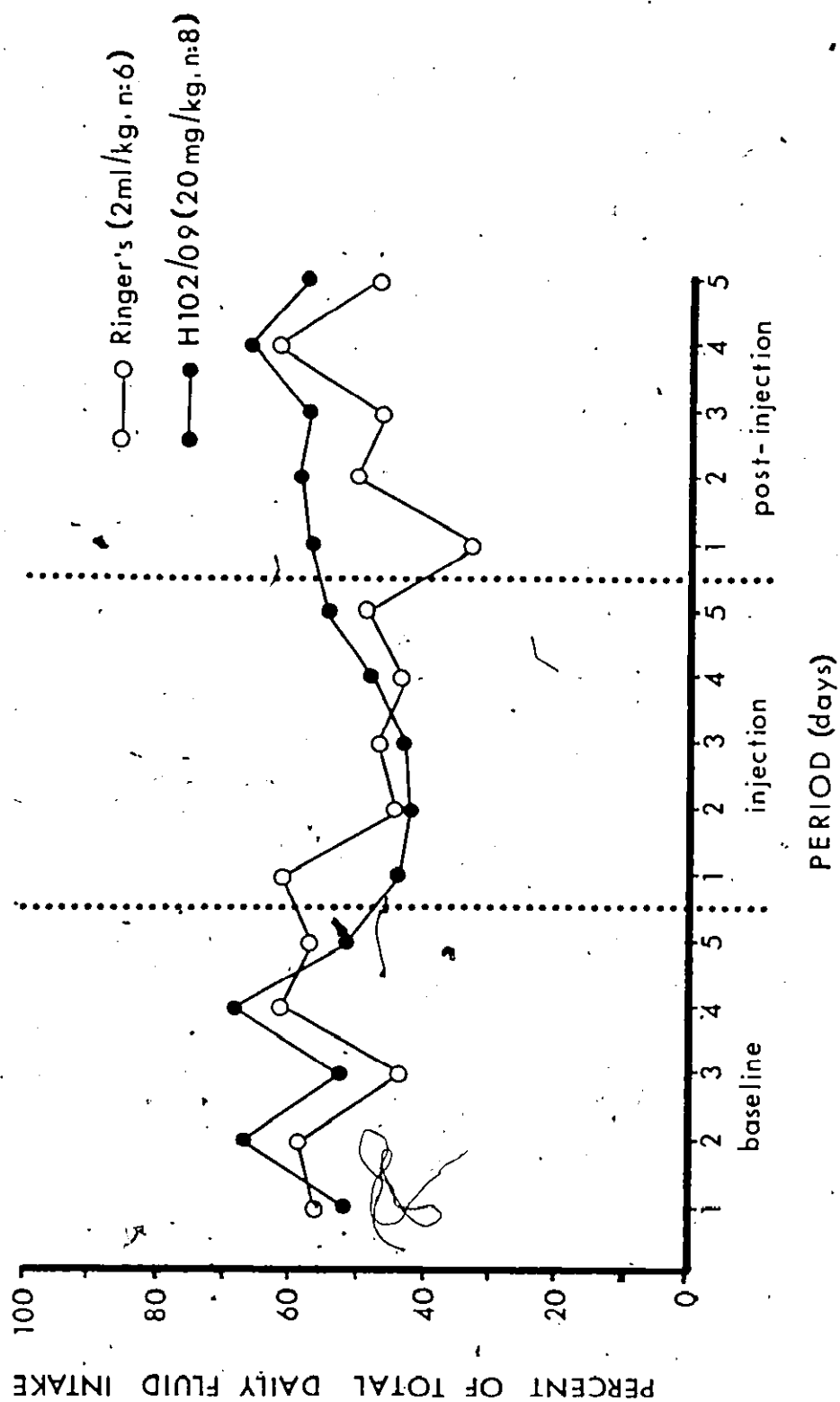

Figure 4. Quinine-Sucrose consumption in terms of mean percent
of total daily fluid intake in rats treated with
H102/09.

500 400 300 200 100 0

MEAN ACTIVITY COUNTS

Ringer's (2ml/kg, n:7)

H102/09 (20mg/kg, n:7)
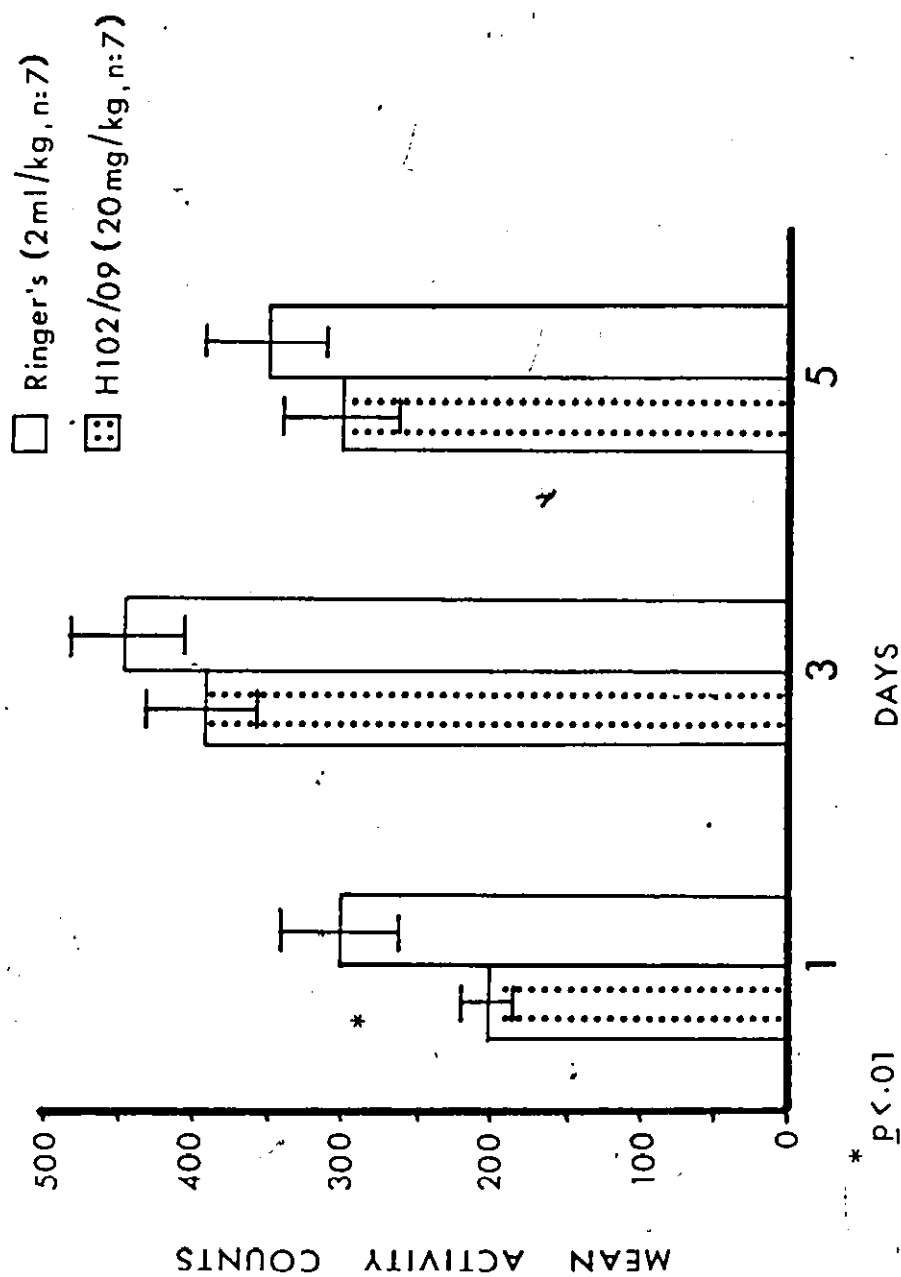
1   3   5

DAYS

* p < .01

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

Treatment	n	SEROTONIN		NOREPINEPHRINE		DOPAMINE	
		Levels (ng/g)	% Control	Levels (ng/g)	% Control	Levels (ng/g)	% Control
Controls 1 x (2ml/kg Ringer's)	4	422.09	-	444.71	-	539.86	-
H102/09 1 x (20 mg/kg H102/09)	4	468.21	110.92	425.35	95.6	619.84	114.80
Controls 3 x (2 ml/kg Ringer's)	4	393.71	-	432.73	-	613.99	-
H102/09 3 x (20 mg/kg H102/09)	4	412.29	104.72	436.00	100.7	631.22	102.8
Controls 5 x (2 ml/kg Ringer's)	4	439.77	-	446.05	-	693.78	-
H102/09 5 x (20 mg/kg H102/09)	4	387.02	88.0	461.38	103.4	654.16	94.3

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; Rosloff & 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, Nyback & 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 extin-
guish the ethanol drinking response. In order to ensure
the performance of the response in absence of reinforce-
ment, 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 al-
ternate days according to procedures described in Ex-
periment 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 consist-

ed of 5 consecutive alternate days of forced ethanol

consumption and 10 daily injections. Animals were in-

jected 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 al-

ternate days without injections for all animals.

Results

Daily ethanol consumption expressed in terms of per-

cent 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 (reacquisi-

tion), both the experimental groups significantly re-

duced their ethanol intake in relation to baseline ($p < .01$)

and control levels ($p < .01$). During days 10-15 of reac-

quisition 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 ($p > .05$).
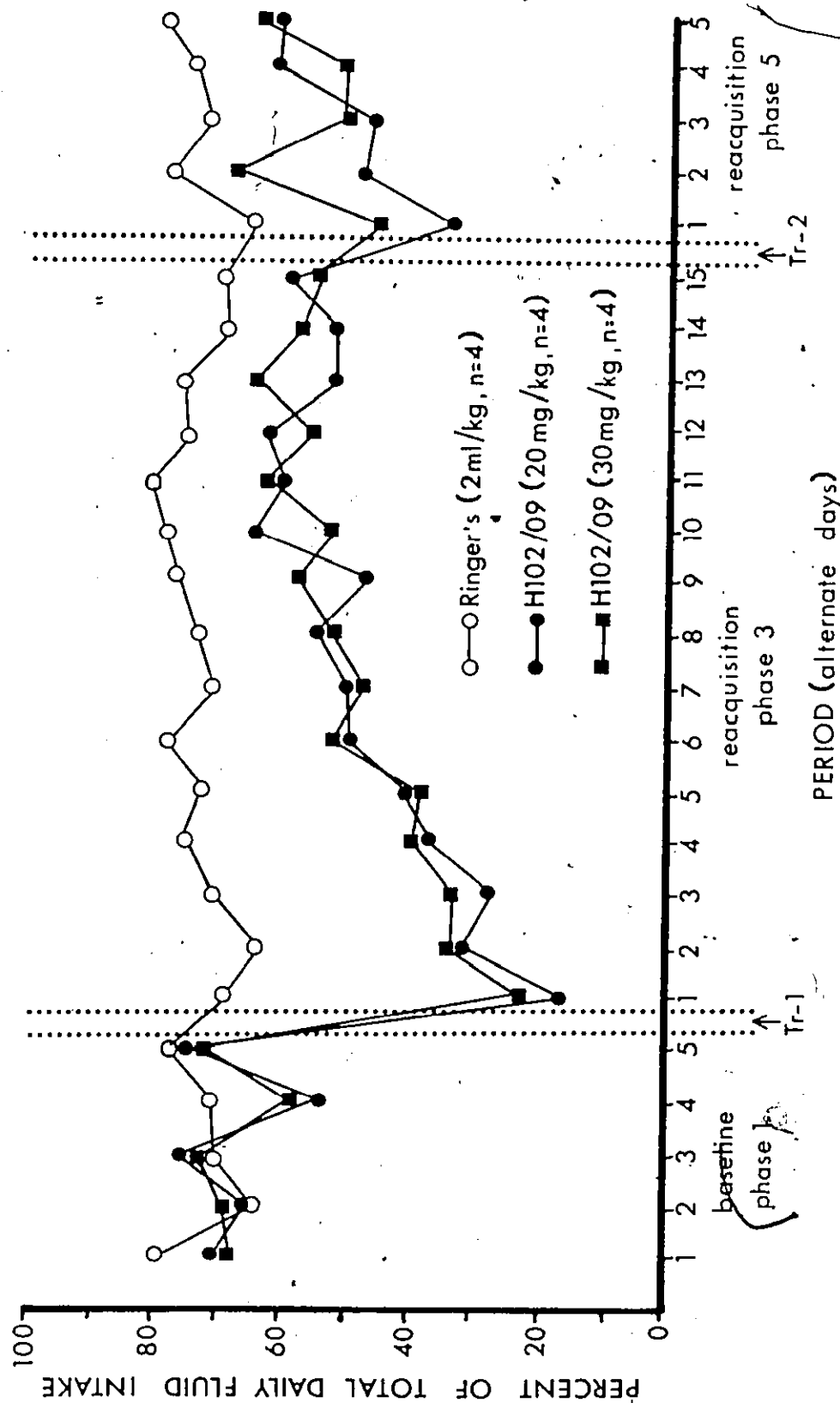
Figure 6. Ethanol consumption in terms of mean percent of total daily fluid intake in rats subjected to extinction treatments (Tr) with H102/09.
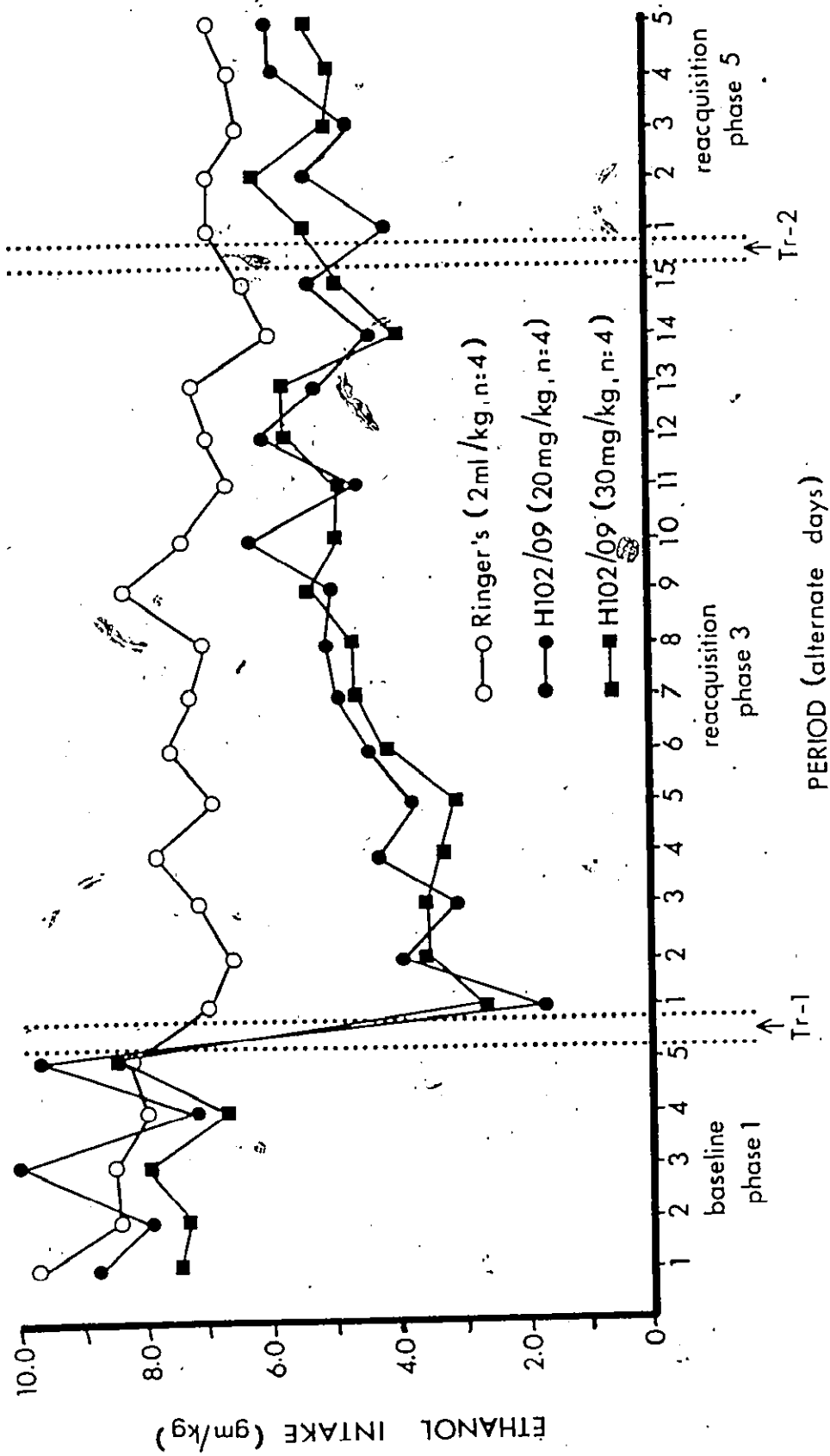
Figure 7. Ethanol consumption in terms of mean grams per kilogram in rats sub-jected to extinction treatments (Tr) with H102/09.

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 re-
sponse, that response must be performed in absence of
reinforcement. When reinforcement is once again availa-
ble, a characteristic reacquisition pattern reliably
occurs (Thompson & Pickens, 1975; Mackintosh, 1974).
In the present experiment, when ethanol was again avail-
able 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 consis-
tent with the report that following a second extinc-
tion 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). Shaskan 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 desmethylimipramine (DMI), a

selective neuronal NE reuptake blocker (Carlsson, Corrodi,

Fuxe & Hokfelt, 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 pre-

sented 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 al-

ternate 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-

tween 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 ($p > .05$).
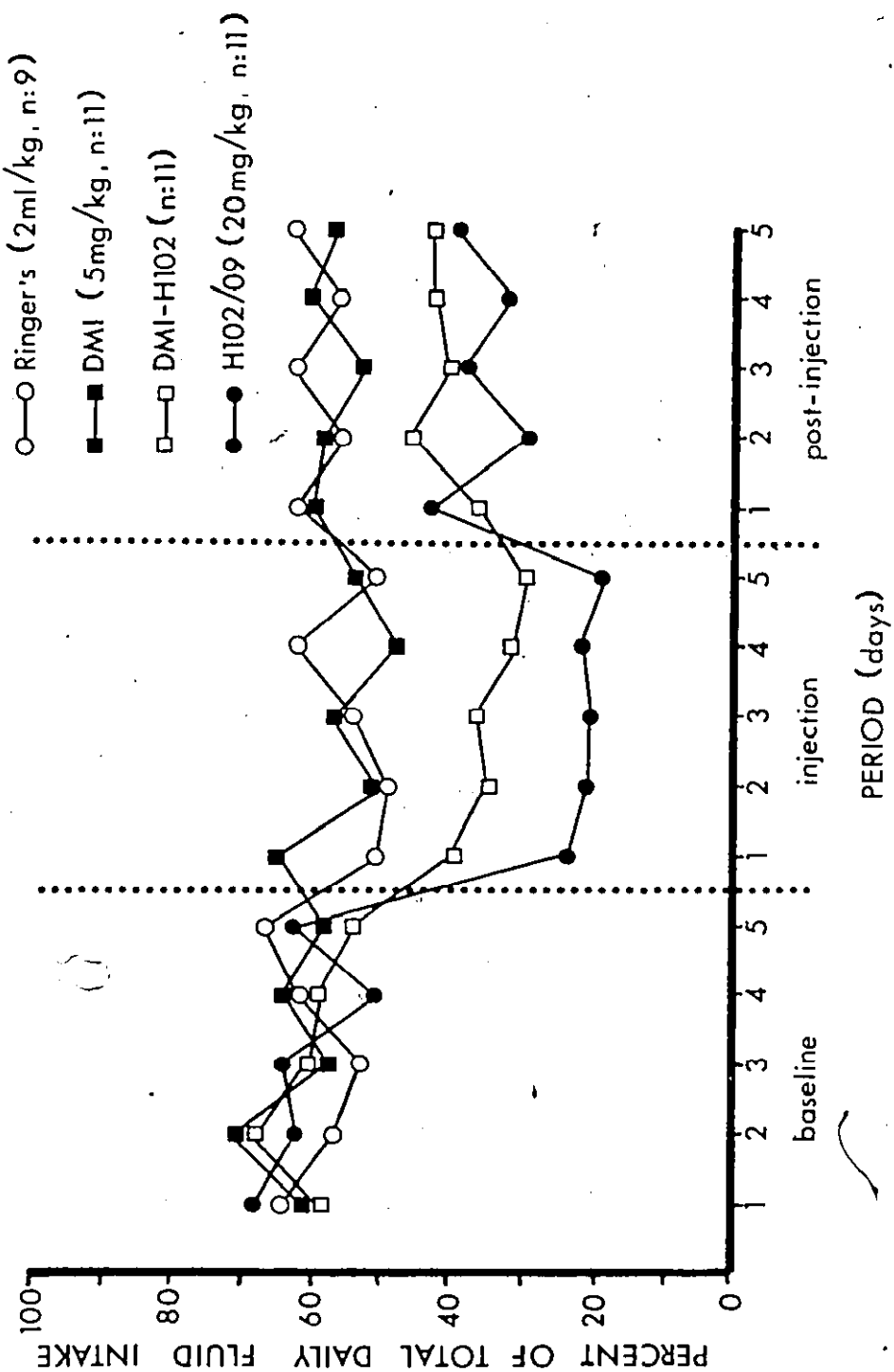
Figure 8. Ethanol consumption in terms of mean percent of total daily fluid intake in rats given DMI prior to H102/09 treatment.
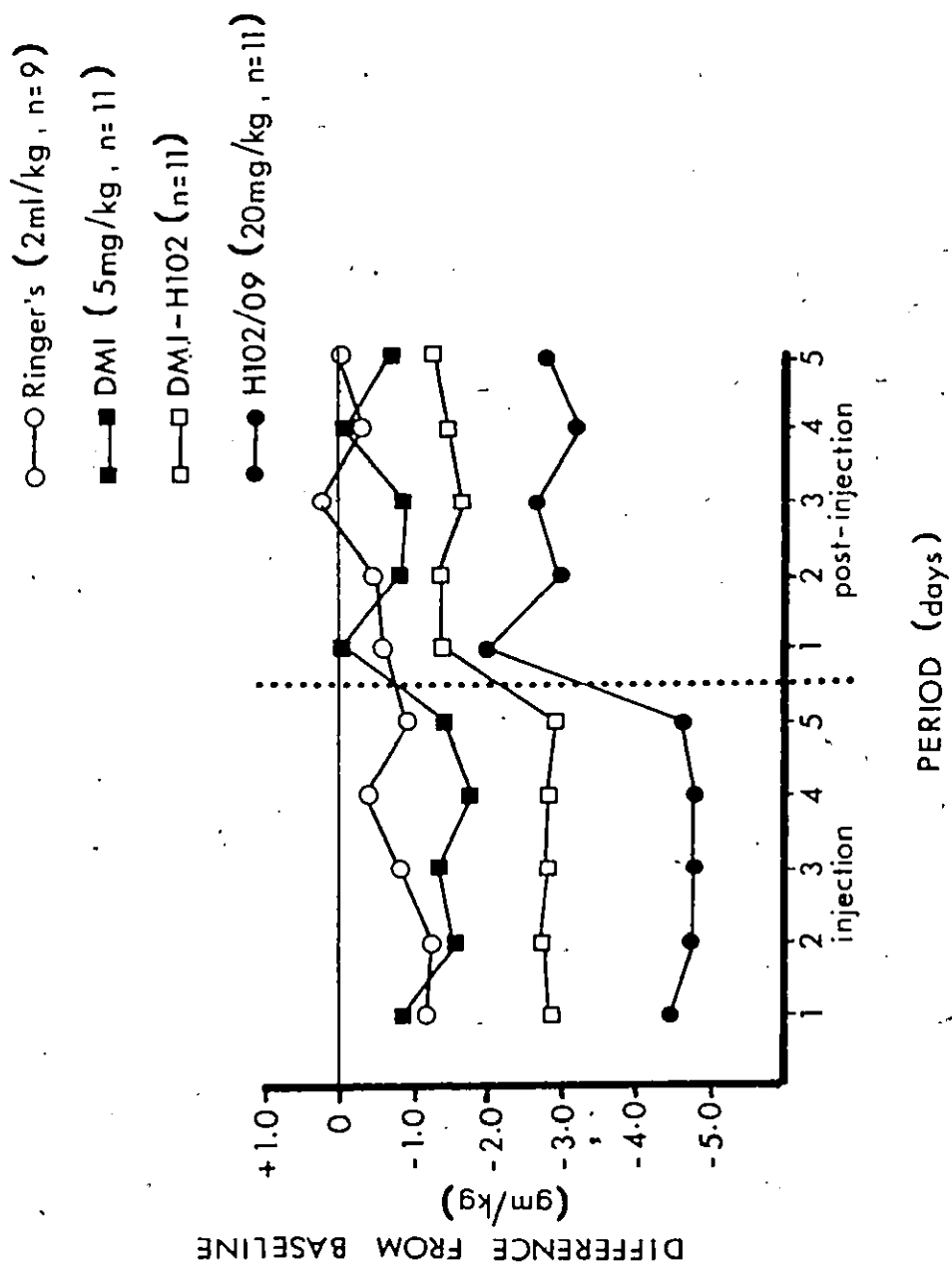
Figure 9. Ethanol consumption in terms of difference from baseline (gm/kg) in rats given DMI prior to H102/09 treatment.

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. How-
ever, due to toxicity problems with higher doses of DMI,
this at the present time cannot be done.

GENERAL DISCUSSION

Several investigators have demonstrated that in-
creasing the central availability of serotonin in the
synaptic gap produces an attenuation of ethanol con-
sumption in laboratory rats (e.g. Geller, 1973). Con-
sistent with this observation as described in Experi-
ment 1, H102/09, a selective central 5-HT reuptake in-
hibitor, produced an attenuation of ethanol self-admin-
istration. 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 ef-
fect 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 in-
creased upon the termination of treatment, a treatment
procedure producing longer lasting effects was desir-
able. 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, in-
dicates 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 de-

scribed in Experiment 2, may have possible clinical ap-

plications. 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 second-

ary reinforcing properties of the drug-taking behavior.

Although, as previously mentioned, there is some

experimental evidence implicating serotonin in the phar-

macological 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 avail-

ability 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 nor-

epinephrine could be involved in ethanol self-adminis-

tration. However, Shaskan and Snyder (1970) have sug-

gested an interaction between surplus central 5-HT and

the norepinephrine neurons. This possibility was ex-

amined in Experiment 3, where it was found that by block-

ing 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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