Pharmacology Biochemistry & Behavior, Vol 24, pp 463–468, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Conditioned Taste Aversion to Ethanol Induced by Zimeldine

K. GILL, K. SHATZ, Z. AMIT AND S O OGREN*

Centre for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University
1455 de Maisonneuve Blvd W., Montreal, Quebec H3G 1M8
**Astra Lakemedel AB, Sodertalje, Sweden*

Received 4 June 1984

GILL, K , K SHATZ, Z AMIT AND S O OGREN *Conditioned taste aversion to ethanol induced by Zimeldine* PHARMACOL BIOCHEM BEHAV **24**(3) 463–468, 1986 —Conditioned taste aversions (CTA) were induced to both ethanol and saccharin solutions with the serotonin uptake inhibitor Zimeldine When animals were pretreated with Zimeldine prior to the presentation of a novel flavour a CTA did not result However, there is evidence that Zimeldine induces an unconditioned suppression of drinking These results are discussed in terms of their relevance to Zimeldine's effects on voluntary ethanol consumption

Conditioned taste aversion Zimeldine Ethanol Serotonin Serotonin uptake blockade Ethanol consumption

A number of investigators have attempted to alter voluntary ethanol consumption in rats through manipulations of central serotonin levels Those manipulations which tend to decrease brain serotonin, i e , p-chlorophenylalanine or 5,6-dihydroxytryptamine have met with little consistent success [7, 11, 12, 15, 17] Studies which have employed agents such as 5-hydroxytryptophan (5-HTP) to raise brain serotonin levels, on the other hand, have consistently shown that such treatment decreased voluntary free-choice ethanol consumption [7, 8, 9, 16]

In addition, several studies have shown that treatment with drugs such as Zimeldine, Fluoxetine or Indalpin which block the reuptake of serotonin (5-HT), thereby increasing synaptic availability of 5-HT, reduced the voluntary oral consumption of ethanol [13, 20, 21, 22] While the effect of these drugs is consistent, a clear uncerstanding of the mechanisms underlying this phenomenon has not been elucidated (for a review of this literature see [1])

In 1978, Zabik, Liao, Jeffreys and Maickel [25] reported that a single dose of 5-HTP (50–200 mg/kg) administered to rats chronically consuming alcohol as their only source of fluid significantly reduced ethanol consumption In addition the 5-HTP administration resulted in a pronounced rejection of ethanol even to the point of death in 25% of the rats tested Zabik and Roache [26] further reported a study using a conditioned taste aversion (CTA) paradigm in which 5-HTP (100 mg/kg) was administered to rats following the consumption of novel tasting saccharin, tartaric acid or ethanol solutions 5-HTP produced a CTA to all the novel flavours used, however only those rats which received the novel ethanol solution paired with the 5-HTP injection exhibited persistent refusal to consume the paired fluid on subsequent test days These authors stated that this particularly strong CTA may not be a function of merely the associative learning mechanisms at work in the CTA paradigm but may involve some as yet undefined drug-drug interaction between 5-HTP and ethanol

These studies by Zabik *et al.* [25,26] were seen as being particularly relevant to the ongoing research project in our laboratory examining the effects of 5-HT uptake blockers on voluntary ethanol consumption The following experiments were carried out to determine whether (1) Zimeldine is capable of producing a CTA and (2) whether CTA induction may contribute to the reduction in voluntary free choice ethanol consumption following treatment with Zimeldine.

In the CTA paradigm fluid deprived rats are injected with a drug following the ingestion of a novel tasting fluid The CTA is measured by comparing the fluid consumption on the drug pairing day with the consumption on subsequent presentations of the fluid. CTA can be produced by a wide variety of emetic and psychoactive agents and is presumed to be a result of an association between the novel tasting fluid and some "aversive" introceptive cues produced by the drug state [2]

In an attempt to answer the above questions the present series of experiments were carried out in which Zimeldine treatment was paired with a series of novel tasting fluids including ethanol solutions

EXPERIMENT 1

As reported above, other investigators [26] have shown that the serotonin precursor 5-HTP appears to induce a strong CTA to ethanol solutions. These authors have suggested that there may be a unique interaction between serotonergic drugs such as 5-HTP and ethanol which result in particularly strong CTAs Therefore, in this study the CTA induced by Zimeldine was examined using both ethanol (8%) and saccharin (0.1%) as the novel flavours A dose of 20 mg/kg Zimeldine was chosen since it has been shown to be

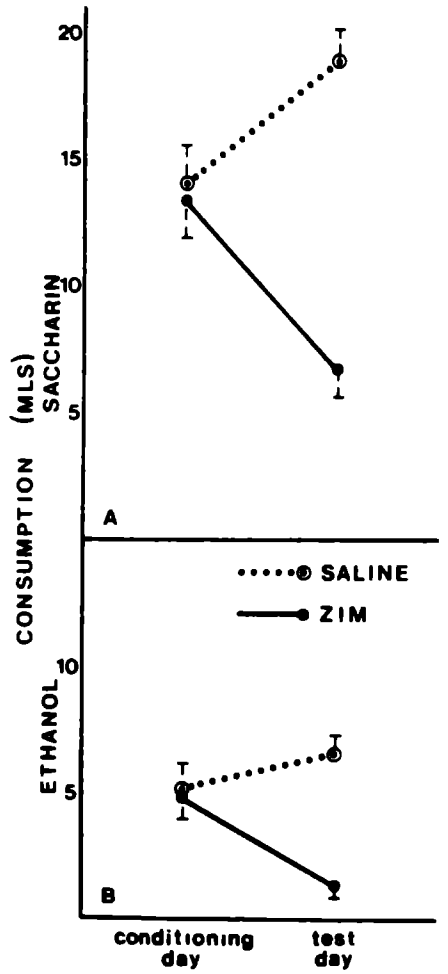

FIG 1 (A) Mean saccharin consumption for saline (n=7) and Zimeldine (n=8) treated groups on conditioning and test days (B) Mean consumption of an 8% ethanol solution for saline (n=8) and Zimeldine (n=8) treated groups on conditioning and test days

an effective dose in reducing voluntary free-choice ethanol consumption [20]

Animals

Thirty-one male Long Evans (Charles River, Canada) rats weighing 225–250 g at the start of the experiment were used Rats were housed singly in stainless steel cages in our animal colony under conditions of controlled humidity and temperature with a 12 hour dark-light cycle Food was available ad lib

Procedure

Following a 5 day acclimatization period, a water deprivation schedule was initiated where rats had access to water in their home cages for 30 minutes per day Water was presented in plastic stoppered drinking tubes fitted with steel ball-bearing spouts This schedule was maintained for 14 days and water consumption was recorded daily Rats were handled and weighed every 5 days. On the 15th day (Conditioning Day) the rats were randomly assigned to one of four groups Two groups of rats received saccharin (0 1%, w/v) as the novel fluid and were injected intraperitoneally with either Zimeldine (20 mg/kg, n=8) or saline (2 ml/kg, n=7) immediately following the 30 minute drinking period The remaining two groups received ethanol (8% v/v, prepared from 95% ethanol diluted with tap water) as the novel fluid and were injected IP with either Zimeldine (20 mg/kg, n=8) or saline (2 ml/kg, n=8) Zimeldine was dissolved in saline immediately prior to injection and was administered in a volume of 2 ml/kg Fluid consumption was measured and was considered as baseline consumption for subsequent comparisons For the following 5 days, rats received water in their drinking tubes On the 6th day following Conditioning Day rats again received their respective flavoured solutions for a 30 minute period (Test Day)

RESULTS

Figure 1 shows the mean saccharin and ethanol consumption on conditioning day and test day For the saccharin consuming group (Fig 1A) a two-way ANOVA with repeated measures revealed a significant drug effect, $F(1,13)=22\ 45$, $p<0\ 001$, as well as a significant drug × days interaction, $F(1,13)=29\ 65$, $p<0\ 001$ Analysis of the ethanol consuming (Fig 1B) group also revealed a significant drug effect, $F(1,14)=10\ 31$, $p<0\ 01$, and drug × days interaction, $F(1,14)=9\ 95$, $p<0\ 01$

DISCUSSION

The results of this experiment demonstrated that a single administration of Zimeldine produces a CTA to both novel saccharin and ethanol solutions There were large differences in baseline intake of the two solutions as measured on Conditioning Day however (i e , 14 04±1 16 ml saccharin compared to 5 18±0 98 ml ethanol), which make any comparisons of the CTAs difficult In the study reported by Zabik and Roache [26], the 5-HTP induced CTA to an ethanol solution was found to be stronger and longer lasting than that induced to a saccharin solution. In order to examine the possibility that Zimeldine may also produce differential CTAs to ethanol containing solutions the following experiment was carried out However, the solutions used for conditioning were matched in terms of their initial baseline consumption prior to the conditioning trial, in order to rule out possible confounds due to palatability and/or salience of the novel flavours

EXPERIMENT 2

The relative strength of CTAs induced by Zimeldine was examined in this study An effort was made to equate the initial palatability of the two novel flavours used for conditioning, one of which contained ethanol This was attempted by mixing ethanol with a "palatable" flavour

Animals

Thirty-one male Long Evans rats (Charles River, Canada) weighing approximately 300 g at the start of the experiment were used Rats were housed and maintained as per Experiment 1

Procedure

Following a 14-day water deprivation period rats were divided into two groups and were subjected to four preliminary preference tests on alternate days The aim of this preliminary testing was to determine a pair of flavoured solutions (one containing ethanol) which were comparable in

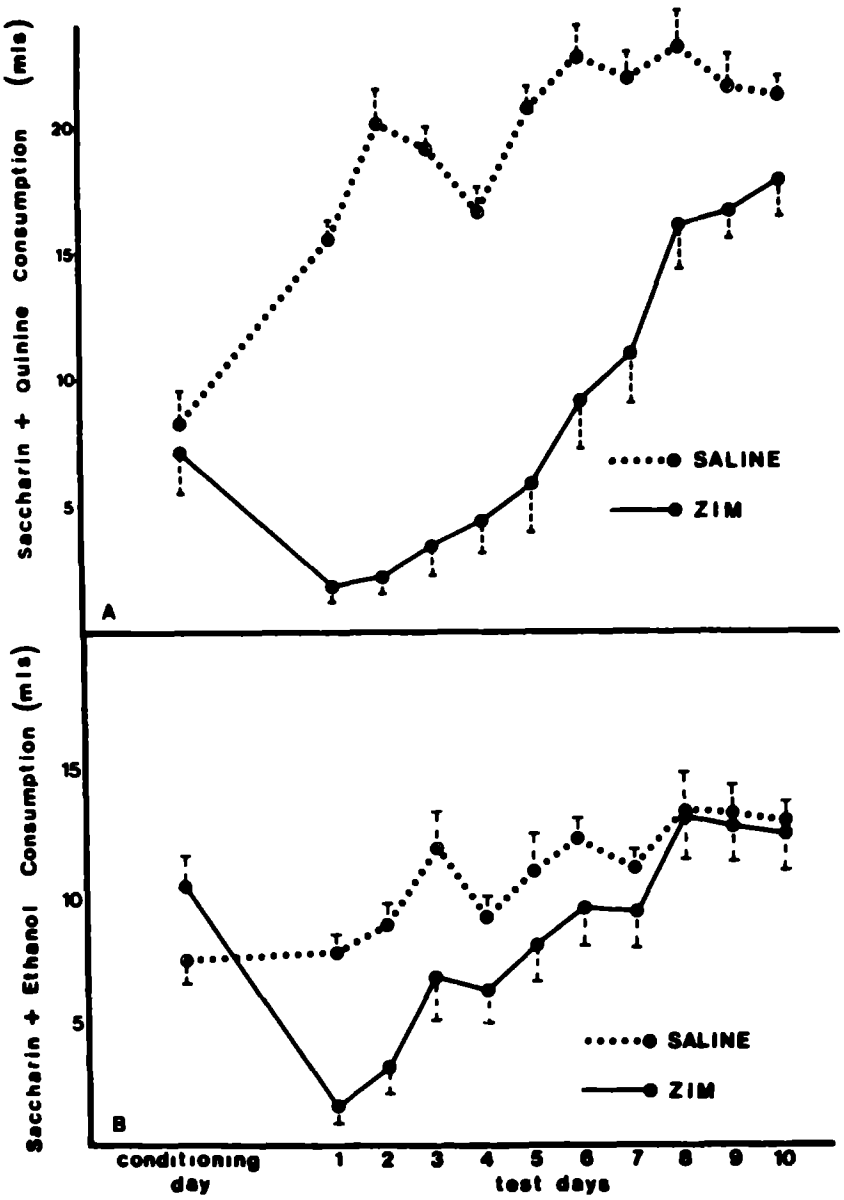

FIG 2 (A) Consumption of the saccharin + quinine (S+Q) solution for those animals treated with saline (n=8) or Zimeldine (n=8) (B) Consumption of the saccharin + ethanol (S+E) solution for those animals treated with saline (n=7) or Zimeldine (n=8)

terms of preference, thus ensuring approximately equal baseline consumption on conditioning day A total of eight different flavour combinations were tested, 4 in each group of animals Half of these solutions contained ethanol (8%, v/v) Both groups had the same exposure to ethanol prior to Conditioning Day and neither group was conditioned with a flavour that it had previously experienced during the preliminary testing The flavoured solutions were presented in the drinking tubes for 30 minutes at the regular drinking time On the intervening days between presentations of the flavoured solutions water was available in the drinking tubes Following the preliminary preference testing it appeared that of all the different solutions tested, saccharin (0.1%) + ethanol (8%) (S+E) and saccharin (0 1%) + quinine (0 005%) (S+Q) solutions were the most equally preferred Therefore, on Conditioning Day the two groups were presented with either the S+E or S+Q solution and were further subdivided so that immediately following the 30 minute drinking period they were injected with either saline (2 ml/kg) or Zimeldine (20 mg/kg) Following five intervening water days the four groups were again presented with their respective solutions for 10 consecutive test days

RESULTS

The mean consumption for those rats consuming the S+Q solution is shown in Fig 2A A two-way ANOVA with repeated measures indicated that there was a significant drug effect, $F(1,14)=62.42$, $p<0\ 001$, and a significant drug × days, $F(10,140)=16\ 25$, $p<0\ 001$, interaction Post-hoc (Tukey) tests revealed that the fluid consumption of the Zimeldine treated rats remained significantly ($p<0\ 01$)

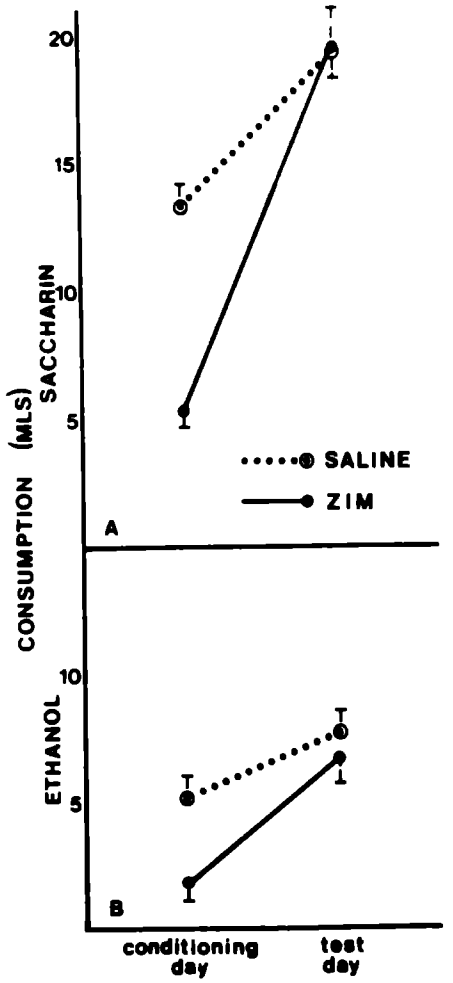

FIG 3 (A) Mean saccharin consumption for saline (n=6) and Zimeldine (n=7) treated animals (B) Mean ethanol consumption for saline (n=7) and Zimeldine (n=7) treated animals Animals received injections 4 hours prior to fluid presentation

below that of the saline treated controls for the first 8 test days When comparing Test Day intake to baseline consumption it appeared that the Zimeldine treated rats had returned to their own baseline on Test Day 4

Figure 2B indicates that rats consuming the S+E solution also show a CTA following Zimeldine treatment A two-way ANOVA with repeated measures indicated that there was no significant drug effect, $F(1,13)=2\ 25$, $p>0\ 05$, but there was a significant drug × days, $F(10,130)=5\ 93$, $p<0\ 001$, interaction Post-hoc (Tukey) tests indicated that the fluid consumption of the Zimeldine treated rats remained significantly ($p<0\ 01$) below control levels for the first three test days Comparisons of Test Day intake to baseline intake in the Zimeldine treated group indicated that these animals resumed drinking baseline levels of the S+E solution on Test Day 5

DISCUSSION

In this study the CTAs induced to both novel flavours were of comparable strength and duration The results of this study indicate, therefore, that there does not appear to be any particular interaction between Zimeldine and ethanol in terms of CTA The fact that the CTAs to both the S+Q and S+E solutions extinguished at comparable rates (on Test Days 4 and 5, respectively) lends credence to this notion

EXPERIMENT 3

In order to examine whether Zimeldine can induce a CTA when given as a pretreatment (i e , backward conditioning) the following experiment was carried out using a design in which Zimeldine or saline pretreatment was paired with novel saccharin or ethanol solutions on one conditioning day Animals were pretreated 4 hours prior to fluid presentation since Zimeldine has been shown to be maximally effective in blocking brain 5-HT uptake 2–4 hours following administration [23]

Animals

Twenty-seven male Long Evans rats (Charles River, Canada) weighing 225–250 g at the start of the experiment were used Rats were housed and maintained under the conditions described in Experiment 1

Procedure

Following the acclimatization and 14-day water deprivation period as described in Experiment 1, rats were randomly assigned to one of four groups Animals were pretreated (IP) with either saline (2 ml/kg) or Zimeldine (20 mg/kg) four hours prior to the presentation of novel saccharin (0 1%) or ethanol (8%) solutions For the next five days water was presented to the rats during the drinking period On the sixth day (Test Day) all groups were pretreated with saline 4 hours prior to presentation of their respective flavoured solutions

RESULTS

Figure 3 presents the Conditioning and Test Day fluid intake for the saccharin and ethanol consuming groups Both Zimeldine pretreated groups exhibited reduced fluid consumption on Conditioning Day as compared to their respective saline pretreated controls On the other hand, Test Day intake of the saccharin and ethanol solutions increased in all groups, indicating that a CTA had not developed Figure 3A presents the Conditioning and Test Day intake for the saccharin consuming groups A two-way ANOVA with repeated measures revealed significant drug, $F(1,11)=11\ 14$, $p<0\ 01$, day, $F(1,11)=96\ 03$, $p<0\ 001$, and drug × day interaction, $F(1,11)=16\ 62$, $p<0\ 01$, effects Figure 3B shows fluid intake on Conditioning and Test Days for the ethanol consuming groups Analysis revealed significant drug, $F(1,12)=7\ 32$, $p<0\ 05$, and day, $F(1,12)=41\ 95$, $p<0\ 001$, effects

DISCUSSION

It appears from these data that pretreating animals with Zimeldine prior to the presentation of an ethanol or saccharin solution did not result in a CTA These data indicate that since a CTA did not develop it appears unlikely that the reduction of voluntary free-choice ethanol consumption normally seen following pretreatment with Zimeldine [1] is due to this mechanism Of interest is the data from Conditioning Day which show that Zimeldine was capable of producing an unconditioned suppression of both ethanol and saccharin intake These findings must be further explored,

particularly considering the fact that Zimeldine has previously been shown to have no effect on the consumption of fluids other than ethanol solutions [20] (See note added in proof)

GENERAL DISCUSSION

The results of this series of experiments demonstrated that Zimeldine is capable of inducing a CTA to a number of novel flavours However, when animals were pretreated with Zimeldine in a manner similar to that employed in a voluntary consumption paradigm no CTA developed This indicates that CTA induction does not account for the action of Zimeldine on voluntary ethanol consumption This was evident upon examination of Experiment 3, where reduced fluid intake was exhibited only on Conditioning Day where Zimeldine injections actually preceded the presentation of the flavoured solutions rather than on the Test Day This pretreatment-induced suppression of drinking is not readily explained considering that Zimeldine is not known to possess sedative or general depressant effects on behavior [18,19] There is considerable evidence in the literature that 5-HT plays an inhibitory role in regulating consummatory behavior [10,24] Manipulations which increase central serotonin (i e , 5-HTP) or block the reuptake of serotonin (i e , Fluoxetine) have been shown to produce anorexia in rats [10] It is possible, therefore, that Zimeldine possesses an anorectic action similar to that reported for Fluoxetine Another possible explanation for this unconditioned suppression of saccharin intake is that Zimeldine alters taste perception This will require further investigation to substantiate

Another point concerns not only the results of Experiment 3 but also the effects of serotonergic manipulations in general which result, for example, in CTAs [26], anorexia [10,24] or decreases in voluntary ethanol consumption [9, 13, 22] There is considerable new evidence which suggests that peripheral serotonin should be considered as a possible mediator of these effects [3, 5, 6] Therefore, before ascribing an exclusive role to cerebral serotonin in the effects of 5-HTP [9,26] or Zimeldine [1] we should examine the possible role of peripheral serotonin in the actions of these compounds

The results of Experiment 2 indicate that Zimeldine, unlike 5-HTP [26], induced CTAs of approximately the same strength and duration to alcoholic and non-alcoholic novel flavours It is apparent, however, that when an ethanol containing solution is used as the CS, it is more salient and possesses aversive taste and post-ingestional pharmacological properties [4,14] which may have served to limit consumption even in saline treated controls That taste and orosensory factors are particularly important in the self-selection of ethanol solutions is well known [4,14] and is evident when examining the baseline intake of the ethanol containing solutions across all groups used in the present studies We must conclude, therefore, that CS-UCS associative learning mechanisms are responsible for the observed CTAs induced by Zimeldine

In conclusion, it appears unlikely that reduction in voluntary ethanol consumption produced by Zimeldine [20, 21, 22] is due to the induction of a CTA It may, however, affect general sensitivity to taste or produce anorexia which resulted in the suppression of fluid intake in Experiment 3 Further research is necessary to determine whether this phenomenon is particular to water deprived animals and whether it plays a role in Zimeldine's effects on the voluntary consumption of ethanol

REFERENCES

1 Amit, Z , E A Sutherland, K Gill and S O Ogren Zimeldine A review of its effects on ethanol consumption *Neurosci Biobehav Rev* **8**. 35–54, 1984

2 Berger, B Conditioning of food aversions by injections of psychoactive drugs *J Comp Physiol Psychol* **81**. 21–26, 1972

3 Carter, R B , L A Dykstra, J D Leander and J B Appel Role of peripheral mechanisms in the behavioral effects of 5-hydroxytryptophan *Pharmacol Biochem Behav* **9**: 249–253, 1978

4 Eckart, M J Conditioned taste aversion produced by the oral ingestion of ethanol in the rat *Physiol Psychol* **3**: 317–321, 1975

5 Ervin, G N , R B Carter, E L Webster, S I Moore and B R Cooper Evidence that taste aversion learning induced by 1-5-hydroxytryptophan is mediated peripherally *Pharmacol Biochem Behav* **20**: 799–802, 1984

6 Fletcher, P J and M J Burton Effects of peripheral serotonin on feeding and drinking in the rat *Pharmacol Biochem Behav* **20**: 835–840, 1984

7 Geller, I Effects of para-chlorophenylalanine and 5-hydroxytryptophan on alcohol intake in the rat *Pharmacol Biochem Behav* **1**· 361–365, 1973

8 Geller, I , R Purdy and J H Merritt Alterations in ethanol preference in the rat The role of brain biogenic amines *Ann NY Acad Sci* **215**. 54–59, 1973

9 Geller, I , R J Hartmann and F S Messiha Blockade of 5-HTP reduction of ethanol drinking with the decarboxylase inhibitor, Ro 4-4602 *Pharmacol Biochem Behav* **15**: 871–874, 1981

10 Goudie, A J , E W Thornton and T J Wheeler Effects of Lilly 110140, a specific inhibitor of 5-hydroxytrypatmine uptake, on food intake and on 5-hydroxytryptophan-induced anorexia Evidence for serotoninergic inhibition of feeding *J Pharm Pharmacol* **28**: 318–320, 1976

11 Kiianmaa, K Evidence for involvement of noradrenaline and against 5-hydroxytryptamine neurons in alcohol consumption by rats In *The Effects of Centrally Active Drugs on Voluntary Alcohol Consumption Satellite Symposium, 6th International Congress Pharmacology*, edited by J D Sinclair and K Kiianmaa *The Finnish Foundation for Alcohol Studies* **24**: 73–84, 1975

12 Kiianmaa, K Alcohol intake in the rat after lowering brain 5-hydroxytryptamine content by electrolytic midbrain raphe lesions, 5,6 dihydroxytryptamine or p-chlorophenylalanine *Med Biol* **54**: 203–209, 1976

13 Le Bourhis, B , G Aufrere and C J P Eriksson Role de la serotonine cerebrale dans la consommation voluntaire d'alcool chez le rat *Cahiers Ireb* **5**· 19–23, 1981

14 Lester, D , M Nackman and J Le Magnen Aversive conditioning by ethanol in the rat *Q J Stud Alcohol* **31**: 578–586, 1970

15 Myers, R D and W L Veale Alcohol preference in the rat Reduction following depletion of brain serotonin *Science* **160**: 1469–1471, 1968

16 Myers, R D , J E Evans and T L Yaksh Ethanol preference in the rat Interactions between brain serotonin and ethanol, acetaldehyde, paraldehyde, 5-HTP and 5-HTOL *Neuropharmacology* **11**· 539–549, 1972

17 Nackman, M , D Lester and J Le Magnen Alcohol aversion in the rat Behavioral assessment of noxious drug effects *Science* **168**· 1224–1246, 1970
18 Ogren, S O , J M Cott and H Hall Sedative/anxiolytic effects of antidepressants in animals In *Recent Advances in the Treatment of Depression*, edited by A Carlsson *et al* Copenhagen Munksgaard, 1981
19 Ogren, S O , S B Ross, H Hall, A C Holm and A L Renyi The pharmacology of zimelidine A 5-HT selective reuptake inhibitor *Acta Psychiatr Scand* **63**· Suppl 290 127–151, 1981
20 Rockman, G E , Z Amit, G Carr, Z W Brown and S O Ogren Attenuation of ethanol intake by 5-hydroxytryptamine uptake blockade in laboratory rats I Involvement of brain 5-hydroxytryptamine in the mediation of the positive reinforcing properties of ethanol *Arch Int Pharmacodyn Ther* **241** 245–259, 1979
21 Rockman, G E , A Amit, G Carr and S O Ogren Attenuation of ethanol intake by 5-hydroxytryptamine uptake blockade in laboratory rats II Possible interaction with brain norepinephrine *Arch Int Pharmacodyn Ther* **241** 260–265, 1979
22 Rockman, G E , Z Amit, Z W Brown, C Bourque and S O Ogren An investigation of the mechanisms of action of 5-hydroxytryptamine in the suppression of ethanol intake *Neuropharmacology* **21** 341–347, 1982
23 Ross, S B and A L Renyi Inhibition of the neuronal uptake of 5-hydroxytryptamine and noradrenaline in rat brain by (Z)- and (E)-3-(4-bromophenyl)-N, N-dimethyl-3(3-pyridyl) alylamines and their secondary analogues *Neuropharmacology* **16** 57–63, 1977
24 Wurtman, J J and R J Wurtman Fenfluramine and fluoxetine space protein consumption while suppressing caloric intake by rats *Science* **198:** 1178–1180, 1977
25 Zabik, J E , S S Liao, M Jeffreys and R P Maickel The effects of DL-5-hydroxytryptophan on ethanol consumption by rats *Res Commun Chem Pathol Pharmacol* **20:** 69–78, 1978
26 Zabik, J E and J D Roache 5-Hydroxytryptophan-induced conditioned taste aversion to ethanol in the rat *Pharmacol Biochem Behav* **18**· 785–790, 1983
27 Gill, K , Z Amit and S O Ogren The effects of Zimeldine on voluntary ethanol consumption Studies on the mechanism of action *Alcohol* **2**· 343–347 1985

NOTE ADDED IN PROOF

Subsequent to the submission of this manuscript additional data relating to this issue has been collected, see [27]