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Tetrahydro-β-Carbolines: Effect on Alcohol Intake in Rats

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AIRAKSINEN, M. M., M. MÄHÖNEN, L. TUOMISTO, P. PEURA AND C. J. P. ERIKSSON. *Tetrahydro-β-carbolines: Effect on alcohol intake in rats.* PHARMACOL BIOCHEM BEHAV **18**: Suppl. 1, 525–529, 1983.—Some β-carbolines, such as tetrahydro-β-carboline (THBC) and 6-methoxy-THBC, occur normally in mammalian tissues, and 1-methyl-THBC has been found in human blood after alcohol intake. Continuous intraventricular (ICV) infusion of THBC and 1-methyl-THBC for 14 days was shown to increase voluntary alcohol intake in rats during the second week of infusion. In this study the experimental arrangement was slightly modified. Alcohol was offered for 7 days before the start of the 14 days of ICV infusion with Alzet® minipumps and alcohol concentration (3–30% v/v) was increased every second day. The rats consumed less alcohol in the second day with the same concentration. Also, the dose of 47 nmoles/hr of 1-Me-THBC increased the voluntary alcohol intake over the controls, but only during the last 7 days. The same dose of 6-MeO-THBC, a serotonergic β-carboline, was ineffective. Neither drug changed the total fluid intake. This study suggests that the increased voluntary alcohol intake by THBC's is not due to their serotonergic effect. A hypothesis concerning a possible involvement of opiate receptors is presented.

Alcohol preference Rat 1-Methyl-tetrahydro-β-carboline 6-Methoxy-tetrahydro-β-carboline Intracerebroventricular infusion

SEVERAL β-carbolines have been found in mammalian tissues and urine [2]. Some of them, like 1,2,3,4-tetrahydro-β-carboline (THBC, tryptoline, noreleagnine), and 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC, 5-methoxytryptoline), the condensation products of tryptamine and 5-methoxytryptamine with formaldehyde, respectively, are normal body constituents [8, 13, 14, 15, 17, 28]. 1-Methyl-THBC (1-Me-THBC, methtryptoline, tetrahydroharmane) is formed by condensation with acetaldehyde, and thus it occurs in plasma and platelets of healthy persons after alcohol intake [24]. In human urine, 1-Me-THBC has been reported to be present normally, though it seems to be increased in alcoholics and after a load with alcohol [6,27]. Many effects of β-carbolines resemble those of alcohol withdrawal [3].

THBC and some tetrahydroisoquinolines (TIQs) have been reported to increase alcohol preference in rats when they were repeatedly injected intracerebroventricularly (ICV) [9, 10, 19, 20, 21]. There are also reports indicating that TIQs have had no effect on alcohol preference [30,32]. In an earlier study we gave THBC and 1-Me-THBC to rats as continuous ICV infusions with Alzet® minipumps and found an increase in alcohol consumption [35]. We have now repeated the experiment with 1-Me-THBC with slightly modified experimental arrangements and, in addition, used 6-MeO-THBC.

METHOD

1-Me-THBC in artificial cerebrospinal fluid (CSF) containing ascorbic acid was infused into male Kuo:WIST rats for 14 days as described in our earlier paper [35]. The ICV cannula was connected to an Alzet® 2002 minipump situated under skin of the neck. Water, an empty bottle, and alcohol in increasing concentrations (3–30% v/v) were available. In our earlier experiments, the alcohol concentration was increased daily, starting from the time of operation for cannula implantation. In the present study, alcohol was offered for 7 days before the operation and the start of infusion, after which the alcohol concentration was increased only every 2nd day (see Fig. 1). Blood samples for blood alcohol determination [11] were taken from the tail during the last night when 30% ethanol was offered (between 0000 and 0100). The position of the cannula in the brain ventricle was checked at the end of the infusion as described earlier [35].

1-Me-THBC and 6-MeO-THBC left in the minipump were extracted into alkaline diethyl ether and the stability of the compounds was verified by chromatographing the extracts on silica gel (TLC plates Silica gel 60 GF-254 Merck AG. Darmstadt, Germany; solvent: n-butanol + acetic acid + water, 4:1:1). The spots were made visible with Dragendorf reagent [33]. One Dragendorff-positive spot (R_f 0.53) was found on the thin layer chromatography plate of extracts containing 1-Me-THBC. Two spots were visible on TLC

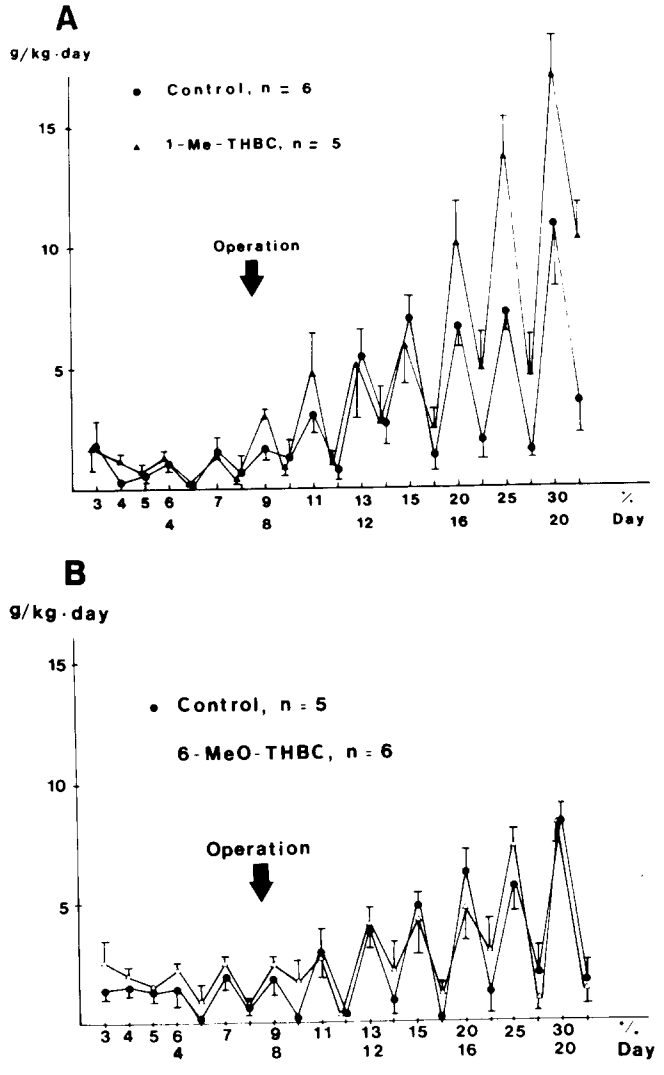

FIG. 1. Intake of alcohol (calculated as 100% ethanol) in rats during ICV infusion of 47 nmoles/hr of 1-Me-THBC (A) or 6-MeO-THBC (B) or of artificial CSF (controls). Insertion of the ICV cannula and minipumps occurred on the eighth day. Means and S.E.M. are indicated.

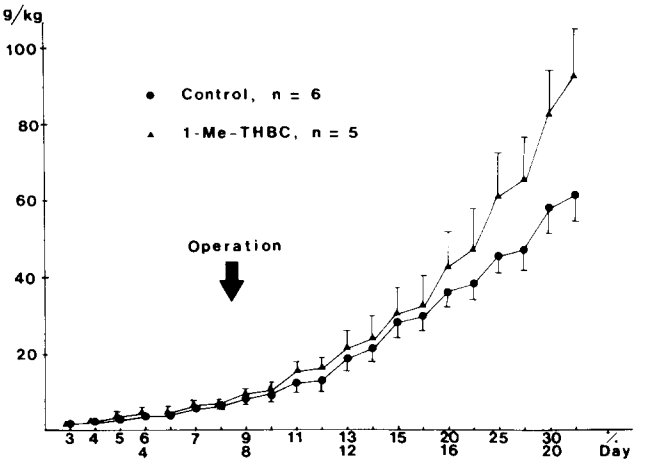

FIG. 2. Cumulative alcohol consumption of rats during ICV infusion of 47 nmoles/hr of 1-Me-THBC or of artificial CSF.

layer of extracts with 6-MeO-THBC; the main compound (R_f 0.44, about 80%) was isolated for mass spectrometry. The extracts were also analyzed with mass spectrometry via direct inlet probe at 70 eV (Jeol 300 D, Japan). The mass spectra, m/z (rel. int. %) of 1-Me-THBC extracts were 186(55), 185(20), 172(10), 171(100), 169(10), 157(40), 156(43), 154(15), and 144(14), and those of 6-MeO-THBC extracts were 202(51), 201(47), 199(13), 174(17), 173(100), 157(11), and 156(56).

1-Me-THBC and 6-MeO-THBC were synthetized by the Pictet-Spengler condensation reaction [12,26] in our laboratory. The mass spectra were consistent with the assigned structures of the synthetized compounds. The purities measured with thin layer chromatography and mass spectrometry were about 99 percent. Other chemicals were commercial and of analytical grade. The doses refer to the free base of the drug.

The consumption of alcohol solution was converted to intake of absolute alcohol. The alcohol intake in grams per weight of the animal is given per day as well as cumulatively during a given period (week). Student's *t*-test was used to evaluate the significance of differences between the means of treated and control groups.

RESULTS

Alcohol Consumption

Alcohol intake increased over that in controls when 47 nmol/hr/rat of 1-Me-THBC was infused, but the same dose of 6-MeO-THBC was ineffective (Figs. 1–3). Total fluid intake did not increase with either drug. It decreased temporarily after the operation in all groups (Fig. 4). The volume of fluid consumed as alcohol solution did not decrease with increasing alcohol concentrations in 1-Me-THBC group as it did in controls.

Contents of the Minipumps

Thin layer chromatography and mass spectrometry revealed no degradation products with 1-Me-THBC in the minipump at the end of the experiment. A small amount (less than 20%) of an unidentified degradation product was found together with 6-MeO-THBC.

Blood Alcohol

Blood alcohol concentration was measurable (≥0.1 mg%) in two controls out of 12 (0.19 and 0.60 mg%) in 3/6 1-Me-THBC rats (0.10, 0.36 and 0.60 mg%), and in 1/6 6-MeO-THBC rat (0.35 mg%). All the high values (≥0.35 mg%) represented those rats which were also drinking more than the others during the last days.

DISCUSSION

Voluntary alcohol intake increased in 1-Me-THBC treated rats as it did in our earlier study [35]. The fact that the effects were found only during the last 6 days when high alcohol concentrations were offered seems to suggest de-

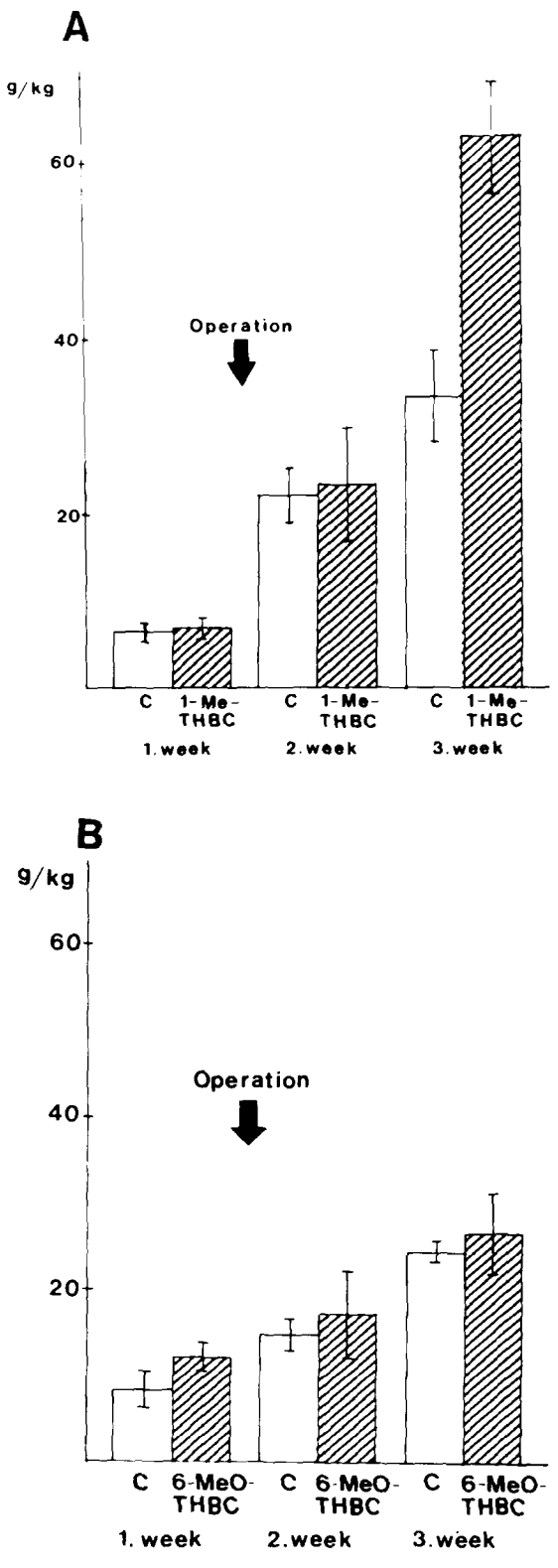

FIG. 3. Weekly consumption of alcohol during three consecutive weeks, the first week without ICV infusion and the second and third weeks with ICV infusion of 47 nmoles/hr of 1-Me-THBC (A) or 6-MeO-THBC (B) or of artificial CSF (C in both experiments).

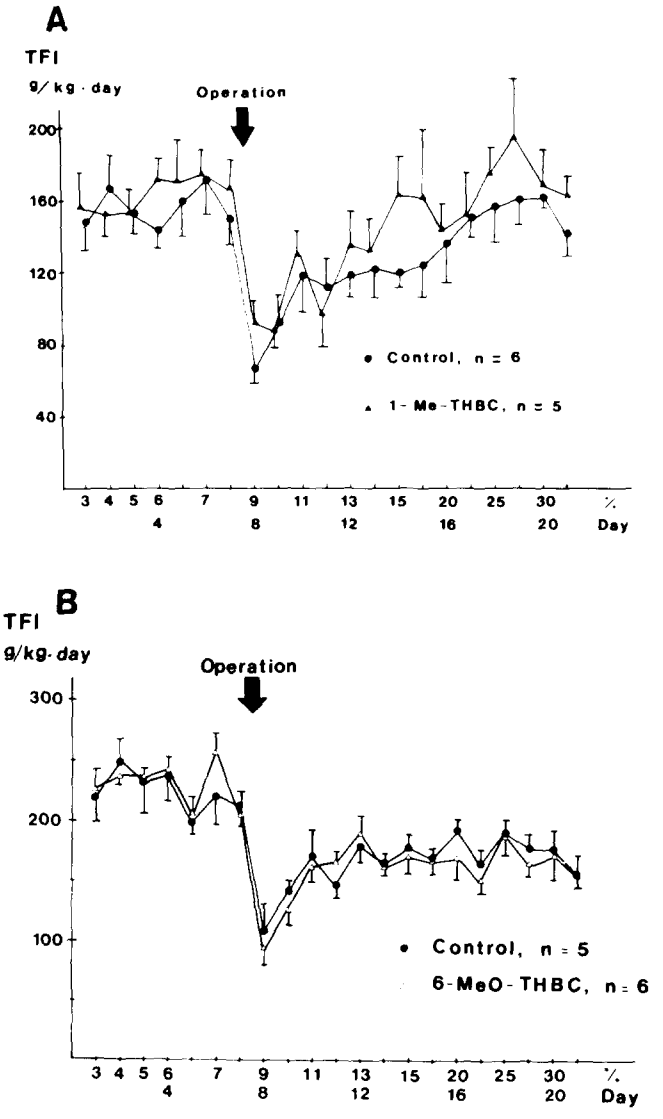

FIG. 4. Total fluid intake (TFI) during ICV infusion of 47 nmoles/hr of 1-Me-THBC (A) or 6-MeO-THBC (B) or of artificial CSF. Insertion of the ICV cannula and minipump occurred on the eighth day.

creased ability to taste of the strong alcohol. Other possibilities are that a certain cumulation of the drug effects or a certain time is necessary, e.g., before the rats learn that alcohol relieves the possible dysforic effects of 1-Me-THBC. Although vomiting and dysphoric effects corresponding to those of nalorphine have been described as parts of the action of harmaline and some other β-carbolines [3,23], the effects of 1-Me-THBC have not been studied in man. A prolonged period of alcohol consumption is not required, because in our earlier study, where alcohol was offered only after the operation, the results were similar to those in the present study: an increase in alcohol consumption over the controls occurred only during the last week of infusion (Figs. 1–3). Some high blood alcohol concentrations were found, suggesting that some rats may have consumed alcohol in order to obtain the inebriating effects of alcohol.

Unexpectedly, the rats regularly consumed alcohol less in the second of two successive days of presentation of a given concentration of alcohol (Fig. 1). We cannot explain this phenomenon. However, increasing the alcohol concentration seems to be useful when inducing "alcoholism" in rats, and high or increasing alcohol concentration may be essential for the effects β-carbolines. In an unpublished study from our laboratory, no clear effect of 1-Me-THBC was seen when a fixed low alcohol concentration (11%) was maintained through the experiment.

Many β-carbolines inhibit MAO-A and amine uptake and show serotonergic effects [3]. However, 6-MeO-THBC, which is more serotonergic than 1-Me-THBC [1, 5, 16], did not change alcohol preference. Actions through other amines or on membranes generally [25] or on specific receptors seem possible. Actions on opiate receptors are particularly interesting, although THBC and 1-Me-THBC showed less affinity for opiate μ-receptors [4] than some other β-carbolines. Affinity for δ-receptors may be more important and with them β-carbolines, including 1-Me-THBC, were found to bind with higher affinity than to μ-receptors (Airaksinen, Steidel and Saano, unpublished). It is interesting that while an acute ethanol administration may increase the affinity of opiate μ-receptors, both acute and chronic ethanol treatment decreases the affinity of δ-receptors [34]. We can speculate that since β-carbolines seem to be antagonists or partial agonists rather than pure agonists of opiate receptors ([4,18], Airaksinen, Steidel and Saano, unpublished), they can decrease the effects of endogenous opioids, thus causing dysphoria which alcohol may relieve. The fact that morphine decreases alcohol intake in rats [29,31], and naltrexone blocks ethanol self-administration apparently by removing the reinforcing properties of ethanol [7], are in favor of this hypothesis, although the results with naloxone are partly conflicting (e.g., [22]). It remains to be shown if this hypothesis can explain the increased voluntary alcohol intake in rats and if the formation of 1-Me-THBC and/or other condensation products of acetaldehyde participate with this or other mechanisms in the development of alcoholism in man.

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