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Differential Actions on Voluntary Alcohol Intake of Tetrahydroisoquinolines or a β-Carboline Infused Chronically in the Ventricle of the Rat

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MYERS, R. D. AND C. L. MELCHIOR. *Differential actions on voluntary alcohol intake of tetrahydroisoquinolines or a β-carboline infused chronically in the ventricle of the rat.* PHARMAC. BIOCHEM. BEHAV. 7(4) 381–392, 1977. – The alcohol drinking pattern of rats of the Sprague-Dawley strain was determined initially in a free-choice situation where water was offered simultaneously with a solution of alcohol, which was increased in concentration from 3 to 30% over a 12-day interval. A special cannula for the chronic infusion of drugs was implanted subsequently in the lateral cerebral ventricle of each animal. Then, one of four tetrahydroisoquinolines (TIQ) or a β-carboline (tryptoline) was infused directly into the ventricle in a dose ranging from 0.0004–4.0 μg per 4.0 μl. Each amine-aldehyde metabolite, dissolved in a CSF vehicle, was delivered over a 58 sec interval every 30 min around the clock throughout a second 12-day alcohol preference test. Control infusions of CSF failed to alter the typical aversion to alcohol exhibited by this strain. TIQ itself, infused similarly into the brain, was also without effect. The chronic intraventricular infusion of 1-methyl-3-carboxy-6,7-dihydroxy-TIQ (1M-3C-6,7DHTIQ) or 4,6,7-trihydroxy-TIQ (4,6,7 THTIQ) produced only a moderate increase in voluntary alcohol intake in terms of both the proportion measure and absolute grams of alcohol ingested. However, salsolinol, 6-methoxy-4,7-dihydroxy-TIQ (6M-4,7DHTIQ) and tryptoline all evoked a significant enhancement in the rats' alcohol preference within 2 to 6 days of the start of the infusions. When a solution of alcohol was offered to the rats in the upper range of concentrations, (i.e., 11 to 30%) the amount of alcohol consumed often reached 10 g per kg per day and in some animals exceeded 15 g per kg per day. Symptoms of intoxication and withdrawal were noted in many of the animals during the course of the infusion of a TIQ or β-carboline. Upon retest for alcohol preference 30 days later, the rats' exaggerated self-selection of alcohol persisted even though the chronic infusions of the amine metabolite had ceased. These findings provide further support for the theory that a family of specific alkaloid conjugates acting within the brain are involved in the etiology of the abnormal drinking of alcohol.

Alcohol self-selection Amine-metabolite theory Cerebral ventricle infusions Drinking of alcohol
Aldehyde condensation product β-carboline in brain Brain alkaloid Tetrahydroisoquinoline infusions
Addiction to alcohol Salsolinol Tryptoline

THE metabolites postulated to be formed in a potentially efficacious amount by a condensation of an amine and aldehyde after alcohol is ingested have been implicated in the biochemical mechanism underlying alcohol addiction [4, 8, 23, 30]. One of the tetrahydroisoquinolines (TIQ) that is produced by the Pictet-Spengler reaction, tetrahydropapaveroline (THP), induces an exaggerated preference for alcohol when the compound is infused chronically into the cerebral ventricle of the rat [30]. Should biological conditions favor such a reaction, several condensation products other than THP may also be synthesized in vivo.

As early as 1961, McIsaac [21] demonstrated that a condensation product derived from an indoleamine, a β-carboline, can be detected in the urine of the rat. Similarly, Cohen and colleagues [4] have reported that TIQ derivatives of norepinephrine and epinephrine are condensed with acetaldehyde or formaldehyde as the adrenal gland is perfused with these aldehydes, or they are found in this gland following systemic injections of methanol [3]. Salsolinol, the condensation product of dopamine and acetaldehyde, has not only been identified in the urine of the human [38], but also in the brain of the rat after an intraperitoneal injection of pyrogallol plus alcohol [6]. In tissue slices or homogenate of rat brain, the uptake of a

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catecholamine is inhibited by a TIQ derived from either dopamine or norepinephrine; serotonin uptake is similarly prevented by a β-carboline [1, 5, 14, 17, 40]. The neuronal effects exerted by these metabolites in vivo and in vitro include the retardation of the degradation of a biogenic amine [2,7], their uptake into nerve terminals [20, 32, 39] and their action in releasing the endogenous amine [14,35].

Overall, however, there is no direct evidence at present to suggest that a condensation product other than THP acts in the CNS to augment the voluntary selection of alcohol. For example, when a β-carboline is injected systemically, the amount of 4% alcohol consumed by a rat is reduced [12]. The purpose of the present study was to determine whether the chronic exposure of the brain to specific TIQs and a β-carboline would alter the pattern of a rat's alcohol intake. In these experiments, 1,2,3,4-TIQ or one of five monoamine-aldehyde condensation products was infused chronically into the ventricle of the rat while it had the choice of alcohol or water in its cage. The metabolites infused were l-methyl-6,7-dihydroxy-TIQ (salsolinol); 4,6,7-trihydroxy-TIQ (4,6,7THTIQ); l-methyl-3-carboxy-6,7-dihydroxy-TIQ (1M-3C-6,7DHTIQ); 6-methoxy-4,7-dihydroxy-TIQ (6M-4,7DHTIQ); and 1,2,3,4-tetrahydro-β-carboline (tryptoline). Individual patterns of alcohol self-selection were obtained not only during 12 days of chronic ventricular infusion, but also following a 30-day interval so that the longevity of a metabolite's initial action could be ascertained.

METHOD

Male rats of the Sprague-Dawley strain weighing 300–500 g were housed in individual cages. They were maintained on powdered Wayne Lab Blox and kept on a 12 hr light–dark cycle at an environmental temperature of 21–23°C. Each rat's volitional selection of alcohol was tested by a standard three-bottle, two-choice technique [29]. For this procedure, three calibrated 100 ml Kimax drinking tubes were attached to the front of an animal's cage with one tube containing a solution of alcohol which was increased in concentration on successive days as follows: 3, 4, 5, 6, 7, 9, 11, 13, 15, 20, 25 and 30%. Each volume/volume solution was prepared in deionized water with 95% ethanol. The second tube was filled with water, whereas the third tube served as a dummy and was empty. The position of the three tubes was interchanged on each day according to a predetermined schedule so that the rat did not develop a position habit.

Chronic Infusion Procedure

Following its 12-day alcohol preference test, each rat was anesthetized with sodium pentobarbital and placed in a stereotaxic instrument in the DeGroot orientation. Following standard procedures [25], a 20 ga guide tube which extended through the base of a Khavari swivel [18] was implanted above the lateral cerebral ventricle at the coordinates of AP +5.8, Lat 1.5 and Hor +3.0. After 2 days had elapsed, a 27 ga injector needle was inserted through the guide tube so that the tip rested in the ventricle. The injector needle was held snugly in place by a cap in such a way that it rotated freely within the guide tube; thus, the rat had complete freedom of movement throughout the experiment [18]. As described earlier [27], the needle was connected via PE tubing to a syringe mounted on a chronic infusion pump.

Each of the compounds used for infusion was dissolved in an artificial cerebrospinal fluid (CSF) which consisted of 7.46 g NaCl, 0.19 g KCl, 0.14 g $CaCl_2$ (anhydrous), and 0.19 g $MgCl_2 .6 H_2O$ per liter of glass distilled water [26]. In order to retard the degradation of the alkaloids, the pH of the solution was lowered to 3.8 with the addition of 0.1 mg/ml of ascorbic acid. After preparation in pyrogen-free glassware, each solution was passed through a 0.22 μm Swinnex millipore filter. The infusion pump was programmed so that the substances were delivered automatically into the rat's lateral ventricle in a 4.0 μl volume every 30 min, during the 12-day alcohol preference sequence. The compounds thus infused were: 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrobromide (salsolinol); 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (1M-3C-6,7DHTIQ); 4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (4,6,7THTIQ); 6-methoxy-4,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (6M-4,7DHTIQ) 1,2,3,4-tetrahydroisoquinoline (TIQ); or 1,2,3,4-tetrahydro-β-carboline hydrochloride (tryptoline, TLN). Salsolinol was purchased from Sigma, TIQ from Aldrich and TLN from K and K Laboratories, whereas the other compounds were generously provided by Dr. M. Collins of Loyola University. For approximately one hour or less each morning, each rat was disconnected from the chronic infusion system in order that the tubing-needle assembly could be flushed with the CSF vehicle and reloaded with a freshly prepared solution.

Taste Tests and Drinking Patterns

Upon completion of their 12-day alcohol test sequence the rats in which 4.0 μg/μl of salsolinol or tryptoline had been infused intraventricularly were given a choice of water and a constant concentration of either 25% or 15% alcohol for six days; again the third tube was empty. Following this interval, the empty drinking tube was replaced with one containing a solution of a palatable solution of Sustagen (Mead Johnson) which was isocaloric with the solution of alcohol presented to the animal. This combination of three fluid choices was presented for four days, after which the Sustagen solution was removed and the empty tube replaced for an additional three days of testing.

Drinkometer records, collected to indicate the temporal distribution of drinking over a 24-hour period, were also obtained for selected animals during a preference test for 25% solution of alcohol and water. Salsolinol in a dose of 4.0 μg/4.0 μl had been infused intraventricularly in one rat during the initial 12-day alcohol preference sequence whereas in two other animals 4.0 μg/4.0 μl of tryptoline had been similarly injected. Drinkometer records were also collected during the entire course of a second alcohol preference test for these three animals as well as for two rats infused with 0.04 μg/4.0 μl of tryptoline and one rat given 6M-4,7DHTIQ intraventricularly during the first preference sequence.

Behavioral Observations and Blood Alcohol Determination

Periodically throughout the day and night, routine observations were made and signs of intoxication and/or withdrawal [11,15] were recorded. Symptoms of particular significance included: "wet-dog shakes," tail stiffness, hyperactivity, compulsive sniffing, weaving, ataxia, teeth

TABLE 1

THE MEAN PROPORTION OF ALCOHOL TO TOTAL FLUID INTAKE AND MEAN G OF ALCOHOL PER KG OF BODY WEIGHT CONSUMED BY EACH GROUP OF ANIMALS DURING THE ALCOHOL PREFERENCE TESTS GIVEN BEFORE (CONTROL) AND DURING THE INTRAVENTRICULAR INFUSION OF 4.0 μl EVERY 30 MIN OF VARIOUS SOLUTIONS

Solution	Dose (μg/4.0 μl)	N	Pre-Infusion Control			During Infusion		
			Proportion	gm/kg	Total Fluid	Proportion	gm/kg	Total Fluid
Salsolinol	4.0	4	0.18	0.7	34.0	0.72	4.9	38.0
	0.04	3	0.09	0.4	29.3	0.37	2.9	33.0
1M-3C-6,7DHTIQ	4.0	3	0.37	1.3	30.7	0.64	3.5	35.3
	0.04	1	0.42	1.4	32.0	0.78	4.7	33.0
4,6,7THTIQ	4.0	4	0.24	0.7	30.5	0.31	1.2	23.3
	0.04	4	0.20	0.6	27.8	0.40	1.9	38.3
6M-4,7DHTIQ	4.0	1	0.50	2.2	33.0	0.60	5.0	35.0
TIQ	4.0	3	0.31	1.0	29.0	0.41	2.0	29.0
Tryptoline	4.0	4	0.16	0.8	34.3	0.58	6.0	52.0
	0.04	3	0.39	1.6	38.5	0.53	5.4	50.7
	0.0004	2	0.14	0.9	31.0	0.61	3.9	67.5
CSF	—	3	0.32	1.3	34.0	0.08	0.6	50.0

chattering, broad-based gait, gnawing, incoordination, twitching, tremor or convulsions.

Representative blood samples were collected for selected rats in the morning hours between 12 p.m. and 3 a.m. during a peak drinking period after alcohol had been ingested during the night. After 25.0 μl of blood were taken in a heparinized capillary pipette from the tip of the rat's tail, the modified enzymatic assay of Roos [37] was used to estimate the level of blood alcohol.

RESULTS

A composite summary is presented in Table 1 of the rat's alcohol drinking in response to the chronic intraventricular infusion of five TIQ compounds, the β-carboline (TLN) and the CSF control vehicle. The results are expressed in terms of means of the proportion of alcohol to total fluid intake, the actual amount of alcohol consumed in g per kg, and the total fluid ingested (water plus alcohol). Values for the 12-day 3 to 30% alcohol preference sequence prior to infusion (control) as well as during the 12 days of chronic infusion also are given.

The greatest increase in alcohol drinking was produced by salsolinol and tryptoline with a virtual seven-fold increase in g per kg intake occurring with the 4.0 μg dose of each compound. Even the infusion of the lower doses of these two metabolites caused a substantial elevation in alcohol ingestion. During the 12 days of chronic infusion, the rats in both the tryptoline and control groups increased their total daily volume intakes by as much as 12 to 35 ml, but there were essentially no significant changes in the total fluid intakes in the other groups. Although the other TIQs also evoked a rise in alcohol preference, this shift was not as great as that produced by salsolinol or β-carboline, and seemed to be dependent upon the structural configuration of the infused compound.

Salsolinol

The changes in the preference for alcohol during the infusion of either dose of salsolinol given in the 4.0 μl volume were significantly different ($p<0.01$, Newman-Keuls tests) from the CSF controls. As illustrated in Fig. 1, the 4.0 μg dose of salsolinol induced a greater shift in proportion of alcohol to total fluid ingested than the 0.04 μg dose ($t = 3.34; df = 11; p<0.01$). In fact, it was not until the sixth day of chronic infusion that the lower dose of salsolinol elicited the sharp shift in alcohol drinking.

With respect to the actual intake of alcohol, Fig. 2 shows that the 4.0 μg group consistently drank close to 5 g per kg per day of the fluid, or a greater amount throughout the course of the 12-day preference sequence. Although the differences between the doses were significant ($t = 3.01; df = 11; p<0.01$), the lower dose of salsolinol did not increase the intake to a corresponding level until the latter half of the sequence when the higher concentrations of alcohol were offered.

Upon re-test 30 days after the chronic infusions of the 4.0 μg dose of salsolinol had been terminated, the rats nevertheless exhibited a nearly identical pattern of alcohol drinking. Figures 3 and 4 present the concordant values for the proportion of alcohol to total fluid as well as the actual intake in terms of g per kg during the repeated 12-day sequence. This result shows that the effect of salsolinol on the internal structures of the brain persists long after the exposure to the condensation product is discontinued.

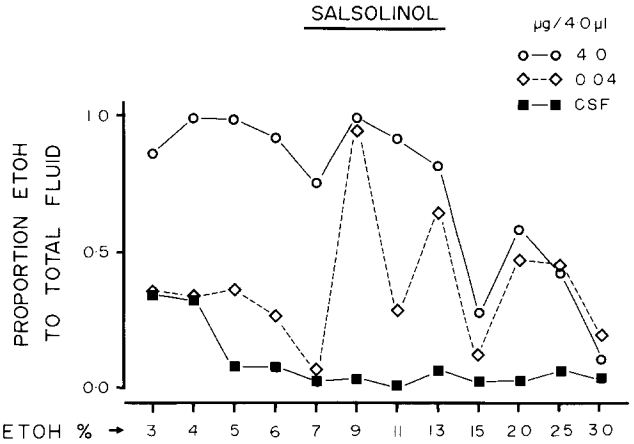

FIG. 1. The proportion of alcohol (ETOH) to the total fluid intake (alcohol plus water) plotted against the concentration of alcohol offered on each day. An infusion in a volume of 4.0 μl of CSF (N = 3), of 4.0 μg (N = 4) or of 0.04 μg (N = 3) of salsolinol was given every 30 min throughout the 12 days.

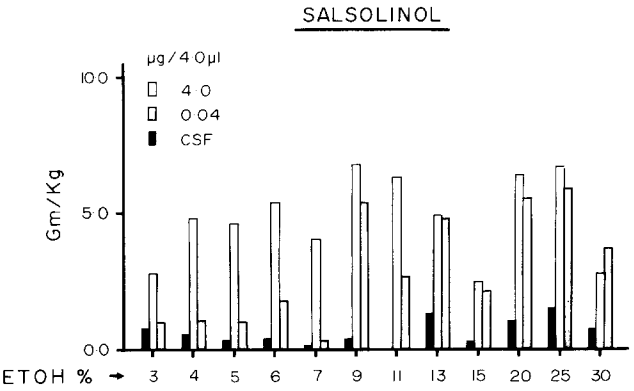

FIG. 2. G alcohol per kg body weight consumed at each concentration of alcohol offered during the chronic infusions of CSF or salsolinol for the three groups of rats as described in Fig. 1.

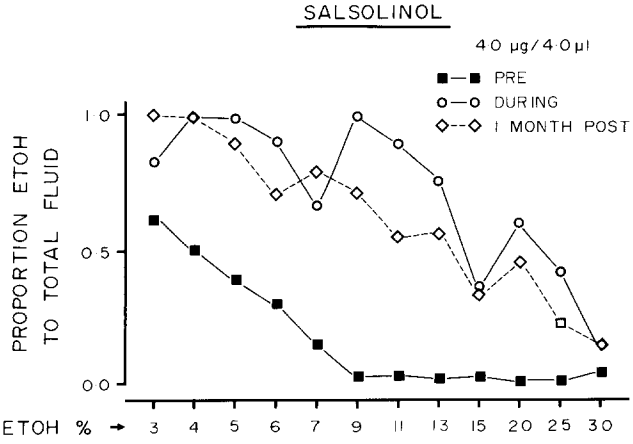

FIG. 3. The proportion of alcohol to total fluid intake plotted against the concentration of alcohol offered before (PRE), during, and after (1 month POST) chronic intraventricular infusions of the 4.0 μg dose of salsolinol (N = 3).

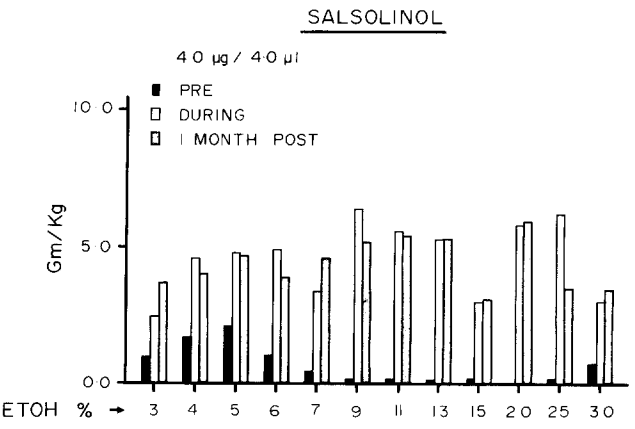

FIG. 4. G alcohol per kg body weight consumed at each concentration of alcohol offered before (PRE), during, and after the salsolinol infusions for the 4.0 μg group of rats as described in Fig. 3.

l-methyl-3-carboxy-6,7-dihydroxy-TIQ (1M-3C-6,7-DHTIQ)

As presented in Table 1, the animals in which 0.04 or 4.0 μg per 30 min of 1M-3C-6,7-DHTIQ was infused intraventricularly consumed more alcohol than the control animals given the CSF vehicle. Figure 5 illustrates the proportion curve for the three rats in which the higher dose was infused. During the first six days of the test sequence during which the 3–9% concentrations of alcohol were offered, nearly the entire fluid intake was in the form of alcohol.

As portrayed in Fig. 6, the corresponding intake of alcohol in g per kg generated a somewhat unusual pattern. A progressive increase in alcohol consumption occurred over the first six days, through the 9% concentration, and then this stabilized at about 4.2 g per kg for several days. During this interval, each animal drank 5.0 g per kg or more on at least one day of the preference test sequence.

4,6,7-Trihydroxy-TIQ (4,6,7-THTIQ)

The chronic intraventricular infusion of either 4.0 or 0.04 μg of 4,6,7-THTIQ caused very little change in alcohol selection. As shown in Fig. 7, the proportional intake of both groups of rats barely exceeded the 0.5 proportion value during the initial part of the preference test. Thereafter, the proportion approximated that of the control group. Overall, there were no statistical differences between the curves of proportion for these three groups.

As depicted in Fig. 8, the g per kg intake of the rats given the higher dose of 4,6,7-THTIQ never rose above the 2.4 g per kg level. From this peak value recorded on the fifth day of the test sequence, the consumption of alcohol fell to a level of approximately 1.0 g per kg for the remainder of the preference test. Again, the differences were not significant. It should be noted that the slightly higher alcohol intake of the four rats which received the lower dose of 4,6,7-THTIQ was attributed to the drinking response of one animal. In this case, the rat displayed the progressive increase in alcohol consumption typical of the other alkaloids in that a mean of 6.0 g per kg of alcohol was ingested during the latter half of the preference session.

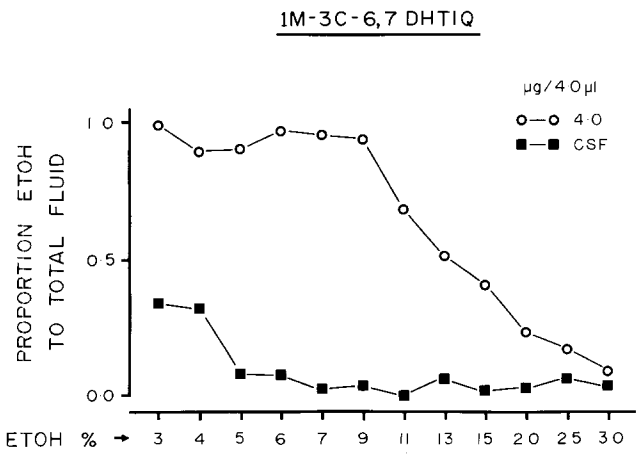

FIG. 5. The proportion of alcohol to total fluid intake plotted against the concentration of alcohol offered on each day. An infusion in a volume of 4.0 μl of CSF (N = 3) or 4.0 μg of 1M-3C-6,7DHTIQ (N = 3) was given every 30 min during the 12 days.

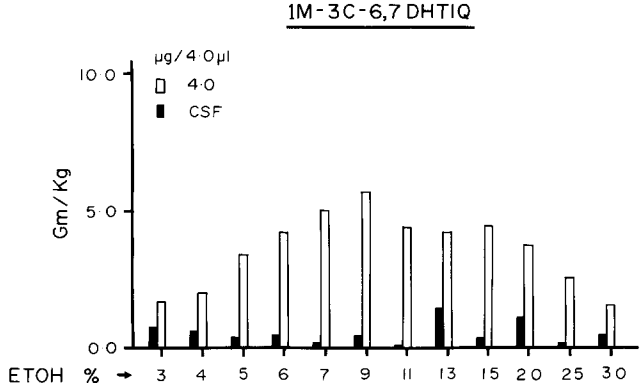

FIG. 6. G alcohol per kg body weight consumed at each concentration of alcohol offered during the chronic infusions of CSF or of 1M-3C-6,7DHTIQ for the two groups of rats as described in Fig. 5.

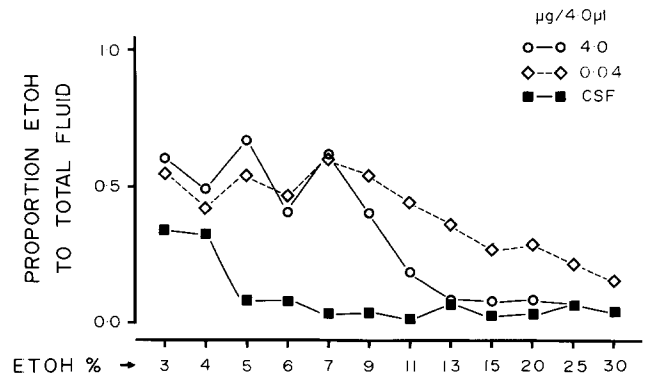

FIG. 7. The proportion of alcohol to total fluid intake plotted against the concentration of alcohol offered on each day. An infusion in a volume of 4.0 μl of CSF (N = 3), or of 4.0 μg (N = 4) or 0.04 μg (N = 4) of 4,6,7THTIQ was given every 30 min during the 12 days.

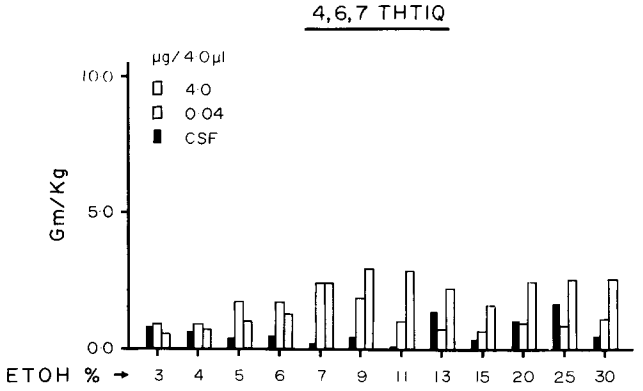

FIG. 8. G alcohol per kg of body weight consumed at each concentration of alcohol offered during the chronic infusions of CSF or of 4,6,7THTIQ for the three groups of rats as described in Fig. 7.

6-Methoxy-4,7-Dihydroxy-TIQ (6M-4,7DHTIQ)

Of the three rats in which a 4.0 μg dose of 6M-4,7DHTIQ was infused intraventricularly, in only one animal (Table 1) did this TIQ penetrate the ventricular cavity as revealed by the dye verification analysis [27]. The relatively high preinfusion control values for its proportion and g intake was enhanced substantially by the infusion of the TIQ derivative. Fig. 9 (TOP) shows that the proportion of alcohol consumed was sustained at the 0.5 level or greater throughout the last 10 days of the preference sequence (5% through 30% concentrations). In contrast to the pre-infusion control test, the steady rise in alcohol consumption is illustrated in Fig. 9 (BOTTOM). Of interest here is the fact that although the mean intake of alcohol for the first half of this test sequence was 2.8 g per kg, the average over the last six days was 7.2 g per kg with a level of 9.1 g per kg of the 30% solution consumed on the final test day. When solutions of 35, 40, 45 and 50% were offered (not shown), the animal continued to drink alcohol in the same amount, i.e., 8.3, 7.2, 5.6 and 10.3 g per kg, respectively.

Tetrahydroisoquinoline (TIQ)

In three rats in which a 4.0 μg dose of TIQ was infused chronically into the cerebral ventricles, there were no noteworthy changes in alcohol drinking. As shown in Table 1, only a slight increase above the pre-infusion level occurred in terms of both proportion and g per kg.

Figure 10 reveals that during the intermediate stage of TIQ infusion, the proportional intake surpassed the 0.5 value at the 7%, 9% and 11% concentrations of alcohol. This transitory preference is likewise reflected in the g per kg measures, for the same days, as portrayed in Fig. 11. Over days, however, the changes in alcohol consumption were not statistically significant.

Tryptoline (TLN)

Of the group of amine-aldehyde condensation products tested in these experiments, the β-carboline, tryptoline, exerted an exceedingly potent effect on the alcohol consumption of the rat. Infused chronically into the

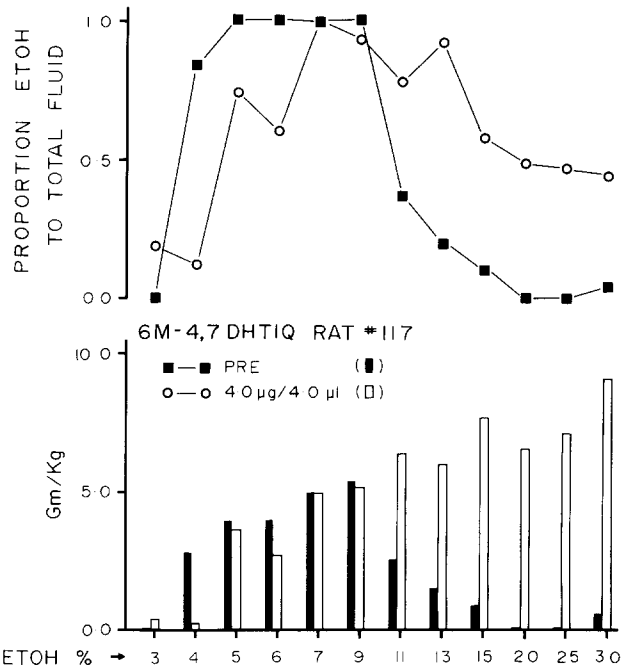

FIG. 9. The proportion of alcohol to total fluid intake (TOP) and g of alcohol per kg body weight (BOTTOM) plotted at each concentration of alcohol offered to Rat No. 117 before infusion (PRE) and during the chronic infusions of 4.0 µg of 6M-4,7DHTIQ given in a volume of 4.0 µl every 30 min to this rat during the 12-day preference test.

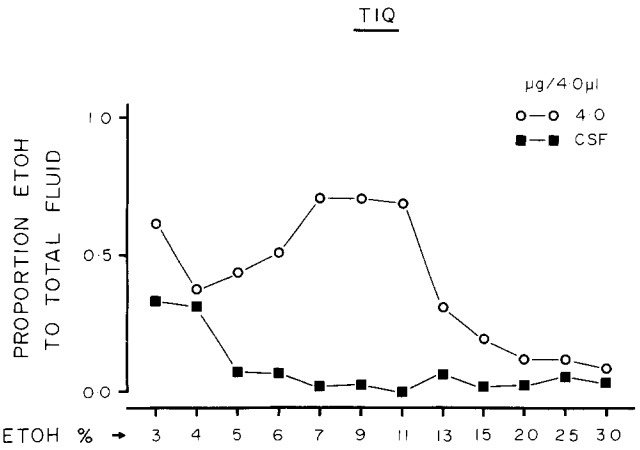

FIG. 10. The proportion of alcohol to total fluid intake plotted against the concentration of alcohol offered during the chronic infusions of CSF (N = 3) or 4.0 µg TIQ (N = 3) given in a 4.0 µl volume every 30 min.

cerebral ventricle in doses ranging from 400 picograms to 4.0 µg every 30 min, tryptoline evoked a preference for alcohol that exceeded the proportion value of 0.5 at every concentration of alcohol offered except the two highest. This shift in preference is illustrated in Fig. 12. An increase in the proportional intake of approximately four-fold (Table 1) over the pre-infusion control levels (t = 3.3 and 30.7, respectively; df = 11; $p<0.05$) occurred in the rats in which the highest and lowest doses were infused. The

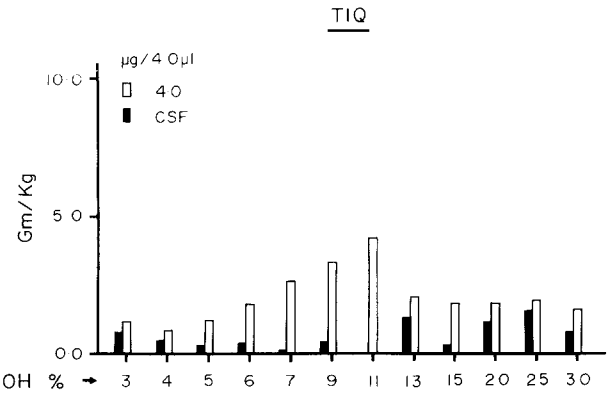

FIG. 11. G of alcohol per kg of body weight consumed at each concentration of alcohol offered during the chronic infusions of CSF or 4.0 µg of TIQ as described for the groups in Fig. 10.

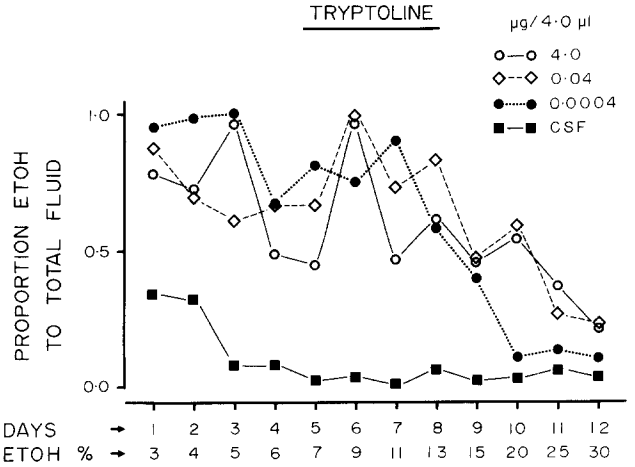

FIG. 12. The proportion of alcohol to total fluid intake plotted against the concentration of alcohol offered on each day. An infusion in a volume of 4.0 µl of CSF (N = 3), or of 4.0 µg (N = 4), 0.04 µg (N = 3) or 0.0004 µg (N = 2) of tryptoline was given every 30 min during the 12 days.

progressive enhancement in the absolute intake of alcohol during the tryptoline infusions is portrayed in Fig. 13. Doses of tryptoline of 4.0 and 0.04 µg induced the most intense drinking particularly in the concentrations ranging from 9% to 30% alcohol. Nevertheless, the group of rats in which a 400 picogram dose was given every 30 min likewise drank 4.0 to 6.0 g per kg per day.

The amount of alcohol imbibed by one rat was particulary remarkable. In Fig. 14, the striking increase above the pre-control level is shown for both the proportion and g per kg values. At the 15 and 20% concentration, the quantity of alcohol consumed by this rat exceeded 10 g per kg per day (Fig. 14 BOTTOM). On the eleventh day of the sequence, when the 25% solution of alcohol was offered, this tryptoline-infused rat drank 16.6 g per kg during the 24 hour interval. When this animal was re-tested for its alcohol preference 30 days after tryptoline infusions had ended, the level of alcohol ingested had stabilized at approximately 3 to 6 g per kg per day (Fig. 14 BOTTOM).

All of the rats given the 4.0 and 0.04 µg doses of

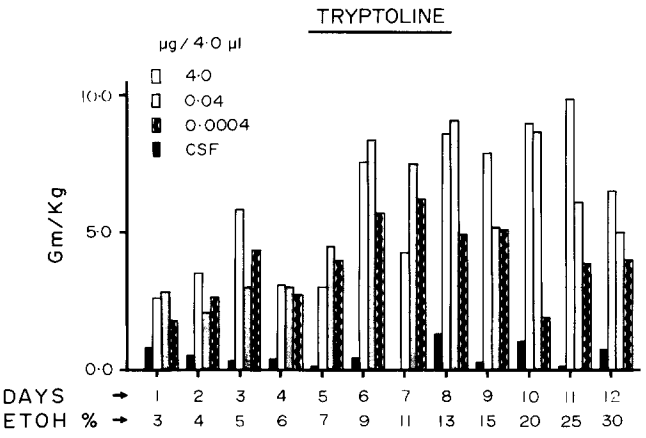

FIG. 13. G alcohol per kg body weight consumed at each concentration of alcohol offered during the chronic infusions of CSF or three doses of tryptoline as described for the groups in Fig. 12.

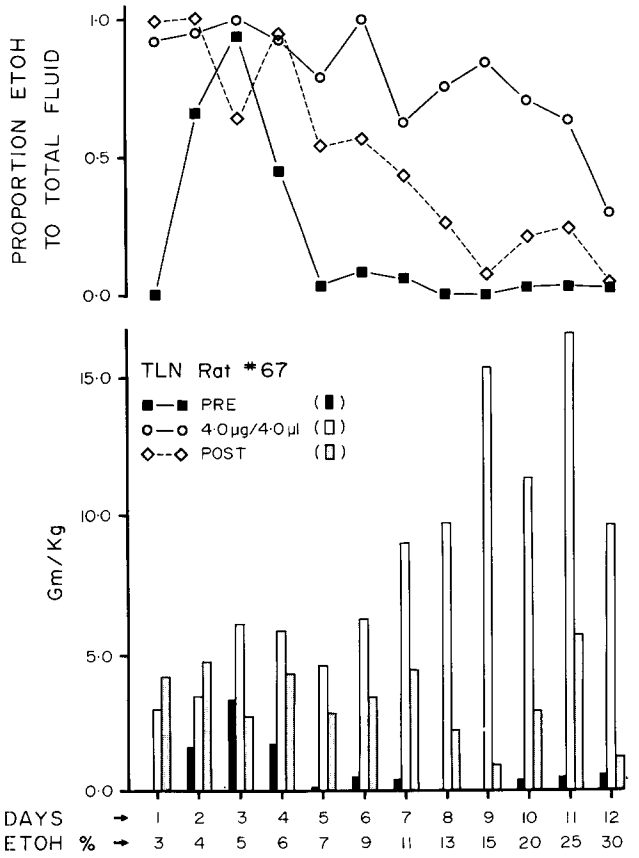

FIG. 14. The proportion of alcohol to total fluid intake (TOP) and g of alcohol per kg body weight (BOTTOM) plotted at each concentration of alcohol offered to rat No. 67 before infusion (PRE), during the chronic infusions of 4.0 µg/4.0 µl of tryptoline, and one month after the infusion sequence (POST).

tryptoline were retested 30 days after the discontinuation of the chronic infusion of the β-carboline. Figure 15 reveals that the proportional change over the precontrol level continued ($p<0.05$, Newman-Keuls tests). Correspondingly, the g per kg intake also persisted (Fig. 16) at a higher level than that of a control ($p<0.01$ Newman-Keuls tests) with the intakes of alcohol approaching or exceeding the 5.0 g per kg level at several concentrations.

Sustagen Test and Drinking Patterns

In the eight animals in which either salsolinol (N = 4) or tryptoline (N = 4) was infused intraventricularly in the 4.0 µg dose, alcohol in concentrations of either 15% or 25% was consumed in spite of the presence of the highly palatable mixture of Sustagen which was isocaloric to the alcohol solution offered. As shown in Table 2, the proportion of alcohol to water remained relatively constant during the four-day period when Sustagen was presented. However, because of the enormous increase in the total fluid consumed, which ranged from 96 to 226 ml of fluid per day, the overall proportion of alcohol to total fluid (alcohol solution plus water plus Sustagen solution) declined sharply. In one animal given tryptoline (No. 68) the g per kg intake of alcohol rose from 4.8 prior to the Sustagen test to 6.1 g per kg per day while Sustagen was given. During the additional three days after the Sustagen test had been completed (Post) the g per kg intake in most of the animals once again returned to the level at which alcohol was consumed prior to the presentation of the Sustagen. These results indicate that the alternative choice of a highly preferred liquid food did not alter the ratio of alcohol to water taken and in several cases, the amount of alcohol drunk was somewhat greater than that observed when the animals were tested prior to the infusion of the metabolites.

Drinkometer records obtained while six rats were either given a preference sequence or offered water and a constant concentration of 25% alcohol indicated that the drinking occurred mainly during the night-time hours; that is, approximately 80% of all alcohol drinking occurred at this time. In Fig. 17, the diurnal intake of alcohol offered on successive days in the 3–30% range of concentrations (oblique axis) is plotted for a representative rat in which the 0.4 µg dose of tryptoline had been infused intraventricularly. It can be seen that this animal drank alcohol usually in two to four bouts during the interval from 7 p.m. to 8 a.m.

In another animal in which 6M-4,7DHTIQ had been infused, most of the alcohol consumption again occurred during the night cycle when the rat was offered a choice of water or 30, 15, 7.5, 15 or 30% alcohol for the respective periods as denoted in Table 3. The proportion of alcohol to water consumed by the animal varied directly with the concentration of alcohol offered, as shown in the Table. Similarly, the g per kg values were related directly to the alcohol concentration presented, although during the last 12 days when the 30% concentration was available, the overall intake decreased. Thus, the consumption of alcohol was generally greater at higher concentrations, but this pattern decreased with time.

Symptoms of Withdrawal and Blood Alcohol

In at least one or two animals of each group given the

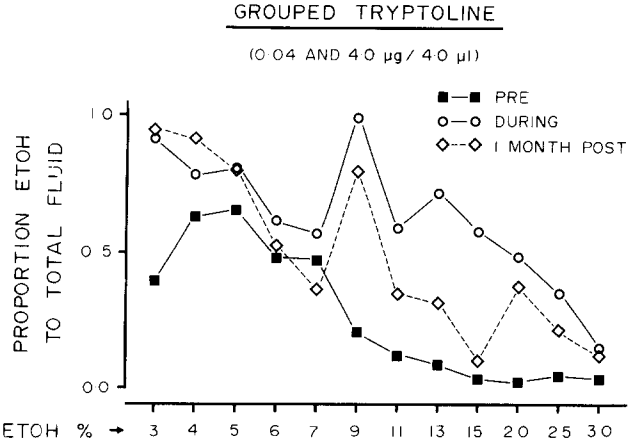

FIG. 15. The proportion of alcohol to total fluid intake plotted against the concentration of alcohol offered on each day before (PRE), during, and after (POST) the chronic intraventricular infusions of tryptoline (N = 5) in doses of 0.04 and 4.0 µg/µl.

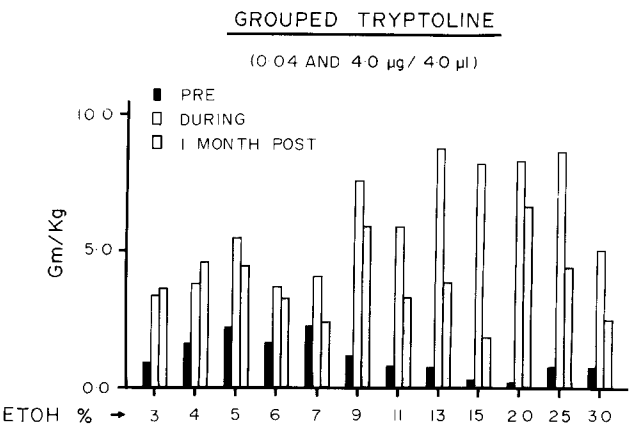

FIG. 16. G alcohol per kg body weight consumed at each concentration of alcohol offered before (PRE), during and after (POST) the chronic infusion of tryptoline for the groups as denoted in Fig. 15.

amine-aldehyde condensation products intraventricularly, signs of withdrawal, ataxia and other behavioral abnormalities were evident. For example, salsolinol induced "wet dog shakes" and hyperactivity within three days after the start of the alcohol test sequence. Following the cessation of 1M-3C-6,7DHTIQ infusions, one rat exhibited convulsions; all other animals given this compound intraventricularly exhibited stiffness of the tail within a few days of the beginning of the alcohol test sequence.

Repeated convulsive episodes were observed in animals in which 4,6,7THTIQ as well as tryptoline were infused; epileptiform activity usually began on the fourth or fifth day after the alcohol drinking had commenced. In relation to this, a given animal often flattened itself on the floor, extended its limbs, elevated the tail rigidly, was hyper-reactive to touch, showed ataxia, sometimes vocalized and displayed tremors of the head muscles. In all rats given 4,6,7THTIQ infusions, teeth chattering also occurred ordinarily by the sixth day of the test sequence. Two of these animals also manifested "wet dog shakes" as well as intermittent convulsions which were characterized by repetitive jerking movements of the forebody as the animal stood in a rearing posture or lay prone on the floor of the cage. Figure 18 (TOP) illustrates the pre-convulsive state of an animal given the higher dose of 4,6,7THTIQ; the animal's body is extended, the tail is elevated and ataxia is prevalent. Subsequent to this, a full-blown convulsion occurred in which hind and forelimb myoclonic movements were noted. This response is portrayed in Fig. 18 (BOTTOM).

During the course of intraventricular infusions of salsolinol (N = 3) or tryptoline (N = 5), during which intervals the animals consistently drank either 20 or 25% alcohol, samples of blood were collected at either 1 a.m. or 2 a.m. Table 4 presents the results of an analysis of blood alcohol levels (BAL) as well as the g per kg intake for selected rats during the 24 hr period. In each case, the plasma levels were below the 0.1% value, presumably because of the rapid metabolism of alcohol in this species [24].

DISCUSSION

These experiments demonstrate that the presence of a minute amount of an amine-aldehyde condensation product in the brain of the rat induces the abnormal drinking of alcohol in preference to water, in a free-choice situation.

TABLE 2

PROPORTION OF ALCOHOL TO TOTAL FLUID INTAKE (PROPORTION), G OF ALCOHOL PER KG OF BODY WEIGHT AND MEAN TOTAL FLUID INTAKE CONSUMED BY RATS INFUSED WITH 4.0 µg/4.0 µl OF EITHER SALSOLINOL (SAL) OR TRYPTOLINE (TLN), BEFORE, DURING AND AFTER THE SUSTAGEN SOLUTION WAS OFFERED IN THE THIRD ("DUMMY") TUBE

Animal	Compound Infused	% ETOH Offered	PRE			Sustagen Test				POST		
			Proportion	g/kg	Mean Fluid	Proportion of ETOH to ETOH plus Water	g/kg	Mean Fluid	Proportion to Total Fluid	Proportion	g/kg	Mean Fluid
60	Sal	25	0.33	7.3	56	0.38	1.8	126	0.03	0.34	5.9	37
61	Sal	25	0.48	8.5	40	0.75	1.7	109	0.05	0.87	4.3	12
63	Sal	15	0.10	1.2	42	0.22	0.4	96	0.02	0.06	0.8	45
64	Sal	15	0.08	3.1	134	0.02	0.5	226	0.01	0.11	4.2	141
65	TLN	15	0.37	5.0	52	0.27	1.8	145	0.04	0.30	4.8	65
66	TLN	15	0.30	4.2	50	0.53	2.6	99	0.08	0.58	6.3	41
67	TLN	25	0.50	11.6	49	0.19	1.1	134	0.02	0.22	3.4	33
68	TLN	25	0.23	4.8	53	0.56	6.1	109	0.11	0.18	4.6	57

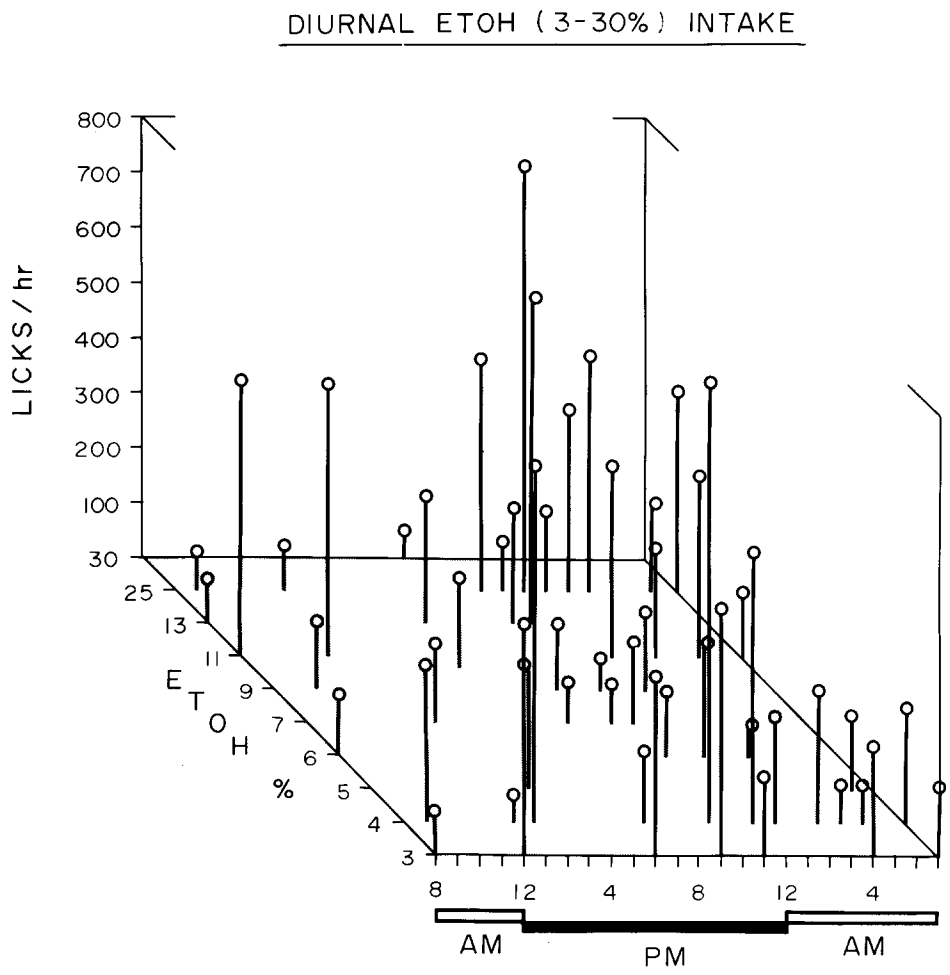

FIG. 17. Drinkometer record of alcohol intake in licks per hr (ordinate) for each hr of the day and night as denoted on the abscissa. The oblique axis denotes the concentration of alcohol presented to the rat on successive days. Tryptoline (0.4 μg every 30 min) was infused intraventricularly in this rat.

Three aspects of this response are notable: first, even though the concentration becomes more gustatorily aversive, the rat nevertheless selects increasing quantities of alcohol with the continuation of intracerebral infusions of an alkaloid; second, symptoms that have been characterized as withdrawal-like [11,15] are exhibited; third, when the rat is once again given the choice of alcohol long after the intraventricular application of a given metabolite has ceased, the rat re-instates its ingestion of an unusual amount of alcohol even when there was no access to the fluid in the interim.

TABLE 3

THE MEAN PROPORTION OF ALCOHOL TO TOTAL FLUID INTAKE AND MEAN G OF ALCOHOL PER KG OF BODY WEIGHT CONSUMED BY A SINGLE RAT, WHICH HAD BEEN INFUSED WITH 6M-4,7DHTIQ, DURING THE PRESENTATION OF CONSTANT CONCENTRATIONS OF ALCOHOL

Concentration of ETOH offered	Duration (Days) on Concentration	Proportion	gm/kg
30	10	0.27	6.3
15	10	0.50	4.9
7.5	10	0.53	2.5
15	16	0.48	4.0
30	12	0.23	3.8

Although THP acting centrally is the most potent of the amine metabolites in inducing alcohol preference [23,30], of the compounds tested in the present experiments, the β-carboline, tryptoline, evoked the most intense drinking. This indole metabolite not only served to augment the imbibition of high concentrations of alcohol, but also enhanced the rat's total fluid intake. This observation coincides with the overall increase in fluid consumption which occurs when the rat is given intraperitoneal injections of tryptoline [12]. In the latter case, alcohol preference was not enhanced by the peripheral treatment with tryptoline, which would indicate that the β-carboline must act directly on structures in the CNS in order to reverse the usual aversion to alcohol.

In contrast to the intensity of drinking produced by the β-carboline, the derivatives of norepinephrine were far less potent when they were given chronically by the ventricular route. Only one of eight rats increased its alcohol intake

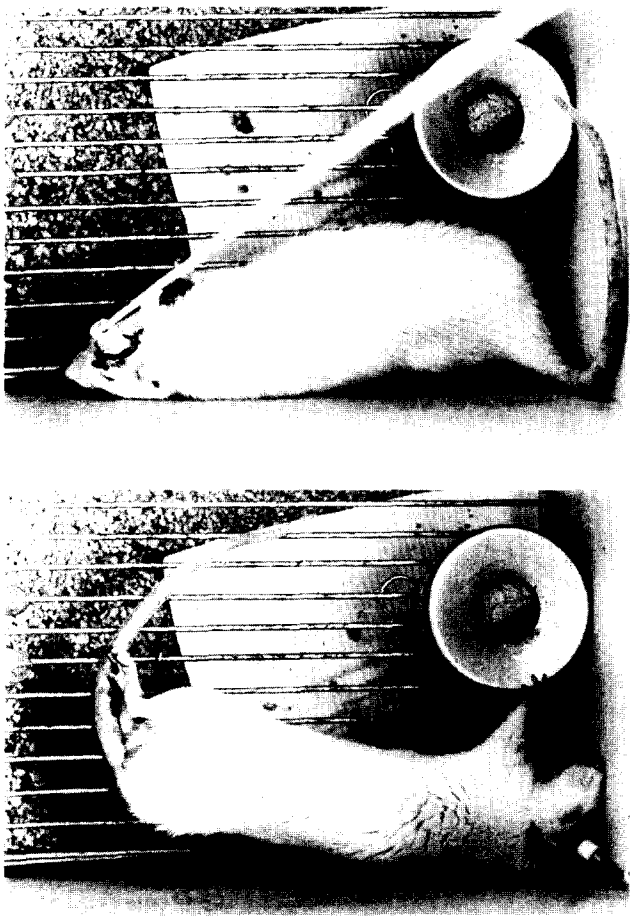

FIG. 18. (TOP) Pre-convulsive prone position of rat in which 4,6,7THTIQ was infused into its lateral cerebral ventricle; the infusion line and swivel as well as food dispenser are shown. (BOTTOM) Same rat one min later undergoing convulsive attack (see text).

TABLE 4

BLOOD ALCOHOL LEVELS ACHIEVED DURING A PEAK DRINKING PERIOD AND 24 HOUR G/KG OF ALCOHOL CONSUMED FOR THE ENTIRE DAY FOR ANIMALS INFUSED INTRAVENTRICULARLY WITH THE HIGH DOSE OF SALSOLINOL OR TRYPTOLINE

Animal	Compound Infused	Time Collected	% ETOH Concentration Consumed	BAL	Daily g/kg
60	salsolinol	2 a.m.	20	0.028	7.9
61	salsolinol	1 a.m.	25	0.087	8.4
62	salsolinol	2 a.m.	20	0.050	7.8
65	tryptoline	2 a.m.	20	0.045	8.3
66	tryptoline	2 a.m.	20	0.025	7.8
67	tryptoline	2 a.m.	20	0.035	12.3
67	tryptoline	1 a.m.	25	0.087	11.0
68	tryptoline	2 a.m.	20	0.044	12.6

during the course of the chronic central infusion of 4,6,7THTIQ. In relation to this, both of the TIQs derived from dopamine, salsolinol and 1M-3C-6,7DHTIQ, did enhance the consumption of alcohol when they were infused chronically into the rat's ventricles. Whether or not this dopa-acetaldehyde condensation product exerts its action on the brain after its decarboxylation to salsolinol cannot be ascertained from these results. Nevertheless, the possibility is raised that should 1M-3C-6,7DHTIQ be formed peripherally, it might cross the blood-brain barrier more readily than salsolinol [22], since dopa itself but not dopamine can penetrate this barrier. Since 6M-4,7DHTIQ was also capable of causing an increase in alcohol preference, a critical chemical variable in the induction of alcohol drinking would not appear to be the compound's availability for 0-methylation [7].

TIQ itself, which contains no substituents on its ring structure, is ineffective, when infused in the brain, in altering alcohol selection. Structurally, this may be indicative of the relative biochemical importance of hydroxyl or other groups in the 6 and 7 positions of the isoquinoline molecule. Alternatively, the potency of the β-carboline analog, the unsubstituted tryptoline molecule, in enhancing alcohol consumption suggests that such a substitution is not essential for the indole structure to be active in the CNS. Because such a wide spectrum of condensation products derived from the biogenic amines may differentially influence alcohol intake as well as withdrawal, each one may contribute in some unique fashion to the etiology of the addictive state. Interestingly, the condensation product yielded by the reaction of dopamine with acetaldehyde, salsolinol, has a different central effect than its analog condensed from formaldehyde, 6,7DHTIQ [7,14].

How the amine-aldehyde condensation products function to produce the withdrawal-like signs in the rat is not clear. Surprisingly, substances as diverse in chemical structure as narcotic antagonists, thyroid releasing hormone, and a tetrahydropyrimidine are all capable of inducing the shaking, tremor and motorial responses characteristic of withdrawal [41]. Although catecholaminergic neurons are already implicated in the withdrawal state [13, 15, 19, 34, 36], the stimulation of serotonin receptors in the brain of the rat or a rise in serotonin content, results in a syndrome resembling the stereotypical responses which we have observed following the administration of the condensation products [16]; these include tremor, rigidity, head shaking and hyperactivity. In view of the influence of these alkaloids on serotonergic pathways in the CNS [40], the possibility now exists that the indoleamine system is another neurochemical avenue through which they operate. Again, such withdrawal-like behavior elicited by the amine-aldehyde condensation products acting directly within the brain offers some substantiation for the functional commonality between the addictive *sequelae* of morphine and alcohol [4,8].

In agreement with our previous finding that an acute intraventricular injection of several alkaloids provokes alcohol preference, it is envisaged that a family of amine metabolites may in part be responsible for alcohol's addictive effects [31]. That is, if a condition exists in vivo which favors the formation of a condensation product via the Pictet-Spengler reaction, it is likely that compounds of this class are actually formed in amine-rich regions of the limbic-forebrain system. The kinetics of the reaction of the parent compounds as well as the availability of substrate

would seem to be principal determinants of the quantity of condensation product, such as salsolinol or THP, that may be synthesized after alcohol is ingested [9,42].

Theoretically, it is not necessary to assume that one or more of the alkaloids must be synthesized in the brain in order to be biologically efficacious [10,22]. Indeed, it is reasonable to assume that a compound could be metabolized peripherally to its active form and then reach the brain parenchyme following its passage through the blood-brain barrier. As postulated previously, a difference in the penetrability of the blood-brain barrier, which would selectively act to prevent or permit the entry of such a metabolite into the brain, could be a crucial factor in the development of the addictive state [28]. With the prolonged drinking of alcohol in near toxic quantities, pathological "holes" in the blood-brain barrier could develop which would permit the influx of the highly potent amine-aldehyde condensation products into certain regions of the CNS [28]. Paralleling this possibility is the viewpoint that the drinking of alcohol at the same time that the circulating level of an amine is high, would favor the formation of a condensation product which could be transported to specific receptor sites in the CNS. In the case of prolonged stress and the consequent adrenal activation which ensues [33], this state in combination with immoderate drinking of alcohol could theoretically facilitate the synthesis of an amine metabolite.

Because of the variations seen in the response of an individual rat to the chronic infusion of the metabolites, it is likely that a certain region of the brain is more reactive to the condensation product than another, presumably because of the presence of specific receptor material. Future research, therefore, should be devoted to identifying the anatomical locus of action of the family of amine metabolites with respect to the evocation of alcohol drinking. Given such a localization, it would then be possible to examine in vivo the functional nature of that structure in terms of its unique chemical properties.

ACKNOWLEDGEMENTS

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