Pharmacology Biochemistry & Behavior, Vol. 22, pp. 205–208, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Isolation and Striatal (^{3}H) Serotonin Uptake: Role in the Voluntary Intake of Ethanol by Rats

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Received 13 July 1983

DAOUST, M., P. CHRETIEN, N. MOORE, C. SALIGAUT, J. P. LHUINTRE AND F. BOISMARE. *Isolation and striatal (^{3}H) serotonin uptake: Role in the voluntary intake of ethanol by rats.* PHARMACOL BIOCHEM BEHAV 22(2) 205–208, 1985.—Ethanol preferring rats were selected and showed a constant voluntary intake of a 12 percent ethanol solution during 14 days (about 5 g/kg body weight daily). Analysis of ^{3}H serotonin uptake by striatal synaptosomes showed that steady state ^{3}H serotonin synaptosomal levels were lower in alcohol preferring rats. Grouping these rats (5 per cage) reduced both voluntary intake of ethanol and synaptosomal ^{3}H serotonin uptake. Furthermore, blocking the serotonin uptake by clomipramine 5 mg·kg^{-1} or 10 mg·kg^{-1} also reduces voluntary intake of ethanol. These data are in agreement with the hypothesis of a modulation of the voluntary intake of ethanol both by chemical and housing stimulation of striatal receptors for serotonin.

Serotonin Drinking rats Voluntary intake of ethanol Striatum Clomipramine

SEVERAL neurotransmitter systems, namely dopamine, norepinephrine, and serotonin, are thought to be involved in the effects of ethanol and/or in voluntary ethanol intake. Several reports suggest their involvement in alcohol sensitivity and addiction [14, 21, 26]. Neurochemical correlates have been sought for on selectively bred lines of animals with alcohol preference or non preference [9,14]. Of these, four strains (P and NP lines [20] and Alko Alcohol (AA) and non Alcohol (ANA) [1] exhibit differences in brain monoamines contents.

Experimental studies on such strains have shown the existence of internal hereditary factors regulating alcohol intake [7]. Moreover, some current theories of the determinants of animal and human ethanol consumption have suggested stress as a possible factor [2, 13, 28]. A number of studies have examined the relations of ethanol consumption with individual or collective housing in rats [6, 7, 16]. Factors such as isolation and deprivation, associated with stress, may increase or decrease spontaneous intake water and ethanol consumption [12,18].

On the other hand, experiments have been performed in order to establish relations between the effects of housing (isolation or grouping) and serotonin metabolism. The data obtained have shown that isolation modified the serotonin brain levels and pargyline, a MAO inhibitor, increased this level more in grouped than in isolated mice [29]; it has also been shown that isolation increased brain serotonin turnover [10].

In the present work, we have studied the effects of the inhibition of serotonin uptake by clomipramine (an antidepressant drug) on voluntary ethanol intake and the serotonin uptake into rat striatal synaptosomes in isolated and grouped alcohol preferring rats and non preferring rats to see whether some relations can be established between these three parameters (i.e., serotonin uptake, housing, and ethanol intake).

METHOD

Behavioral Studies

Adult male Long Evans rats, weighing 180±20 g at the beginning of the experiment were obtained from Janvier (France). The rats were housed individually and had free access to food (UAR France). They were kept in a laboratory with an ambient temperature of 21°C and a 12 hr/12 hr light-dark photoperiod. During the initial selection period, the rats' only drinking fluid was a 12% (vol/vol) ethanol solution prepared from 95% ethanol and water, for 14 days. During the next 2 weeks the rats had the choice between water and the ethanol solution. The two fluid three bottle choice method [24] was used to prevent rats from selecting a fluid based on a position preference. Every other day the amount of fluids and the body weight of animals were measured, the drinking bottles were refilled and randomly rotated.

Animals for which the ethanol solution made up 60% or more of total fluid intake during these two weeks were

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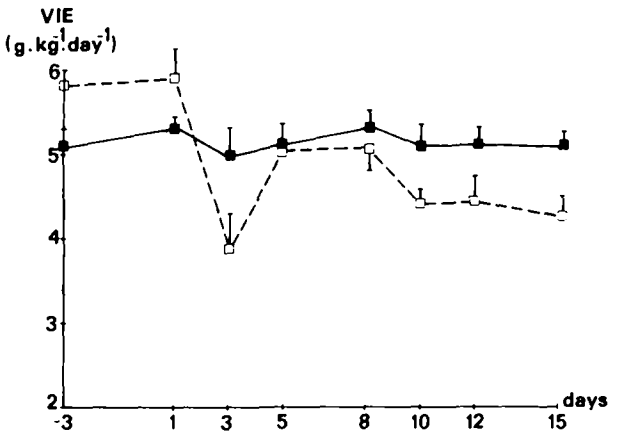

FIG. 1. Voluntary intake of ethanol (V.I.E.) by isolated drinking rats (■) or by grouped drinking rats (□). The results are given in g of absolute alcohol drunk kg^{-1} during pre-treatment (days −3 and 1) and treatment period (days 3, 5, 8, 10, 12, 15), mean±SEM. Statistical analysis: one way analysis of variance; isolated D.R., $F(7,152)=0.7$, $p<0.5$; grouped D.R., $F(7,66)=12$, $p<0.0001$.

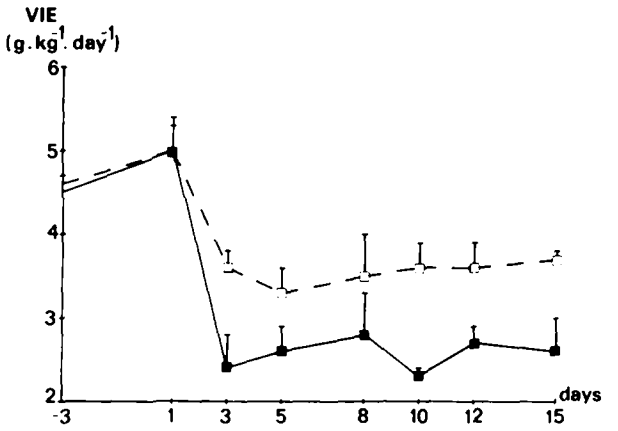

FIG. 2. Voluntary intake of ethanol (V.I.E.) by D.R. daily treated with clomipramine 5 (■) or 10 (□) mg·kg $^{-1}$. The results are expressed in g of absolute alcohol drunk kg $^{-1}$, during pre-treatment (days −3 and 1) and treatment period (days 3, 5, 8, 10, 12, 15). Statistical analysis: one way analysis of variance; $F(7,32)=6.4$, $p=0.0001$ for clomipramine 5 mg·kg $^{-1}$; $F(7,24)=4.57$, $p<0.005$ for clomipramine 10 mg·kg $^{-1}$.

selected as alcohol preferring rats (24% of the rats). Rats for which the ethanol solution made up less than 40% of total fluid intake were considered as non preferring animals.

Influence of Isolation on the Voluntary Intake of Ethanol

Preferring rats, selected as above, were either isolated or grouped in four cages, each with five animals. All the animals were given the choice between water and the 12% ethanol solution. The intakes of water and ethanol solution were noted for fourteen days.

Aversion Test

A conditioned taste aversion towards saccharin was done: the animals were individually housed with food freely available; the rats were adapted to a regimen of water availability which consisted in the presentation of a saccharine 0.005% in water solution for 30 min every other morning; the rats had several days to adapt to this procedure and to establish stable rates of drinking before the treatment began. The rats were then injected with clomipramine. If clomipramine produced an aversion towards saccharin, they would not drink the saccharin solution when offered the saccharine solution on the following days.

Study of (^{3}H) Serotonin Uptake by Striatal Synaptosomes

Rats were killed by cervical dislocation and synaptosomes were prepared from rat corpora striata [4,11]. The striata were weighed and added with a 0.32 M saccharose solution (20/1 v/v) and homogenized with a potter (850 rpm).

After centrifugation (1000 g/10 min) the supernatant synaptosomal preparation [5] was pre-incubated during 5 minutes with nialamide (4 mM) and incubated in the presence of (^{3}H) serotonin (s.a.: 20 Ci/mmol) (20 nM) (CEA Saclay France) in oxygenated Krebs medium (O_2 95%, CO_2 5%). The radioactivity was then determined in a liquid scintillation counter (KONTRON). Non specific uptake of (^{3}H) 5HT was determined by incubating control samples at 0°C instead of 37°C. Specific amine uptake was computed by the equation: uptake at 37°C (fmoles/mg protein) minus uptake at 0°C (fmoles/mg protein). Incubation was stopped at 1.30; 2.30; 5; 10; 15 and 30 min by adding 5°C Krebs medium and centrifugation. The protein concentration of the synaptosomal suspension was determined with cristalline bovine serum albumin as standard [19].

Treatment

Rats were daily IP injected with 5 mg·kg $^{-1}$ or 10 mg·kg $^{-1}$ clomipramine as a solution in 0.9% saline (1 ml/100 g body weight) or with the same volume of saline for control rats.

Statistical Analysis

Statistical analysis was performed by one and two way analysis of variance.

RESULTS

(1) Voluntary ethanol intake remained constant for isolated preferring rats (Fig. 1) during the 14 days of the experiment ($F(7,152)=0.69$, $p>0.5$).

(2) Grouping the preferring rats decreased their ethanol intake (Fig. 1) ($F(7,66)=12$, $p<0.0001$).

(3) Clomipramine decreased ethanol intake of isolated preferring rats (Fig. 3) ($F(7,32)=6.4$, $p<0.0001$ for 5 mg·kg $^{-1}$ and $F(7,24)=4.57$, $p<0.005$ for 10 mg·kg $^{-1}$) though there was no difference between the two doses, (Fig. 2).

(4) The initial rates of serotonin uptake in the isolated preferring and non preferring rats were similar but at steady state serotonin content in synaptosomes isolated from preferring rats was higher (Fig. 3) ($F(1,24)=13.3$, $p<0.001$).

(5) The initial rates of serotonin uptake in the isolated preferring and grouped preferring rats were similar but at steady state, serotonin content in synaptosomes isolated from isolated preferring rats was higher (Fig. 4) ($F(1,24)=8.9$, $p<0.01$).

(6) The aversion test was negative for clomipramine. Its positivity for naloxone validates our procedure (Table 1) [17].

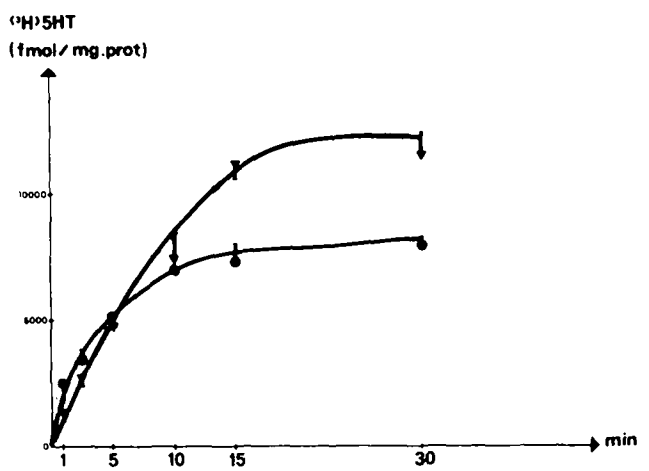

FIG. 3. Time course of (^{3}H) 5HT uptake into rat purified striatal synaptosomes. Synaptosomes were incubated in the presence of (^{3}H) 5HT 20 nM at 36°C for 1.30; 2.30; 5; 10; 15; 30 min. Uptake is expressed as f·moles/mg protein and is shown as the mean±SEM of 3 independent determinations. (▼) isolated drinking rats; (●) isolated non drinking rats; $F(1,24)=13.3$, $p=0.001$.

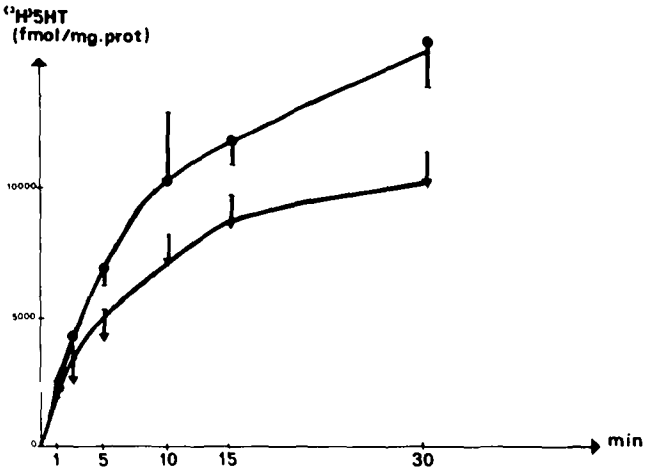

FIG. 4. Time course of (^{3}H) 5HT uptake into purified rat striatal synaptosomes. Synaptosomes were incubated in the presence of (^{3}H) 5HT 20 nM at 37°C for 1.30; 2.30; 5; 10; 15; 30 minutes. Uptake is expressed as f·moles/mg protein and is shown as the mean±SEM of 3 independent determinations. $F(1,24)=8.9$, $p<0.01$; isolated drinking rats (●); grouped drinking rats (▼).

TABLE 1

SACCHARIN SOLUTION INTAKE BY DRINKING RATS BEFORE AND AFTER CLOMIPRAMINE OR NALOXONE INJECTION

	Before Injection	After Injection
Clomipramine 5 mg·kg^{-1}	11.25 ± 0.55	10 ± 0.9*
clomipramine 10 mg·kg^{-1}	7.55 ± 0.9	8.4 ± 0.9*
naloxone 1 mg·kg^{-1}	12.5 ± 0.8	9 ± 1*

The results are expressed in ml of solution drunk and shown as the mean ± SEM of five rats; the t test between consumption before and after injection has been employed.

*$p>0.5$.

**$p=0.005$.

TABLE 2

BODY WEIGHT INCREASE (IN G) OF CLOMIPRAMINE-TREATED AND SALINE RATS DURING TREATMENT (TWO WEEKS)

saline	32 ± 7 g
clomipramine 5	29 ± 4 g
clomipramine 10	30 ± 8 g

The results are given as mean ± SEM, and the t test showed no difference between saline and treated rats.

TABLE 3

MEAN TOTAL FLUID INTAKE BY ISOLATED DRINKING RATS TREATED WITH CLOMIPRAMINE 5 AND 10 mg·kg^{-1} AND BY GROUPED DRINKING RATS

	D −3	D 1	D 3	D 5	D 8	D 10	D 12	D 15	
Grouped D.R.	61	61	82	81	80	81	80	91	$F(7,32)=6.9$ $p<0.001$
Isolated D.R.	67	63	63	67	66	67	65	62	$F(7,47)=0.5$ $p>0.5$
Clomipramine 5 mg·kg^{-1}	61	65	60	59	57	59	62	66	$F(7,25)=0.4$ $p>0.5$
Clomipramine 10 mg·kg^{-1}	61	67	64	67	66	63	65	63	$F(7,35)=0.4$ $p>0.5$

The results are expressed in ml/kg body weight/24 hours of total liquid (water + ethanol): D: days from beginning of treatment.

(7) Clomipramine had no effect on body weight (Table 2) or total fluid intake (Table 3).

DISCUSSION

The first point in this discussion is the stability of the drinking behavior in our selected preferring rats: they maintain a steady rate of alcohol intake over the 14 days following the selection process. This agrees with other published results [22].

The second point is that there seems to exist a relation between ^{3}H serotonin uptake and alcohol intake: both are lower in non preferring rats and in grouped preferring rats than in isolated preferring rats.

This agrees with previous results [16, 23, 27] and is further supported by the fact that clomipramine, which inhibits serotonin uptake [25], also reduces alcohol intake in isolated preferring rats.

This increased ^{3}H serotonin uptake in preferring rats is not a consequence of alcohol ingestion since other authors [23] have shown that serotonin brain levels were 14 to 22% lower in the corpora striata preferring rats than in those of non preferring rats, both never previously exposed to alcohol.

Alcohol preferring rats' preference for alcohol therefore seems to be related to changes in serotonin brain levels or uptake capacities, related either to genetic factors [25] or to environmental stress [16].

The fact that in another study [15] it was shown that tricyclic anti-depressants (which decrease ethanol intake in preferring rats) also increase the release of GABA from rat thalami and striata suggests an involvement of GABA and serotonin in the positive reinforcing properties of ethanol.

Further studies are needed to confirm whether serotonin uptake alone is involved in ethanol intake by preferring rats or whether other receptors, such as GABA for instance [3], are also involved, and to study ^{3}H serotonine uptake in other rat brain areas of preferring and non preferring rats.

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