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A Homotaurine Derivative Reduces the Voluntary Intake of Ethanol by Rats: are Cerebral GABA Receptors Involved?

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BOISMARE, F., M. DAOUST, N. MOORE, C. SALIGAUT. J. P. LHUINTRE, P. CHRETIEN AND J. DURLACH. *A homotaurine derivative reduces the voluntary intake of ethanol by rats: Are cerebral GABA receptors involved?* PHARMACOL BIOCHEM BEHAV **21**(5) 787–789, 1984.—The effects of some derivatives of homotaurine (3 APS), the well known GABA agonist, were tested on the voluntary intake of ethanol by rats. Spontaneously ethanol drinking rats (DR) were selected and had a constant voluntary intake of a 12% ethanol solution (VIE) during 14 days (about 5 g/kg body weight daily). Calcium acetylhomotaurine* (0.26 and 0.52 mmol/kg daily IP) significantly reduced VIE and this was inhibited by the GABA antagonist bicuculline (2 mg/kg IP). The conditioned aversion test to saccharin was negative. Bicuculline alone did not affect VIE. Other homotaurine (3-APS) derivatives: sodium acetyl homotaurine (Na AOTA), homotaurine (OTA), sodium acetyltaurine (Na A TA) and calcium chloride ($CaCl_2$) did not affect VIE. These data suggest that the gabaergic system could be implicated in VIE. *MERAM Lab. patent.

GABA Drinking rats Voluntary intake of ethanol Homotaurine

SEVERAL neurotransmitters have been implicated as mediators of the effects of ethanol and alcohol drinking behavior. Norepinephrine (NE), dopamine (DA), and serotonin (5HT) have received much attention in this regard. Some results suggest that various aspects of these neurotransmitter systems may be related to alcohol sensitivity, tolerance [4,10] and alcohol drinking behavior [6,8]. There is also some evidence for a role of GABA in ethanol's action and withdrawal symptoms. GABA agonists antagonize and GABA antagonists potentiate the convulsions occurring during ethanol withdrawal [2]. Ethanol withdrawal symptoms are also inhibited by benzodiazepines [3] which increase the binding of GABA to its receptors. These experiences led us to suppose that a strong stimulation of brain GABA receptors might reduce the voluntary intake of ethanol (VIE) by rats.

We have therefore studied the effects of homotaurine (3 APS), a potent and stable GABA receptor ligand [1,7]. An acetylated form of this compound, salified by Ca^{++}, calcium acetylhomotaurine (Ca AOTA) was used to increase the brain penetration of the amino-acid. Its effects on the VIE were compared to those of sodium acetylhomotaurine (Na AOTA), homotaurine (OTA), sodium acetyltaurine (Na ATA) and calcium chloride ($CaCl_2$). Bicuculline, a GABA antagonist, was also given with or without Ca AOTA to explore the involvement of GABA receptors in the VIE and to precise the action mechanism of Ca AOTA.

METHOD

Behavioural Studies

Adult male Long-Evans rats weighing 180±20 g at the beginning of the study were obtained from Janvier (France). The rats were housed in individual cages and had free access to food (UAR France standard diet). They were kept with an ambient temperature of 21°C and a 12 hr/12 hr light-dark photoperiod. During the initial selection period they only had access to a 12% (v/v) ethanol solution, prepared from 95% ethanol and water, as drinking fluid for 14 days, followed by another two-week period where they had a free choice between the ethanol solution and water. The two fluids three bottles method was used to prevent fluid selection on the basis of bottle position. Every other day the fluid intakes and body weight were measured, the drinking bottles were refilled and randomly rotated.

Animals preferring the ethanol solutions as 60% or more of their total fluid intake during these last two weeks were selected as drinking rats (24% of the rats), and used for the study.

The results are given as amount of absolute ethanol drunk in g/kg^{-1} and global amount of fluid intake in $ml/kg^{-1}/day^{-1}$. Statistical analysis was done with analysis of variance (one and two-way).

Treatments

All the drugs were prepared as saline solutions. They were administered IP each morning for two weeks, after the 28 day selection period. Eleven groups of drinking rats were studied; each group received one of the following daily treatments: Ca AOTA 0.026 $mmol/kg^{-1}$ (10 mg/kg^{-1}), 0.13 $mmol/kg^{-1}$ (50 mg/kg^{-1}), 0.26 $mmol/kg^{-1}$ (100 mg/kg^{-1}), 0.52 $mmol/kg^{-1}$, (200 mg/kg^{-1}) Na AOTA, OTA, Ca ATA, $CaCl_2$

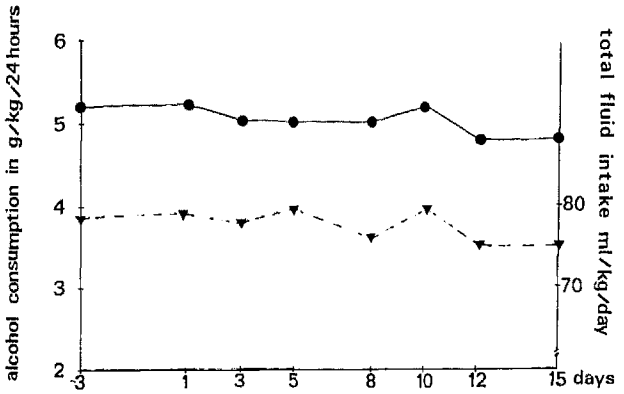

FIG. 1. VIE (●) and total fluid intake (ethanol solution + water) (▼) by the control group during pretreatment— (D −3 and D 1)—and treatment (D 3 to 15). The results are given as mean±SEM of absolute alcohol drunk in g/kg body weight/24 hours (scale on the left) and in mean±SEM of ml of liquid intake/kg body weight/24 hours (scale on the right).

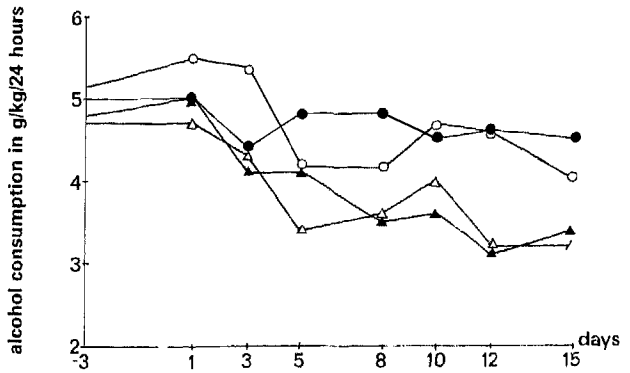

FIG. 2. VIE by drinking rats daily treated with Ca AOTA 0.26 mmol/kg (▲), Ca AOTA 0.52 mmol/kg (△), bicuculline 2 mg/kg (●) or bicuculline 2 mg/gk + Ca AOTA 0.26 mmol/kg (○). The results are expressed in mean absolute alcohol drunk in g/kg body weight/24 hours.

TABLE 1

MEAN OF DAILY ABSOLUTE ALCOHOL INTAKE BY RATS DAILY INJECTED WITH Ca AOTA 0.026 mmol/kg OR Na AOTA, Ca ATA, OTA, Ca C12 0.26 mmol/kg

	D −3	D 1	D 3	D 5	D 8	D 10	D 12	D 15	
Ca AOTA 0.026 mmol/kg	4.8 ± 0.5	4.5 ± 0.5	3.3 ± 0.6	4.3 ± 0.2	4.0 ± 0.4	4.3 ± 0.8	4.0 ± 0.4	3.9 ± 0.3	F(7,28)=1.7 p=0.1
Na AOTA 0.26 mmol/kg	4.3 ± 0.4	4.6 ± 0.3	4.6 ± 0.3	4.5 ± 0.2	4.5 ± 0.2	4.4 ± 0.5	4.2 ± 0.3	4.8 ± 0.3	F(7,72)=0.2 p>0.5
Ca ATA 0.26 mmol/kg	4.2 ± 0.2	4.3 ± 0.3	5.5 ± 0.3	4.7 ± 0.2	4.1 ± 0.3	4.4 ± 0.2	4.3 ± 0.2	4.1 ± 0.4	F(6,56)=0.9 p=0.5
OTA 0.26 mmol/kg	5.5 ± 0.4	5.1 ± 0.3	4.6 ± 0.4	4.7 ± 0.3	4.7 ± 0.3	4.7 ± 0.3	4.4 ± 0.4	4.5 ± 0.5	F(6,63)=0.3 p>0.5
Ca C12 0.26 mmol/kg	4.9 ± 0.4	4.2 ± 0.2	4.5 ± 0.3	3.9 ± 0.4	4.4 ± 0.3	4.4 ± 0.3	3.8 ± 0.3	4.2 ± 0.2	F(6.63)=0.8 p=0.5

The results are expressed in g of absolute alcohol drunk/kg body weight/24 hr ± SEM. Statistical analysis by analysis of variance between D −3–D 1 and D 3–D 15.

all 0.26 mmol/kg^{-1}, bicuculline 2 mg/kg^{-1} alone or associated with Ca AOTA 0.26 mmol/kg^{-1} and finally the same volume of 0.9% saline for controls.

For each drug a conditioned aversion test towards saccharin was done: the animals were individually housed with free access to food; they were adapted to a regimen of water availability which consisted in the presentation of a 0.0005% saccharin in water solution for 30 minutes every other morning. Several days were allowed for the rats to adapt to this regimen and establish stable drinking rates. The rats were thereafter injected with the drugs: had the drugs produced an aversion to saccharin, they would have refused the saccharin solution, which was never the case.

RESULTS

VIE and total fluid intake did not vary in the control group during the two weeks of the study, F(1,52)=0.69 and 0.8, p=0.6 and 0.9 respectively (Fig. 1).

After treatment with Ca AOTA (Fig. 2), except at the lowest dose, (Table 1) VIE significantly decreased from the third to the fifteenth day (one way analysis of variance between the pretreatment (days −3 and 1)) and treatment periods: Ca AOTA: 0.52 mmol/kg: F(7,72)=2.9, p=0.01; Ca AOTA 0.26 mmol/kg; F(7,64)=3.4, p<0.005; Ca AOTA 0.026 mmol/kg: F(7,28)=1.7, p=0.1.

No other treatment significantly influenced VIE (Table 1).

Bicuculline alone had no effect on VIE F(7,32)=1.09, p>0.05) but inhibited Ca AOTA (0.26 mmol/kg)'s effects on VIE (Fig. 2): there was no significant reduction of VIE in this group, F(6,63)=0.5, p>0.5.

Total fluid intake in Ca AOTA 0.52 and 0.26 mmol/kg treated rats did not vary between pre-treatment and treatment period (Table 2).

Taste aversion towards saccharin remained negative. The consumption of solution was respectively 9.5 and 8.5 ml before and 10 and 9.4 after treatment with 0.26 mmol/kg and 0.52 mmol/kg of calcium acetyl homotaurine.

TABLE 2

TOTAL FLUID INTAKE IN Ca AOTA 0.52 AND 0.26 mmol/kg DAILY TREATED DRINKING RATS

	D −3	D 1	D 3	D 5	D 8	D 10	D 12	D 15	
Ca AOTA 0.26 mmol/kg	68	74	67	67	67	69	63	76	$F(7,47)=0.51$ $p>0.5$
Ca AOTA 0.52 mmol/kg	67	69	80	78	65	62	62	59	$F(7,72)=1.6$ $p=0.1$

The results are expressed as mean of ml of liquid intake (water + alcohol)/kg body weight/day.

Ca AOTA did not affect the health of treated rats: their body weight increased during the experiments (35±3) but did not differ from that of control rats (32±7 g).

DISCUSSION

The behaviour we observed was an alcohol preference and not a dependence, since the total amounts of alcohol ingested were not large enough to induce dependence, and blood ethanol levels were never detectable.

The drinking behavior of the control rats was very regular (Fig. 1). Ca AOTA was the only treatment with which a decrease of VIE was noted (Fig. 2), and this happened without any change in the total fluid intake (Table 2). The negativity of the saccharin aversion test enables us to conclude that Ca AOTA had a selective effect on alcohol preference in these rats (Table 2).

The inefficacy of OTA, Na AOTA, Na ATA and $CaCl_2$ (Table 2) shows that both acetylation which is thought to increase the blood-brain barrier penetration and Ca^{++} are necessary for the drug to act, each alone being ineffective.

One could relate this fact to the recent demonstration of two types of brain GABA receptors: the first, $GABA_A$ is stimulated by homotaurine (3 APS) and isoguvacine and blocked by bicuculline, the other, $GABA_B$ is insensitive to bicuculline and stimulated by baclofen, whose binding is modulated by divalent cations such as Ca^{++} [5,9]. The decrease in VIE would then be due to a simultaneous (possibly sinergistic?) stimulation of both types of receptors by Ca AOTA, suggesting that VIE itself could be due to a hypostimulation of these GABA receptors. Inhibition of one of the two receptors subtypes by bicuculline did indeed decrease the effects of Ca AOTA without totally suppressing them even though bicuculline by itself had no effect on VIE. This would fit in with the above mentioned hypothesis of GABA receptor hypostimulation as a source of VIE, further blockade of the receptors having no effect if the hypostimulation is already maximal. Another possible explanation for the inefficacy of bicuculline on VIE could be that a blockade of both receptor subtypes would be necessary to cause an increase of VIE.

Further studies comparing the effects of baclofen and bicuculline alone or together on VIE and its inhibition by Ca AOTA must be done to confirm and precise this theory.

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