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Noradrenergic System: Effect of DSP4 and FLA-57 on Ethanol Intake in Ethanol Preferring Rats

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DAOUST, M., P. PROTAIS AND P. LADURE. *Noradrenergic system: Effect of DSP4 and FLA-57 on ethanol intake in ethanol preferring rats*. PHARMACOL BIOCHEM BEHAV **36**(1) 133–137, 1990.—Ethanol preferring rats (male Long-Evans; n = 6) were selected as drinking rats (DR) and treated with DSP4 (50 mg·kg^{-1} IP) at the end of the preference selection. Two more groups received DSP4 (50 mg·kg^{-1} IP) + the inhibitor of dopamine beta-hydroxylase FLA-57 (1 mg·kg^{-1}·d^{-1} during two weeks), IP (n = 5) or FLA-57 alone (1 mg·kg^{-1}·d^{-1} during two weeks IP) (n = 5). The control DR group (n = 6) received NaCl 0.9%. ^{3}H-Noradrenaline uptake was studied at the 17th day of treatment in DR, treated or not with DSP4, and in ethanol naive rats treated (n = 6) or not (n = 6) with DSP4 (50 mg·kg^{-1}, IP) DSP4 does not modify ethanol intake in DR, and in both treated groups (DR or ethanol naive rats). ^{3}H-Noradrenaline uptake was decreased (about 60%), both in cortex and hippocampus. But the association of FLA-57 and DSP4 decreases both ethanol and fluid intakes. It was suggested 1) that the 40% of intact neurons was able to compensate the DSP4-induced noradrenergic neurons destruction, 2) that the destruction of noradrenergic pathways (FLA-57 + DSP4) is associated with a decrease in ethanol intake but also in fluid intakes, suggesting finally 3) that the modulation of ethanol intake by the noradrenergic system was partial or indirect.

Noradrenaline DSP4 Ethanol intake ^{3}H-Noradrenaline uptake FLA-57

THE possible involvement of catecholamines (CA) neurotransmitter systems in the modulation of voluntary intake of ethanol (EtOH) has been suggested by a number of previous reports (3, 6, 10). Manipulations of CA systems may result in modifications of animals EtOH behavior, but the literature is replete with contradictory findings: in rats, increased turnover of brain noradrenaline (NA) following acute dose of EtOH has been reported (5,15). It has been shown that neurochemical lesions of the CA system diminished voluntary EtOH consumption (20). The intraventricular administration of 6-OHDA reduced EtOH preference (22), and it was concluded that this reduction in EtOH preference could be specifically linked with NA depletion. Moreover, the use of drugs that decrease whole brain levels of NA by inhibiting the conversion of dopamine into NA (inhibition of dopamine B hydroxylase; FLA-57) decreased intragastric self administration of EtOH (8).

The NA activity is regulated by regulating presynaptic receptors and, in another interesting work (23), it was shown that administration of FLA 136, an alpha-2 receptor agonist, reduced EtOH intake by 40%, without altering total fluid intake. All these findings indicated that NA decreasing was accompanied by a reduction of EtOH intake. At the opposite, desipramine, an uptake inhibitor that has a greater specificity for NA systems (6) decreased EtOH intake both in Preferring lines of rats (21) and in EtOH naive rats (6) suggesting that an increase in synaptic NA levels decreased EtOH consumption. Another work (2) showed that alcohol intake decreased when the noradrenergic system was stimulated during REM-sleep deprivation by using a selective NA uptake inhibitor: talsupram. The discrepancies between these results can be attributed to differences in animal models of EtOH intake (REM sleep or free choice). More surely to the lack of specificity of different drugs used: 6-OHDA or desipramine.

The aim of the present experiment was to study the exact role of presynaptic NA neurones activity in the regulation of EtOH intake, using a high specific NA central neurotoxin: DSP4 (12). For this purpose, we studied EtOH intake in EtOH preferring rats after DSP4 treatment. ^{3}H-NA uptake in vitro was studied in treated rats as an "index" of neurotoxicity of DSP4. In order to completely deplete NA, the action on EtOH intake was also studied in rats injected with both DSP4 and the dopamine beta-hydroxylase inhibitor FLA-57 (11).

METHOD

Behavioral Studies (6)

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 (U.A.R. France standard diet). They were kept with an ambient temperature of 21°C and a 12 hours/12 hours light-dark photoperiod. During the initial selection period (14 days), they only had access to a 12% (vol./vol.) EtOH solution, prepared from 95% EtOH and water as drinking fluid for 14 days. This period was followed by another two-week period during which they had free choice between EtOH and water. The two fluids three bottles

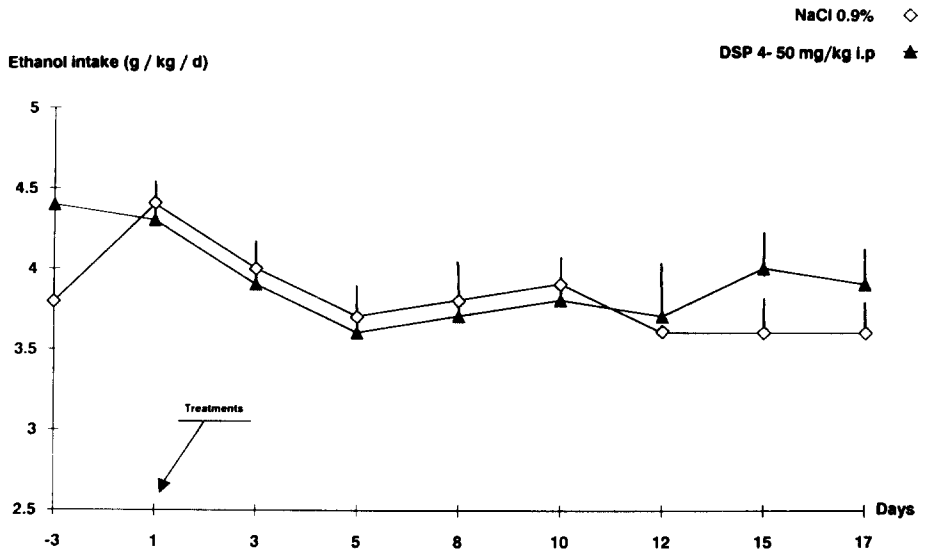

FIG. 1. Daily ethanol intake, expressed as g of absolute ethanol by drinking rats receiving on day 1; DSP4 50 $mg \cdot kg^{-1}$ or saline 0.9%.

method was used to prevent fluid selection on the basis of bottle situation. Every other day, fluid intakes and body weights were measured, the drinking bottles were refilled and randomly rotated.

Animals preferring EtOH as 60% or more of their total fluid intake during these last two weeks were selected as drinking rats (DR) and used for the study. During the study which immediately followed the selection process, there was no change in the liquid intake: water and EtOH solution regimen remained constant.

The results are given as daily absolute EtOH intake in $g \cdot kg^{-1}$ total fluid intake in $ml \cdot kg^{-1} \cdot d^{-1}$ and % of alcohol drunk. Statistical analysis was done with analysis of variance (one- or two-way), or with the Student *t*-test.

Treatments

Twelve DR were divided in two groups of 6 animals. One group received DSP4 (50 $mg \cdot kg^{-1}$ IP) on the first day of the experiment. The control DR group received NaCl 0.9%. They were kept isolated and had free choice between EtOH and water during 16 days. Their fluid intake (water + EtOH) were noted every other day.

Ten DR were also daily IP injected with FLA-57 (1 $mg \cdot kg^{-1}$) (n = 5) or with DSP4 (50 $mg \cdot kg^{-1}$ one day) + FLA-57 (1 $mg \cdot kg^{-1} \cdot d^{-1}$ during two weeks) n = 5. Twelve other EtOH naïve rats were also divided in two groups of 6 animals. One group received DSP4 (50 $mg \cdot kg^{-1}$ IP) and the control group was injected with NaCl 0.9%. These twelve rats were also kept isolated with only water to drink. On day 17, all rats were killed for ^{3}H-NA uptake assay.

^{3}H-Noradrenaline Uptake Assay (18)

On the day 17, rats were killed and their brains were rapidly removed and dissected (13). Uptake studies were done on cortex and hippocampus. Synaptosomes were prepared in 0.32 M saccharose and homogenised with a potter type Elvejhem (850 rpm).

After centrifugation (1000 × g; 10 min) the supernatant synaptosomal preparation was preincubated during 5 minutes with nialamide (4 mM) and incubated in the presence of ^{3}H-NA (45 nM) (Amersham, UK) in oxygenated Krebs medium (O_2 95%, CO_2 5%) during 10 minutes. Incubation was stopped by adding 5°C Krebs medium and centrifugation. The resulting pellet was dissolved in Luma-Solve. The radioactivity was then determined in a liquid scintillation counter (Kontron, efficiency 30%).

Nonspecific uptake of ^{3}H-NA was determined by incubating control samples at 0°C instead of 37°C. Correction for "extra neuronal uptake" was made by subtracting uptake values after incubations with 50 μM desipramine. The protein concentration was determined with crystalline bovine serum albumine as standard (19).

Drugs

DSP4 was obtained from RBI (USA) and ^{3}H-NA (30–50 Ci/mmole from Amersham (UK). FLA-57 was obtained from Astra (Sweden).

RESULTS

Figure 1 shows EtOH intake in DR treated or not with DSP4 (50 $mg \cdot kg^{-1}$; IP) or NaCl 0.9%. EtOH intake was the same in both groups, $F(8,45) = 0.93$, $p = 0.5$, NS, for DSP4 and, $F(8,45) = 1$, $p = 0.4$, NS, for control group. No difference between the two groups: $F(1,64) = 0.1$, $p = 0.7$ (ANOVA, two-way).

Figure 2 shows that DSP4 decreased ^{3}H-NA uptake from about 60%, both in DR and in naïve rats; in cortex (Fig. 2a) and hippocampus (Fig. 2b).

Total fluid intakes remained constant in both groups (Table 1), with no difference between the two groups: $F(1,64) = 0.3$, $p = 0.5$.

Figure 3 shows EtOH intake in DR treated with FLA-57 (1 $mg \cdot kg^{-1} \cdot d^{-1}$; IP) or with FLA-57 (1 $mg \cdot kg^{-1} \cdot d^{-1}$; IP + DSP4 50 $mg \cdot kg^{-1}$ on day 1). n = 5 in each case. EtOH intake decreased in rats injected with the two drugs, when compared to saline group: $F(1,64) = 27$, $p < 0.001$ or to DSP4 group: $F(1,64) = 6$, $p = 0.01$, $p = 0.07$, or to FLA-57 group: $F(1,64) = 3.3$, $p = 0.07$.

Figure 4 shows total liquid intake in DR treated with FLA-57 (1 $mg \cdot kg^{-1} \cdot d^{-1}$; IP) + DSP4 (50 $mg \cdot kg^{+1}$) or with saline. Treatment decrease total liquid intake: $F(1,64) = 32$, $p < 0.001$.

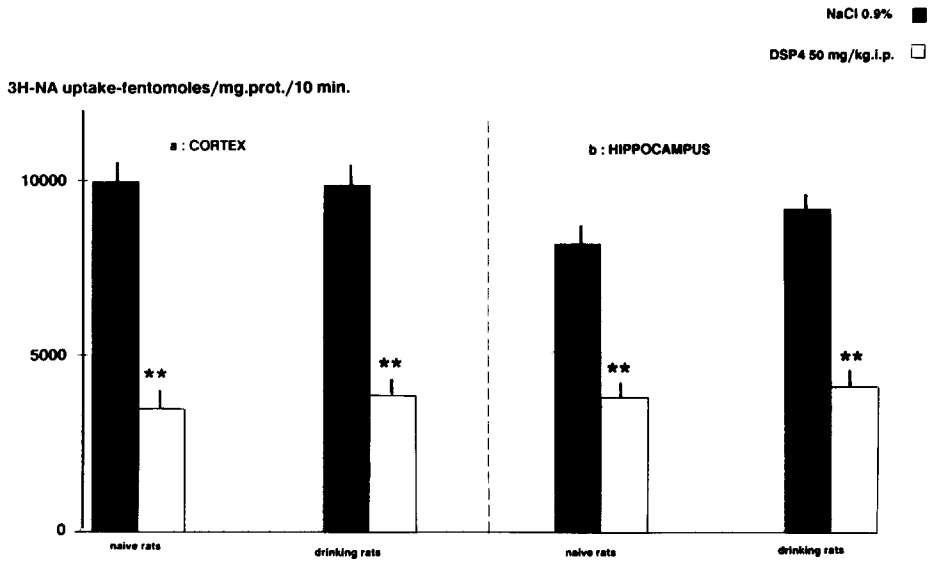

FIG. 2. ^{3}H-NA uptake by synaptosomal preparations. Results are expressed as fentomoles/mg·protein/10 minutes. $^{**}p<0.05$ (Student t-test when DSP4 group is compared to saline group). See the Method section for ^{3}H-NA uptake assay.

Total liquid intake is significantly decreased, compared to DSP4 alone: $F(1,64)=27$, $p<0.001$, but not versus FLA-57 alone: $F(1,64)=1.3$, $p>0.05$. FLA-57 alone decreased total liquid intake, compared to saline group: $F(1,64)=25$, $p<0.001$ (data not shown).

DISCUSSION

Biochemical data of this study show that DSP4 treatment decreased ^{3}H-NA uptake in rats, alcoholised or not. These results agree with others showing that DSP4 caused a selective and lasting depletion of central NA. The selectivity of DSP4 for NA neurons is emphasized by the fact that concentrations of dopamine and serotonin are known to be unchanged after treatment (12). The NA depletion is not complete. We found here a 60% depletion and other authors found 80% or 75% (12,14). It was shown that the selective depletion of NA by DSP4 produced alterations of the alpha-2 receptor and the beta receptor (but not alpha-1), and it was suggested that these alterations can be defined as NA receptor supersensitivity (12).

In our DSP4-treated rats, presenting such a NA receptor supersensitivity, EtOH intake was maintained. Two suggestions can be done: 1) a functional neuronal adaptation occurred to compensate NA depletion. The remaining neurones providing this compensation. After DSP4 treatment, other neurotransmitters are altered, particularly GABA. It has been shown that a functional coupling between noradrenergic and GABAergic systems (25,26), and an increase in GABA B receptors has been demonstrated after DSP4 treatment. The regulation of other neurotransmitters could also be an adaptative way. 2) The maintenance of EtOH intake is not sensitive to DSP4 treatment. Our DR are not genetically selected but chosen as high EtOH preferring rats. In a precedent work (8), we showed that DR were not different from nondrinking rats with regard to their EtOH sensitivity. We also showed that during selection (9), GABA binding was modified both in DR and nondrinking rats and we suggested that DR were more tolerant to EtOH than nondrinking rats since more EtOH was required to altered GABA transmission in the same magnitude.

This EtOH tolerance observed in DR was not altered by DSP4 treatment. These results agree with others (24) showing that in mice selected for EtOH sensitivity, LS (sensitive mice) or SS (insensitive mice), DSP4 treatment did not alter their EtOH sensitivity. The authors concluded that norepinephrine probably does not directly mediate behavioral sensitivity to EtOH in these mouse lines. These data and our present results did not agree with others, including ours (6), showing the involvement of NA transmission in the modulation of EtOH intake. REM-sleep deprivation (1), talsupram (2), desipramine (6.21), U-14 624 (10), or FLA-56 (alpha-2 receptor agonist) (23), or 6-OHDA (16, 17, 20) were all used to manipulate NA system. When NA levels are increased in the synaptic cleft (2, 6, 21), EtOH was decreased. When NA pathways were lesioned with 6-OHDA, an increase in EtOH intake was observed (16), on the third week after injection. But this increase was transient. The authors suggested that the depletion of central NA stores resulted in an increased alcohol consumption via compensatory mechanisms following damage to the ascending NA pathways. This depletion of NA stores was also accompanied by a dopamine depletion. On the other hand, when

TABLE 1

TOTAL LIQUID INTAKE BY DR TREATED WITH DSP4 (50 mg·kg^{-1}, IP), OR WITH NaCl 0.9% ON DAY 1 AND DURING 17 DAYS

Days Treatments	−3	1	3	5	8	10	12	15	17
DSP4	69	72	76	68	64	72	64	63	65
50 mg·kg^{-1}, IP	±5	±4	±13	±4	±1	±3	±3	±2	±3
NaCl 0.9%	89	79	87	77	72	74	75	71	76
	±5	±2	±4	±2	±3	±4	±4	±2	±7

Results are expressed as ml·kg^{-1}·d^{-1} of total liquid (water + ethanol) (mean ± SEM) n=6.

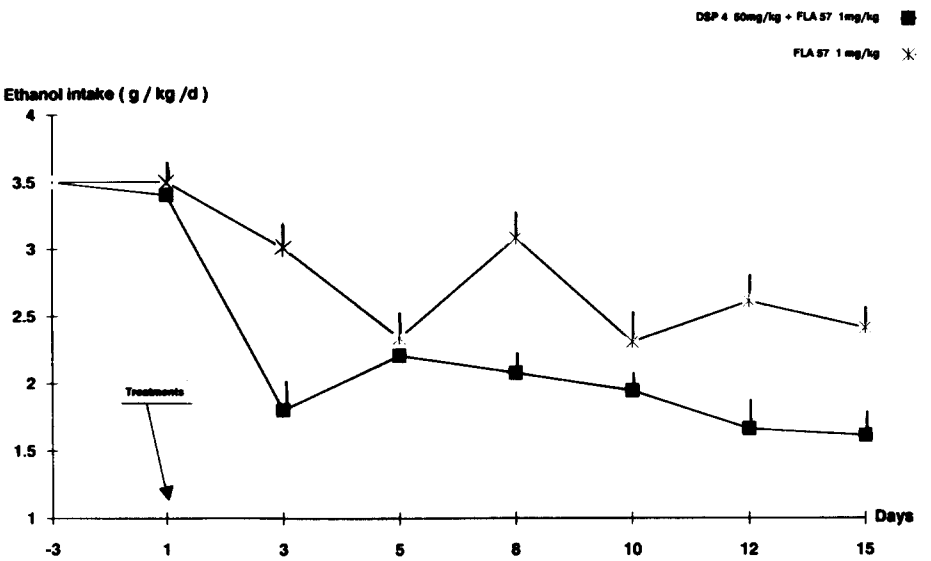

FIG. 3. EtOH intake expressed as g of absolute ethanol by drinking rats IP injected with FLA-57 (day 1 to 15), 1 $g \cdot kg^{-1} \cdot d^{-1}$ + DSP4 (on day 1), 50 $mg \cdot kg^{-1}$, or with FLA-57 alone (day 1 to 15), 1 $mg \cdot kg^{-1} \cdot d^{-1}$.

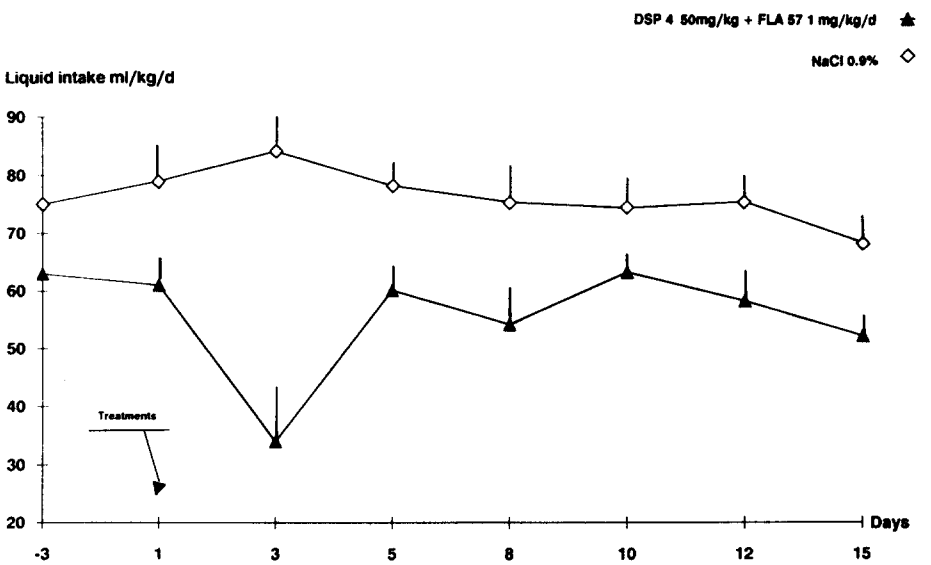

FIG. 4. Total (water + ethanol) liquid intake, expressed as ml of liquid, $kg^{-1} \cdot d^{-1}$, by drinking rats, IP-injected with FLA-57 (day 1 to 15) 1 $mg \cdot kg^{-1} \cdot d^{-1}$ + DSP4 (on day 1) 50 $mg \cdot kg^{-1}$ or with NaCl 0.9% (day 1 to 15).

NA depletion was obtained with REM sleep deprivation (1), EtOH was also increased. In another work (20), NA depletion with 6 OHDA was associated with a reduction of EtOH intake.

The discrepancies between these results could be attributed to the lack of specificity of such depletions. In each case, dopamine being also depleted. At the opposite, results about the increase of NA levels in the synaptic cleft (with uptake inhibitors) seem more coherent. In all studies, (2, 6, 21, 10), an enhancement of NA transmission is accompanied by a decrease in EtOH intake. These data suggest that the reduction of EtOH intake was depending on an increase in NA availability, but that the destruction of NA pathways was not directly involved in the maintenance of EtOH intake.

It is the case in our present work, showing that when NA neurons were totally destroyed or inhibited (FLA-57 + DSP4), EtOH intake decreased, but also total fluid intakes. The partial destruction of NA neurons with DSP4 or the inhibition of NA synthesis with FLA-57 were enable to alter EtOH intake in our animal model. These data show that NA was more probably involved in the regulation of fluid intakes in general; its EtOH behavior modulation being only partial and/or indirect.

Finally the ability of NA to decrease EtOH intake is associated

with its postsynaptic action: increase in NA levels in the synaptic cleft with uptake inhibitors. At the opposite, when NA is decreased, EtOH is not always modified, indicating that EtOH modulation via NA neurons seems to be partial or indirect. The action of other neurotransmitters (GABA, serotonin or both) and their modulation during NA decreasing has to be examined to better understand the exact nature of the interaction NA-EtOH.

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