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Ethanol Effects on Dopaminergic Function: Modulation by the Endogenous Opioid System

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BARBACCIA, M. L., A. REGGIANI, P. F. SPANO AND M. TRABUCCHI. *Ethanol effects on dopaminergic function: Modulation by the endogenous opioid system.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 303–306, 1980.—Different behavioral and biochemical data suggest that ethanol has different effects on central dopaminergic transmission in rat and mouse. We found that ethanol induces an increase of striatal dopamine turnover which does not persist after chronic drinking. Following chronic ethanol treatment, we observed the development of supersensitivity of the striatal dopamine (DA) recognition sites, in terms of an enhanced affinity. We investigated various experimental models to clarify the existence of an enkephalinergic modulation of ethanol effects on the dopaminergic system. We found that in the rat, a pretreatment with naloxone abolishes the striatal DA turnover increase observed after ethanol. DBA 2J mice, which differ from C57 BL/6J and Swiss Albino, by genetically lacking enkephalinergic modulation on dopaminergic activity in the striatum, do not show any change of DA metabolism after acute ethanol. In the rat retina, where we hypothesized a less operant regulation of dopaminergic activity by enkephalins, tolerance does not develop after chronic drinking to the increase in DA turnover as it did in striatum. Our results confirm the importance of the endogenous opioid system in the regulation of the ethanol induced neurochemical and behavioral effects.

Ethanol Dopaminergic system Opioid system Naloxone

IN the last ten years various laboratories have been involved in the study of the mechanism by which ethanol interacts with the central nervous system (CNS).

It is of particular interest to clarify the relationships between alcohol consumption and the so called "rewarding system", whose anatomical bases seem to be the endorphinergic-enkephalinergic neuronal systems. Along this line, recent clinical reports indicate a possible role of endogenous opiates in determining the behavioral changes induced in man by ethanol. Various authors have, in fact, demonstrated the efficacy of naloxone to antagonize ethanol intoxication symptoms in humans [11, 16, 22] and in experimental animals [3]. In our studies on the mechanism of action of ethanol we particularly investigated the dopaminergic-enkephalinergic interactions, whose functional interplay has been demonstrated in some brain areas [17,18]. In fact acute and chronic ethanol consumption affect dopaminergic transmission in various animal species. Acute ethanol administration causes an increase of striatal DA turnover in the rat [14,19] and in the mouse [23]. Moreover, using behavioral and biochemical models, an alteration of DA receptor sensitivity in rats chronically drinking ethanol was reported [15,19]. Our working hypothesis is that these changes may be mediated by other neuronal systems, i.e. the endogenous opioids. Various authors have ascribed an important role in determining ethanol-induced central effects to tetrahydroisoquinolines (T.I.Q.s), a class of compounds deriving from the condensation of acetaldehyde with endogenous amines. T.I.Q.s have been found in cerebrospinal fluid and urine of human alcoholics [21]. The T.I.Q.s interact in central and peripheral nervous systems with opiate receptors as well as with dopaminergic function [6,9].

In our paper we examined some specific experimental conditions, using different models, in which we observed that ethanol alters DA function in the brain by acting through the central opioid system.

Effect of Ethanol on DA Turnover and Receptors

Previously we reported, in accord with other authors [14, 15, 23] that ethanol interferes, in the CNS, with dopaminergic transmission. We found that striatal dopaminergic turnover, measured as dihydroxyphenylacetic acid (DOPAC) levels, is increased by acute ethanol administration in the rat [19]. Interestingly, we observed a correlation between the changes of striatal DOPAC levels and the rate of hepatic metabolism of ethanol. In fact, increasing or decreasing the rate of liver metabolism, we found an earlier or a reduced peak of striatal DOPAC increase. Acetaldehyde is able to induce the same effect on central dopaminergic function as ethanol. The acutely induced increase of DOPAC does not persist after chronic alcohol consumption (Barbaccia *et al.*, to be published). To evaluate the sensitivity of the dopaminergic recognition sites to ethanol action, we measured the ^{3}H-Spiroperidol specific binding in rat striatal tissue after acute and chronic treatment. Figure 1 shows the time course of the modification of the dopamine binding sites at various times after chronic ethanol drinking. After five days of ethanol consumption we observed a supersensitivity of the dopaminergic recognition sites persisting up to 21 days of

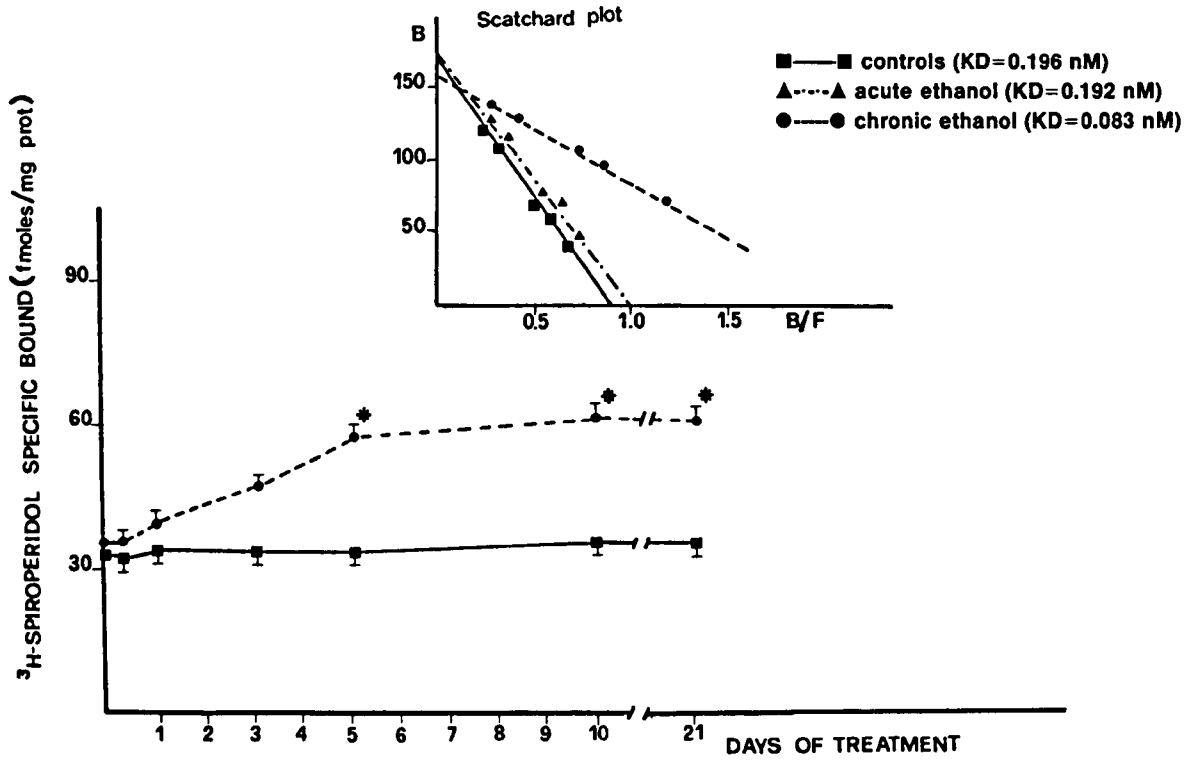

FIG. 1. Time course and Scatchard analysis of ^{3}H-Spiroperidol specific binding in rat striatal membranes after acute and chronic ethanol treatments. *$p<0.01$ in comparison to control animals. The ^{3}H-Spiroperidol concentration was 0.05 nM. In insert: B is the ^{3}H-Spiroperidol specific bound expressed as fmoles/mg protein; F is the concentration of ^{3}H-Spiroperidol expressed as nM. The ^{3}H-Spiroperidol radioreceptor binding was performed according to the method described by Burt *et al.* [4] with minor modifications. Each value represents the mean ± SEM of three experiments run in quadruplicate. The acutely treated rats received ethanol (3 g/kg) intragastrically as an aqueous solution at 20%. The control animals received an equal volume of water. During the chronic treatment lasting 21 days, the rats consumed 9 g/kg/day of ethanol as a solution at 6% in their drinking water. The animals were killed three hours after the last administration.

treatment. The kinetic analysis shows that ethanol induces a significant increase in the affinity of the labelled ligand binding to its receptors.

Opiate Antagonist and Ethanol

Naloxone, as reported above, has been proposed as a treatment for alcoholic intoxication [11, 16, 22]. We tested this opiate receptor antagonist on the striatal dopaminergic function changes induced by ethanol. The pretreatment with naloxone prevents the increase of striatal DOPAC levels induced by alcohol treatment and by morphine injection, indicating that the changes of striatal dopamine turnover observed after ethanol are mediated via an enkephalinergic-dopaminergic interaction whose presence has been demonstrated in the striatum [18,24]. In contrast, naloxone is ineffective in blocking the DOPAC increase after haloperidol (Fig. 2).

Biochemical Effects of Ethanol on Different Strains of Mice

We tested the effects of acute and chronic ethanol on dopaminergic system activity in several strains of mice: Swiss albino, C57BL/6J and DBA 2J. These mice differ because the last strain lacks enkephalinergic receptors upon the nigrostriatal dopaminergic fibers [20]. Interestingly, we found, as is shown in Table 1, that only those mouse strains, whose nigro-striatal dopaminergic firing is modulated by enkephalins, respond to ethanol with an increase of striatal DOPAC levels. The DBA failed to show any effect of ethanol on striatal dopaminergic system. It is important to note that DBA mice do not show modifications of striatal DOPAC levels after either ethanol or morphine [20,24], while they are sensitive, in terms of dopamine turnover changes, to acute haloperidol treatment.

Action of Ethanol on Retinal Dopamine Metabolism

It has been recently reported that the modulation of dopaminergic activity by retinal opiate receptors is less operant in comparison with the striatum (Barbaccia *et al.*, to be published). We investigated the effect of ethanol on the dopaminergic system in the retina. As Fig. 3 shows, we found that in this area acute ethanol, similar to haloperidol, induces an increase of DOPAC levels. Moreover, tolerance does not develop to this event, as indicated by the DOPAC levels which remain higher than controls either after chronic drinking or after chronic haloperidol.

The results support on an anatomical basis the existence of interrelationships between enkephalinergic and dopaminergic neurons, modulating the neurochemical effects of ethanol.

CONCLUSIONS

In agreement with observations of other authors, our re-

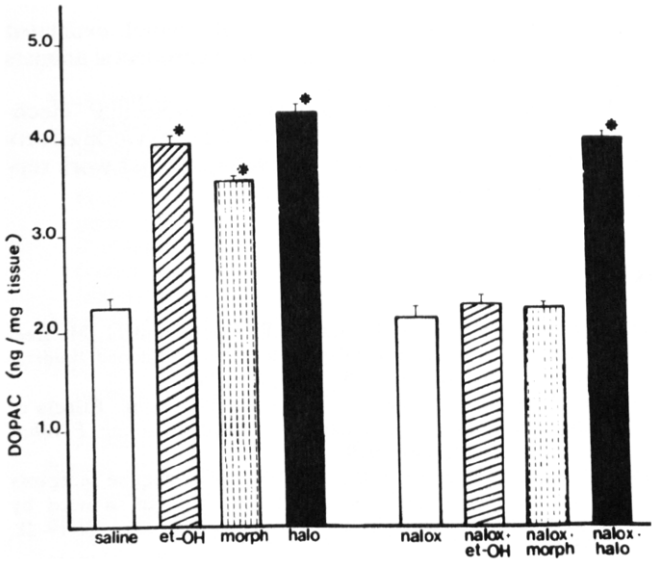

FIG. 2. Effect of Naloxone on striatal DOPAC increase induced by ethanol, morphine and haloperidol. *$p<0.01$ in comparison to saline treated animals. Each value represents the mean ± SEM of three experiments run in quadruplicate. Morphine (10 mg/kg) and haloperidol (1 mg/kg) were administered intraperitoneally. The rats were killed 1 hour after the treatment. Naloxone (3 mg/kg) was administered intraperitoneally 30 min before killing. The control animals received an equal volume of saline and were sacrificed 30 minutes after injection. For acute ethanol (Et-OH) treatment see Fig. 1. Dopac level determinations were performed in accord to the method described by Argiolas *et al.* [2].

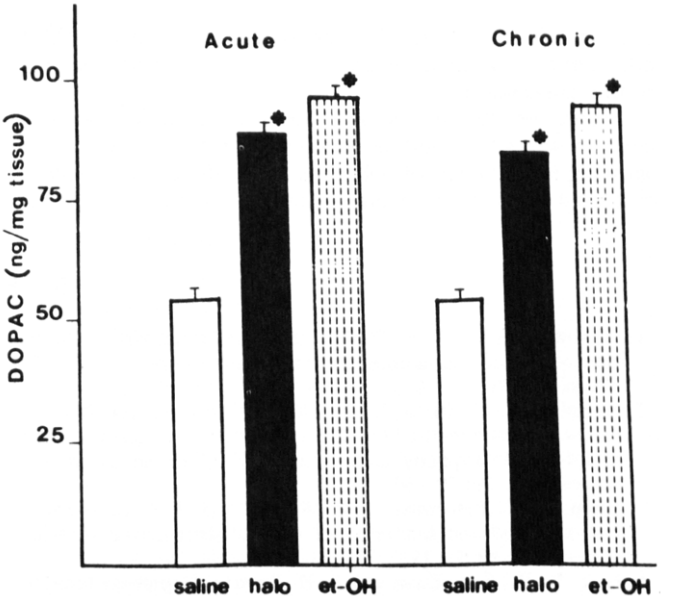

FIG. 3. Effect of acute and chronic treatments with ethanol and haloperidol on DOPAC levels in rat retina homogenate. *$p<0.01$ in comparison to saline treated rats. Each value represents the mean ± SEM of three experiments run in quadruplicate. For treatments and DOPAC levels determination see Figs. 1 and 2.

sults indicate that ethanol interacts with the dopaminergic system, increasing DA turnover in some rat brain areas and inducing a supersensitivity of dopamine recognition sites, after acute and chronic treatment, respectively. Our paper, however, is focussed on the complexity of the mechanism of action of ethanol, whose effects on dopaminergic function cannot be explained only via a direct interaction with this system. The results suggest that the functional relationship between enkephalins and DA may be most important for the development of alcohol's neurochemical effects. The models we used in our study offered significant indications on this point. In particular, while in the striatum the increase of DOPAC levels returns to basal values after chronic drinking, in the retina tolerance does not occur. Hong *et al.* [10] ascribed an important role to striatal enkephalinergic neurons in the development of tolerance to DA turnover changes. Our working hypothesis is that, after chronic ethanol treatment, tolerance may be explained on these bases. In fact we previously suggested that in the retina the modulation of enkephalinergic neurons on dopaminergic fibers is physiologically less operant. Alternatively, recalling the hypothesis of a putative role of T.I.Q.s in ethanol-induced effects [5, 8, 21], it may be hypothesized that a difference in formation or metabolism of these compounds in various cerebral areas may account for the neurochemical phenomena induced by ethanol.

In spite of some observations suggesting the interaction with the lipid component of the cell membrane as the molecular site of action of alcohol [7, 12, 13], our results indicate the specificity of ethanol action in terms of neuronal systems involved.

On this line may be interpreted the data showing the effect of naloxone in blocking the increase in DA turnover after ethanol. The differential effect of ethanol on DA turn-

TABLE 1

EFFECT OF ACUTE AND CHRONIC ETHANOL TREATMENTS ON STRIATAL DOPAC LEVELS OF DIFFERENT MICE STRAINS

	DOPAC (ng/mg tissue)					
Treatment	Albino		C57		DBA	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
Saline	2.31 ± 0.15	2.52 ± 0.18	2.18 ± 0.13	2.25 ± 0.14	3.02 ± 0.21	3.17 ± 0.19
Ethanol	3.25 ± 0.15*	2.45 ± 0.23	3.11 ± 0.11*	2.23 ± 0.12	3.28 ± 0.18	3.11 ± 0.15

*$p<0.01$ in comparison to saline treated animals of the same strain.
Each value represents the mean ± SEM of three experiments run in quadruplicate.
For treatments and DOPAC levels determinations see Figs. 1 and 2.

over in C57 and DBA mice, characterized by a genetically different population of opiate receptors, give further support to this contention.

Moreover, the action of ethanol at the opiate receptor level is again stressed by clinical and behavioral studies showing the efficacy of naloxone and naltrexone to antagonize post-alcohol intoxication states in humans [11, 16, 22] and to alter the reinforcing properties of ethanol, evaluated with intravenous self-administration in experimental animals [1,3].

Although the neuronal circuits and molecular mechanisms at the basis of the neurological and behavioral effects of ethanol are yet to be exactly characterized, our work represents a further step toward this end.

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