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Ethanol and Dopaminergic Systems

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LUCCHI, L., M. LUPINI, S. GOVONI, V. COVELLI, P. F. SPANO AND M. TRABUCCHI. *Ethanol and dopaminergic systems*. PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 379–382, 1983.—Chronic ethanol consumption produces derangements of cell membrane structure, perhaps by changing membrane lipid content. This impairment leads to modification of membrane-related processes. In fact, after chronic ethanol exposure, an increase in striatal adenylate-cyclase activity occurs. On the other hand, dopamine is unable to further potentiate the production of cyclic AMP. This finding demonstrates that the dopaminergic receptor associated with adenylate-cyclase activity is affected by chronic ethanol treatment. In particular, the affinity of the dopaminergic receptor labelled by ^{3}H-Spiperone is enhanced. In addition, the receptor-adenylate cyclase coupling system is impaired after chronic *in vivo* exposure of animals to ethanol.

Ethanol Striatal dopaminergic systems Dopamine receptors Adenylate cyclase Guanyl nucleotides

ETHANOL consumption produces various effects on biological and biochemical structures, in particular in the central nervous system (CNS). The molecular mechanism of ethanol's effects has not been completely clarified; however neurotransmitter systems seem to be the first step in the mechanism of alcohol action in the CNS. In fact, various experimental reports indicate several modifications in catecholaminergic [2, 15, 28] and GABAergic transmission [22,27] after acute or chronic ethanol consumption. Our work has been carried out in order to better clarify the role of neurotransmitters in the mediation of ethanol effects at the central level.

In previous studies it has been demonstrated that DA turnover, measured as 3,4-dihydroxyphenylacetic acid (DOPAC) content, is increased after acute ethanol consumption [2,21], whereas chronic treatment failed to enhance striatal DOPAC concentration. The increase in dopaminergic turnover depends on the rate of ethanol metabolism, i.e., on the formation of acetaldehyde and other products of alcohol metabolism. In this context an important role of acetaldehyde has been demonstrated for several of ethanol's central effects [2,14]. Furthermore, it has been demonstrated that the dopaminergic receptor complex is affected by ethanol administered chronically. Following such treatment, an increased affinity for ^{3}H(−)Sulpiride and ^{3}H-Spiperone has been shown [3,21]. The modifications of the dopaminergic receptor complex induced by chronic ethanol treatment result in an imparied function of adenylate-cyclase catalytic unit activity. On the other hand, the functionality of adenylate-cyclase activity is linked to membrane structure integrity, as proposed by Gordon *et al.* [9]. It has been demonstrated that ethanol modifies cellular membrane structure, for example, by increasing membrane fluidity [5,12]. However, our findings rule out the hypothesis that the biochemical events produced by chronic ethanol consumption may be the result of a primary membrane composition derangement.

METHOD

The experimental procedure of ethanol intoxication was carried out using male Sprague Dawley rats (Charles River, Calco, Italy), under the following conditions. Acute treatment was performed by means of gastric intubation, and ethanol was administered as a 20% (v/v) aqueous solution (corresponding to 3 g/kg body weight of ethanol). Chronic intoxication was carried out by administering a 6% (v/v) aqueous solution of ethanol during 21 days to the rats as the only available beverage. The mean daily ethanol consumption was 9 g/kg/day. Control rats received an equivalent diet in which ethanol was substituted by an equicaloric amount of sucrose in order to avoid caloric imbalance between controls and chronically ethanol-treated rats. A significant difference in body weight between ethanol-fed and sucrose-fed groups was not observed after the chronic treatment (265±21 g and 280±23 g for control and ethanol-treated rats, respectively). Animals were killed by decapitation, brains were removed rapidly and the striata dissected following the method indicated by Glowinski and Iversen [8]. 3,4-Dihydroxyphenylacetic acid (DOPAC) concentration was determined according to the radioenzymatic method of Argiolas *et al.* [1]. ^{3}H-Spiperone (NEN, 27.6 Ci/mmole) specific binding was carried out according to Burt *et al.* [4] with minor modifications. Briefly, the particulate fraction from striatal membranes (0.2–0.25 mg protein/tube) was incubated in the presence of 0.2 nM ^{3}H-Spiperone and increasing concentrations of apomorphine as the displacing drug. Specific binding is defined as the percentage of displacement given by the various apomorphine concentrations with respect to the total binding.

The determination of adenylate cyclase activity was performed according to Clement-Cormier [6]. The particulate fraction from striatal membranes was incubated with a mixture of (8-^{14}C)-Adenosine 5'-triphosphate (ATP) (Amersham, 50 mCi/mmole), containing 0.5 mM cold ATP. The purifica-

TABLE 1

EFFECT OF "IN VIVO" ACUTE AND CHRONIC ETHANOL TREATMENTS ON DOPAMINERGIC TRANSMISSION IN RAT STRIATUM

	Acute Ethanol	Chronic Ethanol
DOPAC Content	↑	no changes
^{3}H-Spiperone Binding	no changes K_d and B_{max}	↓ K_d, no change B_{max}
GTP Effect on ^{3}H-Spiperone Binding	no effect	no effect

SEM was used as index of variability. Statistical analysis was performed using the two-tailed Student's *t*-test [17].

Changes described with respect to the values obtained in control animals. Results are the mean of three different assays carried out in triplicate using 5 rats per group [17].

TABLE 2

EFFECT OF CHRONIC ETHANOL TREATMENT ON BASAL AND DOPAMINE-STIMULATED ADENYLATE CYCLASE ACTIVITY IN RAT STRIATUM

	Control	Chronic Ethanol
	cAMP (pmoles/mg protein/min)	
Adenylate Cyclase (Basal enzyme activation by Mg^{++})	65 ± 3	101 ± 4*
Adenylate Cyclase (DA stimulated)	180 ± 5†	120 ± 9

*$p<0.01$ with respect to control value.

†$p<0.01$ with respect to basal value obtained with the optimal Mg^{++}.

SEM was used as index of variability. Statistical analysis was performed by the two-tailed Student's *t*-test.

Results are the mean ± SEM of three experiments performed in triplicate using 4 rats per group.

tion of cyclic AMP (cAMP) formed was carried out according to Kebabian *et al.* [13].

Protein content was measured by the method of Lowry *et al.* [6]

RESULTS

In Table 1 are summarized the effects of acute and chronic ethanol treatments on the dopaminergic system of rat striatum. The various biochemical parameters demonstrate that ethanol interacts with dopaminergic transmission. In particular, it is shown that dopamine (DA) synthesis is activated after acute ethanol administration, as demonstrated by the increase in DOPAC content. On the contrary, chronic ethanol consumption failed to modify DA turnover [2]. Following chronic ethanol treatment, when DA synthesis apparently becomes tolerant to ethanol's effect, a receptor supersensitivity develops. The affinity of DA receptors preferentially labelled by ^{3}H-Spiperone is enhanced in striatal membranes from rats treated chronically with ethanol. In fact, the K_d values are 3.68±0.3 nM and 1.9±0.09 nM for the low-affinity component, and 0.2±0.01 and 0.12±0.01 nM for the high-affinity component in control and chronically ethanol-treated rats, respectively.

It has been reported that the affinity of DA receptors for agonists is decreased by guanine nucleotides (GTP) [18,20]. This event reflects coupling between the receptor and the effector, adenylate cyclase, as proposed by others [18, 19, 23]. Our results show that in striatal membranes from chronically ethanol-treated animals, 10^{-6} M GTP is unable to produce its effects on DA receptor affinity [17].

Basal adenylate cyclase activity is higher in striatal membranes from rats chronically treated with ethanol than in those from controls. The activation obtained in the presence of 10 mM Mg^{++} is more pronounced in membranes from chronically ethanol-treated rats than in those from controls, as shown in Table 2. On the other hand, the adenylate cyclase stimulation by 40 μM DA is lower in striata from chronically ethanol-treated rats than in control striatal membranes. No changes were observed in adenylate cyclase activity in striatal membranes from acutely ethanol-treated animals. In addition, there was no difference in the stimulation induced by 10 mM NaF in membranes from control and chronically ethanol-exposed animals (data not shown).

DISCUSSION

Recent studies regarding the mechanism of action of ethanol have been focused on its effect on cell membranes. In fact, physiologically relevant concentrations of ethanol may increase membrane fluidity [5, 9, 12], and it was demonstrated that the potency of various alcohol in changing some membrane-related events is proportional to their lipid solubility [10,26].

It has been demonstrated that a large number of membrane-related activities, such as enzymatic and membrane transport processes are affected by membrane lipid content [9,11]. The reported effects of ethanol on brain neurotransmitters [3, 21, 28] may thus be a consequence of these changes in membrane lipid composition. In this paper we have described the enhanced function of the dopaminergic receptor complex during chronic ethanol consumption, at a time when dopaminergic turnover is tolerant to ethanol's effects. In addition to the change in receptor function, there is also a change of adenylate cyclase activity. In striatal membranes from rats treated chronically with

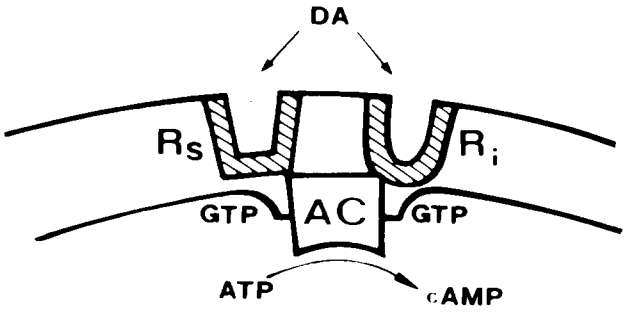

FIG. 1. Schematic representation of interactions between receptor unit (R) and adenylate cyclase (AC) through linkage with GTP.

ethanol, the basal adenylate cyclase activity is enhanced with respect to controls. In *in vitro* studies, ethanol produced a comparable increase in adenylate cyclase activity, as reported by Rabin and Molinoff [20]. The increase in cAMP production was the result of ethanol effects on different metabolic steps responsible for its formation. The lack of stimulation of adenylate cyclase activity by dopamine in membranes from rats exposed *in vivo* to chronic ethanol treatment confirms the previously reported hypothesis that the dopaminergic receptor associated with adenylate cyclase is affected by alcohol [28]. This event was demonstrated in our experiments by the shift of the curve for apomorphine displacement of ^{3}H-Spiperone binding to striatal membranes from chronically ethanol-treated rats [3]. In this study an enhanced affinity for the agonist at the dopaminergic receptor was demonstrated. The agonist portion of the dopaminergic receptor to which ^{3}H-Spiperone binds is the region with high affinity for apomorphine, and is post-synaptic [25]. This agonist receptor is sensitive to guanyl nucleotides and to thermal denaturation, as discussed in a recent paper by Sokoloff *et al.* [24]. The interaction between the receptor with adenylate cyclase is mediated by guanyl nucleotides (GTP) as proposed by various authors [7,23], and the affinity of dopamine receptors for agonists is selectively decreased by GTP [18, 20, 24]. After chronic ethanol exposure we found that GTP is unable to modify the agonistic component of dopamine receptor binding in striatal membranes [17], clearly demonstrating that the receptor-adenylate cyclase coupling system is affected by ethanol.

In Fig. 1 is schematically represented the interactions taking place between the receptor unit (R) and the adenylate cyclase (AC) through linkage with GTP. This process is dependent on membrane integrity and, as reported, is affected at various steps by ethanol modifications of membrane structure. Our data are consistent with the hypothesis that ethanol activates dopaminergic transmission, in particular the adenylate cyclase system, as a consequence of the increase in membrane fluidity.

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