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Ethanol and Receptor Function

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The effects of ethanol *in vitro* (21 and 42 mM) and *in vitro* (10 and 30 days of exposure) on the dose:effect curves for noradrenaline and acetylcholine in isolated auricles and uterus of rats, respectively, have been studied. Likewise, the acetylcholine-atropine interaction was studied in rat uterus under the above-mentioned conditions. Analysis of theoretical curves as well as pD_2 and pA_2 calculations revealed that ethanol causes changes in the tissue responses to the agents assayed which in turn may be an expression of modifications in the receptor function.

In recent years, several authors have pointed out that ethanol causes modifications in biomembrane composition and function (1, 3, 5, 7, 10, 14, 16). As a consequence, the possibility exists that these modifications give rise to subsequent changes in the structure of the so-called «membrane receptors», including those where neurotransmitters are specifically bound. Supporting this hypothesis, CIOFALO (2) found that low concentrations of ethanol *in vitro* increased the total α-adrenergic receptor population in the rat brain. On the contrary, high concentrations of the drug produced a decrease in the receptor population. Likewise, REGGIANI *et al.* (11) observed that chronic administration of ethanol causes a selective increase of specific ^{3}H-spiroperidol binding in rat striatum, kynetic analysis showing a significant increase in the affinity constant. From a theoretical point of view, these receptor changes may in turn result in modifications of tissue responses to neurotransmitters. In the present paper, the dose:response curves for noradrenaline (NA) and acetylcholine (ACh) in isolated auricle and uterus of rats, respectively, were comparatively studied either in the absence or in the presence of ethanol *in vitro* as well as after *in vivo* exposure to the drug. On the other hand, as ethanol has complex actions on the cellular metabolism and on the heart catecholamine storage (9) and inotropism (4, 12) which by themselves may produce alterations in the pharmacological responses, additional experiments were performed in rat uterus to

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assess the influence of ethanol on the cholinergic antagonism by atropine, a ligand whose blocking activity depends only on its receptor affinity.

Materials and Methods

Sprague-Dawley rats, weighing 250-300 g, were used. The animals were housed individually in wire screen cages and allowed free access to food and water. An injection of stilbestrol, 0.1 mg/kg s.c., was given 24 hours before the experiments to the animals designated for uterus preparation.

In vitro *experiments.* The animals were decapitated and the auricle and uterus were suspended in a tissue bath (20 ml) containing Krebs solution (modified by adding Na_2EDTA and atropine) in the case of the auricles, and Jalon's solution for uterus. The bathing medium was continuously oxygenated by dubbling a mixture of 5 % carbon dioxide in oxygen and maintained at 37° C. Auricles and uterus were mounted on glass tissue-holders and attached to the recording instruments. These were comprised of a Statham force-displacement transducer for auricle preparations, and a frontal-writing lever for uterus studies. The preparations were allowed to equilibrate for 30 min with 0.5 g of resting tension or load. Isometric contractile responses of auricles were recorded on a Beckman R-511 dynograph. A Bio-Science Kymograph was used to record the isotonic contractile responses of the uterus.

Appropriate concentrations of ethanol were selected by exposing auricles (n=10), for four minutes, to 21, 42, 84 and 168 mM of the drug. Only the concentrations of 21 and 42 mM which were free from arrhythmic effects (see Results) were employed in the subsequent studies. Control dose: effect curve for NA (Norepinephrine bitartrate, Sigma) was obtained by exposing the auricles (n = 12) to logarithmic increasing non-cumulative doses of this agent for two minutes. Cumulative concentrations of ACh (Acetylcholine chloryde, Sigma) were used in uterus preparation (n = 12) to obtain the dose: response curve for this agent. All these experiments were repeated in presence of 21 and 42 mM of ethanol added to the bath two minutes before either Na or ACh (a separate group of 48 rats was used, twelve animals per dose of ethanol).

In vivo *experiments. In vivo* ethanol administration was carried out by substituting drinking water by a 15 % (v/v) ethanol solution. In the control animals, the alcoholic beverage was substituted by a liquid diet isocaloric with ethanol (sucrose, 90 g/l). One group (n = 12 for each treatment) of these animals was sacrificed ten days after ethanol or sucrose exposure. Their auricles and uterus were prepared and tested for NA and ACh in the way above-mentioned for the *in vitro* experiments. Similar assays were carried out with an equal number of preparations 30 days after ethanol or sucrose exposures. At the end of the two intoxication periods, the amount of ethanol consumed by each animal was noted. Likewise, alcoholemia was measured at the same times by gas liquid chromatography.

Effect of ethanol on atropine-induced antagonism. The action of atropine (Atropine sulphate, Sigma) 10^{-8} M on the dose: response curve for ACh was tested under all the experimental circumstances above described for uterus preparation.

Data analysis. The data obtained in the different experimental series were used to calculate the theoretical log dose: response curves for the compounds studied. In the case of agonists, the equation $E_A = \frac{Emax}{K_A/A + 1}$ was employed, where K_A represents the dissociation constant and

A the dose producing an effect E_A which is a fraction of the maximal effect Emax. For competitive antagonism induced by atropine the following equation was used: $E_{AB} = \frac{E_A max}{1\,(1\,B/K_B)K_A/A}$, where K_B represents the dissociation constant of the antagonist and B the dose used. On the other hand pD_2 values for NA and ACh as well as pA_2 values for atropine (15) were calculated for all the experimental series. These latter parameters were submitted to statistical calculation by using the Student «t» test for comparison between means.

Results

It is necessary to indicate that the data obtained in the organs removed from sucrose-fed rats were entirely similar to those seen in control preparations *in vitro*. Therefore, in order to simplify the graphical exposition of the results, control data of both sucrose treated and untreated animals are presented together.

The effects of increasing doses of ethanol on isolated auricles are summarized in table I. The doses of 21 and 42 mM of ethanol were selected for the subsequent studies because they did not induce either arrhythmia or marked disturbances in the contraction force. The effects of ethanol on the pharmacological response to NA are set forth in figure 1. The presence of ethanol *in vitro* at the dose of 21 nM induced a slight decrease in the maximal chronotropic effect of NA. This effect was accompanied by a marked increase in the maximal inotropic effect. When the concentration of ethanol was augmented to 42 nM a further decrease in the maximal chronotropic effect was observed, the maximal inotropic effect also being reduced. In the animals exposed to ethanol for ten days, a slight increase of both the maximal chronotropic and inotropic effects of NA was seen.

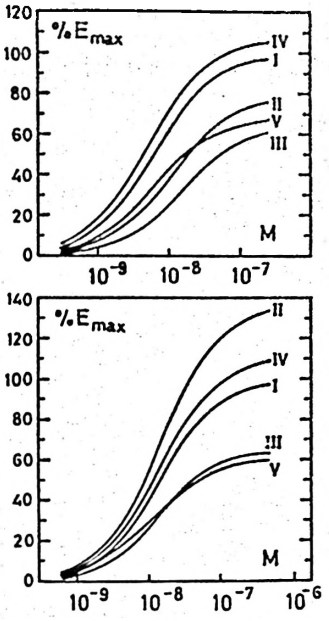

Fig. 1. *Modifications induced by ethanol on the theoretical dose:response curve for the chronotropic (upper part) and inotropic lower part) effects of NA.*
I = Control. II = Ethanol, 21 mM. III = Ethanol, 42 mM. IV = Ten days of ethanol exposure. V = 30 days of ethanol exposure.

After 30 days of alcohol intoxication both parameters were similarly diminished.

Results obtained in isolated uterus are presented in figure 2. The presence of 21 nM of ethanol did not produce any change as compared with the control curve for ACh. Ethanol, 42 mM, gave rise to a displacement to the right of the dose:response curve accompanied by a moderate decrease of the maximum effect. When the animals were submitted to the alcoholic beverage for ten days, the dose:effect curve for ACh exhibited a marked displacement to the left without any change in the maximal effect. However, alcohol exposure for 30 days produced a displacement to the right on the dose:effect curve without any change in the maximal effect.

Table I. *Effect of ethanol on chronotropic and inotropic activity of the isolated auricle of rat.*
The values indicate per cent of change as compared with controls (mean ± S.E.M.). The chronotropic activity at 0 time was 192 ± 8 beats/min, contraction force being 0.76±0.02 g.

Ethanol (mM)	1 min		2 min		3 min		4 min	
	Freq.	Force	Freq.	Force	Freq.	Force	Freq.	Force
21	—0.4±3.5	—3.9±2.5	—0.4±3.5	—4.8±3.9	—0.4±4.0	—7.1±3.5	—0.2±3.4	—7.0±3.4
42	—4.2±5.7	—7.7±3.2	—3.1±4.3	—11.8±6.5	—4.2±5.8	—11.4±6.7	—4.2±5.8	—10.1±9.1
82	Auricle arrest							
164	Auricle arrest							

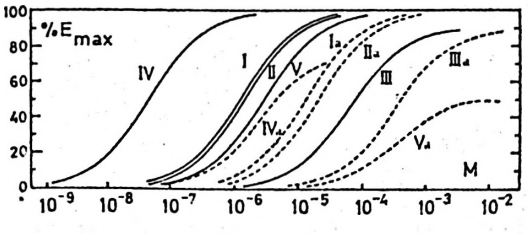

Fig. 2. *Modifications induced by ethanol on the theoretical dose:response curve for ACh (——) and its antagonism by atropine (-----).* I = Control. II = Ethanol, 21 mM. III = 42 mM. IV = Ten days of ethanol exposure. V = 30 days of ethanol exposure. Parallel experimental circumstances for ACh-atropine interaction are indicated by the letter *a* immediately after the Roman numerals.

The experiments in which atropine was used revealed that ethanol, 21 and 42 mM, did permit the normal development of competitive antagonism (as compared with ACh in presence of ethanol). After ten days of alcohol exposure, ACh was unable to overcome the atropine blockade, the maximal effect being lessened about 25 %. This abnormality was even more intense in the uterus from rats exposed for 30 days to ethanol where the maximal effect was diminished about 50 %.

Table II shows the pD_2 and pA_2 values obtained. The pD_2 values for chronotropic and inotropic effects of NA were not modified by ethanol. A decrease in the affinity of ACh was observed in presence of 42 mM of ethanol as well as after 30 days of ethanol intoxication. On the contrary, ten days of ethanol exposure produced an increase in the affinity of ACh. The pA_2 value for atropine was significantly increased after 30 days of ethanol exposure.

The mean ethanol cumulative consumption after ten days of exposure was 11.8 ± 1.2 g/100 g body weight ($\bar{x} \pm$ SEM). After 30 days the amount consumed was 32.4 ± 2.1 g/100 body weight.

Blood ethanol level of rats intoxicated during ten days was 105 ± 9 µg/100 ml. After 30 days the level was 108 ± 13 µg/100 ml.

Discussion

The present results show that ethanol both *in vitro* and *in vivo* produces relevant changes in the responses to NA and ACh in the isolated auricle and uterus of rats. As has been mentioned above, ethyl alcohol might cause these changes through mechanisms other than some action on membrane receptors. However, by taking into consideration the effect of ethanol on the cholinergic antagonism by atropine, an action on membrane receptors can be suggested. In fact, atropine increased its affinity for the cholinergic receptors in such a way that an irrever-

Table II. *Values of pD_2 for NA and ACh, and values of pA_2 for atropine, in presence of ethanol* in vitro *and after the* in vivo *exposure to ethanol (mean ± S.E.M.).*

	Auricle frequency pD_2 for NA	Auricle force pD_2 for NA	Uterus pD_2 for ACh	Uterus pA_2 or atropine
Control	7.1 ± 0.4	6.7 ± 0.6	4.8 ± 0.5	8.9 ± 0.6
Ethanol, 21 mM	6.9 ± 0.7	6.7 ± 0.6	4.7 ± 0.4	9.0 ± 0.7
Ethanol, 42 mM	6.7 ± 0.6	6.7 ± 0.6	3.1 ± 0.2 *	9.0 ± 0.3
10 days ethanol	7.2 ± 0.6	6.7 ± 0.6	6.3 ± 0.5 *	9.7 ± 0.5
30 days ethanol	7.1 ± 0.7	6.7 ± 0.7	3.5 ± 0.3 *	10.4 ± 0.2 *

* $p < 0.05$ as compared with control.

sible blockade occurred after ethanol exposure. As the blocking action of atropine depends only on its ability to occupy the cholinergic receptors, any change in its competitive behavior should be exclusively atrributable to receptor modifications, since chemical alterations in the atropine molecule by ethanol have not been hitherto described. Furthermore, in the experiments performed in organs isolated from animals exposed to ethanol *in vivo*, atropine continued to exhibit abnormal antagonism in spite of the absence of ethanol in the medium bath. The effect of β-blocking agents on NA response in presence of ethanol has not been studied here. Independently of their β-blocking effectiveness, such compounds produce some collateral actions, e.g. membrane stabilization and inhibition of catecholamine release (13) which would be responsible for alterations in the spontaneous activity of auricles, thereby causing possible misinterpretation of the effects of ethanol.

The data here presented do not permit us to establish the biochemical nature of the changes occurring in the biomembranes when the cells are exposed to ethanol, any hypothesis being merely speculative at the moment. Nevertheless, it has been reported that ethanol gives rise to marked changes in the lipid composition of biomembranes (5, 10) and if the membrane lipids play a role in the receptor mechanisms (8), it is easy to understand that ethanol may cause subsequent alterations in such mechanisms. The findings referred by CIOFALO (2) on the ethanol-induced changes in the adrenergic receptor population agree with this hypothesis. Likewise, the data publishew by REGGIANI *et al.* (11) concerning changes in spiroperidol affinity, strongly suggest the occurrence of receptor function alterations. On the other hand both authors found differences in the effects of ethanol depending on its concentration and exposure time. This was equally observed here and well illustrated by the pD_2 and pA_2 analysis. These facts clearly indicate the difficulties existing in extrapolating the data obtained *in vitro* and in acute experiments to long-term experiments.

Blood concentrations of ethanol at the end of the intoxication periods were negligible. These data reflect the high rate of ethanol elimination in the rat (17) and emphasize the relevance of the finding here reported in such a way that low albeit persistent levels of ethanol were able to induce the above-mentioned changes.

In view of the results here presented several topics merit further attention. Firstly, it is necessary to ascertain whether or not ethanol has similar effects on the responses mediated by receptors other than those here investigated. Secondly, information is required about the reversibility of the receptor abnormalities once ethanol exposure is stopped. And thirdly, are these changes present in dependent animals? All these data are necessary to obtain a better knowledge of the role played by receptor alterations in the development and maintenance of ethanol tolerance and dependence.

Resumen

Se estudia el efecto del etanol *in vitro* (21 y 42 mM) e *in vivo* (10 y 30 días de ingesta continuada) sobre las curvas dosis-efecto de noradrenalina y acetilcolina en aurícula y útero aislados de rata, así como la interacción acetilcolina-atropina en útero de rata en las circunstancias arriba señaladas. Del análisis de las curvas teóricas, así como del cálculo de los valores de pD_2 y pA_2 se deduce que el etanol modifica la respuesta de los tejidos a los agonistas estudiados, lo que puede significar una modificación de la función receptorial.

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