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Neurotransmitter-Receptor Binding in Various Brain Regions in Ethanol-Dependent Rats

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HUNT, W. A. AND T. K. DALTON. *Neurotransmitter-receptor binding in various brain regions in ethanol-dependent rats.* PHARMAC. BIOCHEM. BEHAV. **14**(5) 733–739, 1981.—Since chronic ethanol administration has been demonstrated to induce a number of alterations in neurotransmitter utilization, the possibility was investigated that the receptors, on which these transmitters act, are altered because of a modified synaptic input. Male Sprague-Dawley rats were rendered physically-dependent on ethanol by the oral administration of 9–13 g/kg of ethanol each day over a 4 day period. The binding of radioligands specific for α-adrenergic, β-adrenergic, dopaminergic, serotonergic, muscarinic cholinergic, and GABAergic receptors was assessed at various intervals after withdrawal in several areas of the brain. No alteration in receptor binding was observed at any point under the conditions studied. The data suggest that the signs of an ethanol withdrawal syndrome are not mediated through changes in the ability of neurotransmitters to interact with their receptors.

Ethanol　Ethanol-dependence　α-Adrenergic receptors　β-Adrenergic receptors
Dopaminergic receptors　Serotonin receptors　Muscarinic cholinergic receptors　GABA receptors

ETHANOL treatment induces a number of alterations in the activity of several neurotransmitters in the rat brain. Catecholamine and some cholinergic neurons are activated by ethanol [8, 11, 20, 23], while other cholinergic, serotonergic and GABAergic neurons are depressed [12, 19, 23, 24]. When administered chronically, ethanol can continuously disrupt normal synaptic transmission. This might lead to the activation of various compensatory mechanisms that could modify the properties of the receptors on which the transmitters act.

The purpose of the present study was to determine whether chronic ethanol treatment leading to the development of physical dependence might involve changes in receptor properties. Ample evidence exists to suggest that long-term alterations in synaptic input can lead to changes in receptor density. For example, disruption of dopaminergic activity in the nigrostriatal pathway by a lesion with 6-hydroxydopamine results in supersensitivity, accompanied by an increase in the number of dopaminergic receptors [9]. Conversely, long-term treatment with apomorphine, a dopamine agonist, results in a reduction in dopamine receptors [32].

In this study receptor function was assessed in ethanol-dependent rats by measuring receptor binding to appropriate tissue preparations from brain using different radioligands. The receptors studied included α- and β-adrenergic, dopaminergic, serotonergic, muscarinic cholinergic, and GABAergic receptors. Measurements were made at various intervals after withdrawal.

METHOD

Induction of Physical Dependence on Ethanol and General Experimental Design

Physical dependence on ethanol was induced in male Sprague-Dawley rats (150–250 g) by the method of Majchrowicz [29]. This involved the administration of 9–13 g/kg a day for 4 days of a 20% ethanol solution by oral intubation.

After the last dose of ethanol animals were decapitated while still intoxicated (prodromal detoxication phase), during the early stage of the withdrawal syndrome, 1 day or 3 days after withdrawal. The brains were excised and dissected into the following areas: cerebellum, cerebral cortex, caudate nucleus, hypothalamus, thalamus, hippocampus, nucleus accumbens, and substantia nigra.

Receptor binding was assessed in appropriate areas of the brain using established radioligand techniques and employed a concentration of ligand that was less than the dissociation constant. Specific binding was defined as the difference between total binding and that obtained in the presence of an excessive concentration of an unlabeled ligand which binds specifically to the receptor under study.

α-Adrenergic Binding

α-Adrenergic binding was measured by the method of Greenberg and Snyder [16] using ^{3}H-dihydroergokryptine (DHE) as the radiolabeled ligand. ^{3}H-DHE binding was measured in the cerebral cortex, hypothalamus, cerebellum,

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hippocampus and nucleus accumbens. The brain tissue was homogenized in 40 vol of ice-cold 50 mM Tris-Cl buffer (pH 7.4) and centrifuged twice at 50,000×g for 10 min at 4°C. The final pellet was resuspended in 80 vol of Tris buffer containing 0.1% ascorbic acid and 10 μM pargyline. An aliquot of homogenate was incubated in a final volume of 150 μl with 0.37 nM ^{3}H-DHE in the presence and absence of 100 μM (−)norepinephrine for 30 min at 25°C. The mixture was then poured through a GF/B glass fiber filter under vacuum and the filter was washed with five 4-ml portions of ice-cold 50 mM Tris buffer (pH 7.4). Radioactivity on the filter was determined by liquid scintillation spectrometry.

β-Adrenergic Binding

β-Adrenergic binding was measured by the method of Alexander *et al.* [1] using ^{3}H-dihydroalprenolol (DHA) as the radiolabeled ligand. Tissue was prepared in the same manner as for ^{3}H-DHE binding. ^{3}H-DHA binding was determined in the cerebral cortex, hypothalamus, cerebellum, hippocampus and nucleus accumbens. Aliquots of tissue homogenates were incubated in a final volume of 150 μl with 1.8 nM ^{3}H-DHA in the presence and absence of 10 μM D,L-propranolol for 10 min at 37°C. The contents were filtered through GF/B filters which were then washed with five 3-ml portions of ice-cold 50 mM Tris buffer. Radioactivity on the filter was determined as above.

Dopaminergic Binding

Dopaminergic binding was assessed using three different radioligands. They were ^{3}H-apomorphine, ^{3}H-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN), and ^{3}H-haloperidol. These ligands appear to probe different dopaminergic receptors [15, 25, 33].

Tissue from the caudate nucleus was prepared as above. For ^{3}H-apomorphine binding, the final pellet was resuspended in 50 mM Tris buffer (pH 7.4) containing 0.1% ascorbic acid. The binding procedure was similar to that described by Muller and Seeman [32]. An aliquot of the tissue suspension was incubated at 37°C for 10 min in Tris buffer containing 0.5 nM ^{3}H-apomorphine in the presence and absence of 300 nM unlabeled apomorphine. Three ml of ice-cold Tris buffer were added and contents filtered rapidly through a GF/B glass fiber filter. The tube and filter were then washed with five 3-ml portions of Tris buffer. The amount of radioactivity remaining on the filter was determined by liquid scintillation spectrometry.

^{3}H-ADTN binding was assessed in tissue pellets resuspended in 50 mM Tris buffer (pH 7.1) containing 0.1% ascorbic acid and 1 mM $MnCl_2$, using the method of Creese and Snyder [10]. The tissue samples were incubated at 37°C for 10 min in Tris buffer containing 8 nM ^{3}H-ADTN in the presence and absence of 5 μM unlabeled ADTN. The remainder of the procedure was identical to that used for ^{3}H-apomorphine binding.

Tissue pellets for ^{3}H-haloperidol binding were resuspended in 50 mM Tris buffer (pH 7.1) containing 0.1% ascorbic acid, 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ according to the method of Burt *et al.* [6]. Samples were incubated at 37°C for 10 min with 3.0 nM ^{3}H-haloperidol in presence and absence of 100 μM unlabeled dopamine. The remainder of the procedure was identical to that above.

Serotonin Binding

Serotonin binding was measured by the method of Bennett and Snyder [4] using ^{3}H-serotonin as the radioligand. Tissue was prepared as above. ^{3}H-serotonin binding was determined in the caudate nucleus, hippocampus and cerebral cortex. Aliquots of tissue homogenate were incubated in a final volume of 200 μl with 4 nM ^{3}H-serotonin in the presence and absence of 10 μM unlabeled serotonin for 10 min at 37°C. The contents were filtered through GF/B filters and washed with five 3-ml portions of ice-cold 50 mM Tris buffer. Radioactivity on the filters was determined as before.

Muscarinic Cholinergic Binding

^{3}H-quinuclidinyl benzilate (QNB) binding was used to study the muscarinic cholinergic receptor. The tissue samples were homogenized in 20 vol of 0.32 M sucrose and centrifuged at 4°C at 1,000×g for 10 min. ^{3}H-QNB binding was measured according to the method of Yamamura and Snyder [49] in the cerebral cortex, hippocampus and caudate nucleus. An aliquot of the supernatant was incubated at 25°C in 2 ml of medium containing 50 mM sodium phosphate buffer (pH 7.4) and 0.5 nM ^{3}H-QNB in the presence and absence of 100 μM oxotremorine. After 60 min 3 ml of ice-cold buffer were added and the whole mixture was filtered through a GF/B filter. The incubation tube and filter were washed with five 3-ml portions of buffer. Radioactivity on the filters was determined by liquid scintillation spectrometry.

GABA Binding

GABA binding was determined by the method of Zukin *et al.* [50] using ^{3}H-GABA as the radioligand. Brain tissue was homogenized in 25 vol of ice-cold 0.32 M sucrose and centrifuged at 1,000×g for 10 min at 4°C. The supernatant was centrifuged at 20,000×g for 20 min at 4°C. The pellet was then homogenized in distilled water and centrifuged twice at 48,000×g for 20 min and the resulting pellet was stored at −80°C for at least 18 hrs. On the day of assay, the pellet was homogenized in 0.01% Triton X-100 and incubated at 37°C for 30 min. After centrifugation at 48,000×g for 10 min the pellet was resuspended in 25 vol of 100 mM Tris citrate buffer (pH 7.1) and centrifuged twice at 48,000×g for 10 min. The resulting pellet was resuspended in Tris buffer and used for the binding assay. ^{3}H-GABA binding was determined in the cerebellum, cerebral cortex, caudate nucleus, thalamus, nucleus accumbens, and substantia nigra.

An aliquot of the tissue homogenate was incubated in the presence and absence of 1 mM GABA with 6.2 nM ^{3}H-GABA in a final volume of 1 ml for 5 min at 0°C, then centrifuged at 48,000×g for 10 min at 4°C. The pellet was surface-washed twice with ice-cold distilled water. The supernatants were discarded and the tubes were drained to remove excess water. The pellet was then dissolved in 0.5 ml of Protosol and the radioactivity determined by liquid scintillation spectrometry.

Protein Determination

Protein was measured in each tissue sample by the method of Lowry *et al.* [28].

RESULTS AND DISCUSSION

The six receptor systems examined in these experiments were studied in various areas of the brain at different times

TABLE 1

^{3}H-DIHYDROERGOKRYPTINE BINDING IN ETHANOL-DEPENDENT RATS

	^{3}H-Dihydroergokryptine Binding (fmol/mg Protein)				
	Cerebral Cortex	Hypothalamus	Cerebellum	Hippocampus	Nucleus Accumbens
Control	37.2 ± 1.5 (14)	37.7 ± 2.7 (6)	12.3 ± 1.5 (5)	21.4 ± 2.2 (6)	35.4 ± 2.0 (6)
Intoxicated	41.6 ± 2.4 (10)	30.1 ± 2.5 (6)	14.8 ± 1.1 (6)	21.2 ± 1.4 (6)	30.4 ± 4.9 (6)
Withdrawal syndrome	44.2 ± 3.4 (16)	31.9 ± 2.6 (6)	14.8 ± 1.2 (6)	24.4 ± 1.9 (6)	35.3 ± 1.5 (6)
1 day after withdrawal	40.2 ± 2.9 (12)	34.7 ± 2.7 (6)	14.7 ± 1.5 (6)	25.7 ± 2.4 (6)	39.5 ± 1.7 (6)
3 days after withdrawal	33.9 ± 1.3 (11)	31.2 ± 2.1 (6)	12.3 ± 1.3 (5)	22.0 ± 1.8 (6)	34.2 ± 1.1 (6)

Values expressed as the mean ± SEM. Numbers in parentheses refer to the number of animals per group.

TABLE 2

^{3}H-DIHYDROALPRENOLOL BINDING IN ETHANOL-DEPENDENT RATS

	^{3}H-Dihydroalprenolol Binding (fmol/mg Protein)				
	Cerebral Cortex	Hypothalamus	Cerebellum	Hippocampus	Nucleus Accumbens
Control	119 ± 11.0 (6)	127 ± 7.3 (19)	84.6 ± 6.3 (17)	145 ± 14.3 (5)	158 ± 7.4 (7)
Intoxicated	115 ± 7.3 (8)	120 ± 14.7 (5)	102.0 ± 7.9 (8)	158 ± 5.8 (4)	163 ± 9.5 (6)
Withdrawal syndrome	115 ± 3.2 (7)	120 ± 10.5 (5)	84.0 ± 5.2 (10)	160 ± 8.3 (6)	168 ± 13.2 (7)
1 day after withdrawal	102 ± 6.4 (8)	123 ± 6.4 (12)	90.6 ± 12.5 (10)	133 ± 8.4 (7)	178 ± 10.1 (7)
3 days after withdrawal	118 ± 5.8 (7)	122 ± 4.4 (12)	89.0 ± 9.6 (10)	123 ± 5.8 (8)	172 ± 13.8 (7)

Values expressed as the mean ± SEM. Numbers in parentheses refer to the number of animals per group.

after the last dose of ethanol. Measurements were made while animals were intoxicated, during the early stages of the withdrawal syndrome, 1 and 3 days after withdrawal. Behaviorally, intoxicated animals were markedly ataxic and sedated with blood ethanol concentrations of 320–390 mg/dl, while during the withdrawal syndrome typical signs of tremor, rigidity, and convulsions were observed as described previously [29]. At 1 and 3 days after withdrawal the animals appeared normal. The results can be found in Tables 1–6. No alteration was observed in the binding of any of the radioligands at any interval after withdrawal.

It can be argued that the use of a single radioligand concentration might not, under some conditions, detect changes in certain binding properties of receptors. Scatchard plots, employing several concentrations of ligands, allow for the calculation of affinity of the ligand for and density of receptors [3]. Assuming a homogeneous population of receptors and a linear Scatchard plot, the detection of a change in the density of receptors is independent of the ligand concentration, if affinity is uneffected. If a ligand concentration equal to the dissociation constant (K_D) is employed, a change in affinity would be directly related to a change in binding, if the density of receptors is uneffected. The use of a lower concentration of ligand would be more sensitive in detecting affinity changes, whereas a higher concentration would be less sensitive.

In the experiments reported here, a concentration of radioligand equal to or less than the K_D was employed. This should allow for the detection of an alteration in binding attributable to changes either in affinity or density. Furthermore, with the exception of GABA binding, all the Scatchard plots for the various ligands are linear. However, some Scatchard plots were determined in our study as a check and an effort was made to examine receptor subtypes, that are not separated by such plots. For example, α-adrenergic receptors, which have two subtypes, α_1 and α_2, were evaluated using three radioligands, ^{3}H-DHE which binds to both subtypes [36], ^{3}H-WB-4101, and ^{3}H-para-amino-clonidine, which bind to the α_1 and α_2 subtypes, respectively [39,46]. Scatchard plots of these ligands in the cerebral cortex of ethanol-dependent rats were not significantly different from control.

In spite of the many reports suggesting that chronic ethanol treatment disrupts neurotransmitter activity, these changes do not appear to lead, under the conditions studied, to significant alterations in receptor function based on ligand-binding experiments. The development of maximal physical dependence on ethanol can be achieved in only 4 days using an oral intubation model [30]. This may not be long enough to activate the mechanisms that lead to changes in receptor properties. Therefore, it would appear that an impairment of the ability of neurotransmitters to interact with their receptors is not a prerequisite for the development of ethanol dependence.

Several studies have appeared indicating that with longer term treatment with ethanol, receptor function can be al-

TABLE 3

STRIATAL DOPAMINERGIC RECEPTOR BINDING IN ETHANOL-DEPENDENT RATS

	^{3}H-Apomorphine Binding (fmol/mg Protein)	^{3}H-ADTN Binding (fmol/mg Protein)	^{3}H-Haloperidol Binding (fmol/mg Protein)
Control	9.6 ± 0.36 (6)	11.1 ± 1.7 (7)	36.2 ± 2.4 (10)
Intoxicated	10.8 ± 0.45 (6)	14.0 ± 1.3 (6)	39.0 ± 1.6 (6)
Withdrawal syndrome	10.9 ± 0.36 (6)	13.4 ± 1.7 (8)	37.9 ± 3.3 (4)
1 day after withdrawal	10.3 ± 0.68 (8)	10.7 ± 0.6 (5)	43.7 ± 2.3 (6)
3 days after withdrawal	9.5 ± 0.53 (8)	13.1 ± 0.8 (7)	29.0 ± 3.9 (5)

Values were expressed as mean ± SEM. Numbers in parentheses refer to number of animals per group.

TABLE 4

^{3}H-SEROTONIN BINDING IN ETHANOL-DEPENDENT RATS

	^{3}H-Serotonin Binding (fmol/mg Protein)		
	Caudate Nucleus	Hippocampus	Cerebral Cortex
Control	14.6 ± 1.3 (14)	26.7 ± 3.3 (5)	35.0 ± 2.1 (6)
Intoxicated	19.5 ± 2.1 (14)	29.0 ± 2.4 (6)	38.8 ± 3.0 (6)
Withdrawal syndrome	16.0 ± 1.4 (14)	26.1 ± 3.5 (6)	31.8 ± 2.2 (5)
1 day after withdrawal	13.6 ± 1.4 (14)	28.8 ± 1.9 (6)	38.4 ± 3.2 (6)
3 days after withdrawal	16.5 ± 1.1 (14)	25.0 ± 2.9 (6)	32.1 ± 3.4 (6)

Values expressed as the mean ± SEM. Numbers in parentheses refer to the number of animals per group.

TABLE 5

^{3}H-QUINUCLIDINYL BENZILATE BINDING IN ETHANOL-DEPENDENT RATS

	^{3}H-QNB Binding (fmol/mg Protein)		
	Cerebral Cortex	Hippocampus	Caudate Nucleus
Control	460 ± 23.0 (10)	261 ± 18.5 (5)	479 ± 23.5 (5)
Intoxicated	449 ± 28.3 (4)	297 ± 4.0 (5)	435 ± 9.8 (4)
Withdrawal syndrome	479 ± 18.5 (5)	264 ± 23.5 (5)	486 ± 16.8 (4)
1 day after withdrawal	453 ± 30.8 (6)	229 ± 4.9 (6)	456 ± 21.2 (6)
3 days after withdrawal	436 ± 39.3 (9)	231 ± 19.4 (4)	447 ± 20.5 (4)

Values expressed as the mean ± SEM. Numbers in parentheses refer to the number of animals per group.

tered. The effects in some cases have been specific to only certain areas of the brain or the time after withdrawal from ethanol. For example, ethanol administered for 60 days resulted in a reduction in the number of ^{3}H-DHA binding sites in the brain when animals were still intoxicated. Three days later the opposite effect was observed [2]. This time course is identical to that found for the ability of norepinephrine to stimulate adenosine-3′, 5′-cyclic monophosphate formation in brain slices, where sensitivity is reduced on the day of withdrawal but is elevated after 3 days [14]. Because of the coupling between the β-adrenergic receptor and adenylate cyclase [27], these phenomena may both be expressions of the same altered mechanism. When mice consumed a liquid diet containing ethanol for 7 days, ^{3}H-QNB binding was elevated in the hippocampus and cerebral cortex, but not in the caudate nucleus [44]. This effect disappeared within 24 hrs. In another study, where rats were chronically treated with ethanol for 18 weeks on a liquid diet, ^{3}H-QNB binding was considerably enhanced in the caudate nucleus, but not in the hippocampus and cerebral cortex [35]. However, the increased binding was unchanged 4 weeks after withdrawal. In animals treated for 11–15 days, ^{3}H-serotonin binding in the caudate nucleus was increased, but was reduced in the hippocampus [31]. ^{3}H-spiroperidol binding in the caudate nu-

TABLE 6

^{3}H-GABA BINDING IN ETHANOL-DEPENDENT RATS

	^{3}H-GABA Binding (fmol/mg Protein)		
	Cerebellum	Cerebral Cortex	Caudate Nucleus
Control	511 ± 19.1 (12)	124 ± 5.2 (18)	44.9 ± 5.8 (5)
Intoxicated	514 ± 29.1 (5)	135 ± 6.8 (6)	40.9 ± 1.3 (3)
Withdrawal syndrome	506 ± 19.4 (6)	125 ± 7.6 (6)	37.4 ± 4.5 (4)
1 day after withdrawal	562 ± 22.9 (12)	116 ± 9.3 (10)	46.7 ± 4.2 (7)
3 days after withdrawal	532 ± 18.3 (8)	109 ± 6.7 (9)	50.0 ± 3.2 (6)
	Thalamus	Nucleus Accumbens	Substantia Nigra
Control	187 ± 15.0 (11)	86.3 ± 5.7 (8)	48.1 ± 3.2 (7)
Intoxicated	211 ± 13.4 (10)	77.6 ± 3.9 (6)	39.9 ± 2.0 (4)
Withdrawal syndrome	210 ± 9.6 (10)	101 ± 9.2 (5)	56.3 ± 6.4 (6)
1 day after withdrawal	200 ± 14.4 (12)	103 ± 6.0 (7)	43.2 ± 5.4 (6)
3 days after withdrawal	207 ± 14.2 (11)	104 ± 1.8 (5)	47.8 ± 3.2 (9)

Values were expressed as the mean ± SEM. Numbers in parentheses refer to the number of animals per group.

cleus was reported uneffected after 7 days of treatment [42], elevated after 14 days [26], and reduced after 13 months [35]. Low affinity ^{3}H-GABA binding in whole brain was lower after 14 days of ethanol treatment [45].

The relevance of these findings to the development of tolerance and physical dependence on ethanol is difficult to identify. When physical dependence is induced over a 4 day period, ^{3}H-DHA (Table 2), ^{3}H-haloperidol (Table 3), ^{3}H-serotonin (Table 4), ^{3}H-QNB (Table 5), and high affinity ^{3}H-GABA (Table 6) binding are unaffected at any time up to 3 days after withdrawal. It would appear then that the reported alterations in neurotransmitter receptor binding reflect a different aspect of chronic ethanol treatment.

One possibility that could account for the observed alterations in receptor binding reported by others is the development of nonspecific brain damage. In recent experiments that controlled for possible nutritional imbalances, chronic ethanol treatment for 4 months reduced the density of hippocampal dendritic spines [38]. After 5 months of treatment, there was a considerable loss of hippocampal pyramidal and dentate gyrus granule cells [48]. The possible relationship between a reduction in neurotransmitter binding and cell loss is obvious and has been exploited to determine the localization of receptors on certain cell types. For example, striatal lesions with kainic acid, which destroys intrinsic neurons while sparing afferents and fibers in passage, reduce ^{3}H-spiroperidol and ^{3}H-QNB binding and is correlated with the loss of cells in the caudate nucleus [13].

How cell damage can explain alterations in receptor binding with shorter treatment periods is not clear. Simple comparisons of chronic studies are not very easy because of many factors that can contribute to the results [19,22]. For example, the dose of ethanol consumed and the peak blood ethanol concentration obtained per day is not the same for different lengths of treatment. Longer term studies using liquid diets generally obtain lower blood ethanol concentrations than those from short term studies using oral intubation [29,44]. What might be an explanation is that receptor binding is reflecting the initial stages of cell damage before it is apparent morphologically.

The data on the binding of the three dopaminergic ligands may provide further information into the effect of chronic ethanol treatment on dopaminergic function. Dopaminergic mechanisms have been reported to be less responsive to various drug treatments 24 hrs after ethanol withdrawal. Ethanol, haloperidol, opiates, and GABA agonists are all less effective in increasing dopamine synthesis [18, 41, 43, 47]. Also, the dopaminergic agonists apomorphine and piribidil have a reduced ability to induce increases in locomotor activity and hypothermia, respectively [17,43]. These observations led to the suggestion that dopaminergic receptors are not functioning normally. Dopamine-sensitive adenylate cyclase, one index of dopaminergic receptor function, was found to be reduced [42]. However, ^{3}H-spiroperidol binding was unaffected [42]. The conclusion was drawn that there may be an uncoupling of the dopaminergic receptor from the adenylate cyclase.

It was recently demonstrated that there exist multiple receptors for dopamine with different localizations. On postsynaptic sites there are two receptors, designated D_1 and D_2 [25]. The D_1 receptor is associated with the dopamine-sensitive adenylate cyclase, while the D_2 receptor is not. In addition, a presynaptic dopamine receptor has been suggested [33].

Neuroleptics, such as haloperidol and spiroperidol, are believed to bind to D_2 receptors [25]. However, their binding sites may not be exclusively on postsynaptic dopaminergic receptors and may also be localized on presynaptic corticostriatal afferents [40]. Consequently, the use of ^{3}H-neuroleptic binding may not be the best choice for determining whether the effect of ethanol on dopamine-sensitive adenylate cyclase (D_1) activity is related to a disruption in the ability of dopamine to interact with its receptor. ^{3}H-ADTN binding might better reflect changes in dopamine-sensitive adenylate cyclase since both are exclusively localized on intrinsic neurons in the caudate nucleus [15].

The use of ^{3}H-apomorphine, ^{3}H-ADTN, and ^{3}H-haloperidol allowed us to possibly examine separately presynaptic, D_1, and D_2 receptors, respectively, after chronic ethanol treatment. The negative results obtained further support the idea that an alteration in the ability of dopamine to interact with its receptor does not explain the subsensitivity of dopaminergic mechanisms.

The fact that subsensitivity develops to both dopaminergic agonists and antagonists after ethanol withdrawal raises some interesting questions. For example, why are opposite effects on agonists and antagonists not observed? One might expect subsensitivity to agonists and supersensitivity to antagonists, or vice versa. The precedent for this thought comes from the result of chronic treatment with dopaminergic agonists or antagonists. With chronic treatment with the agonists, apomorphine and amphetamine, the presynaptic dopaminergic receptors become subsensitive, as evidenced by a reduction in ^{3}H-apomorphine binding [32]. However, animals treated in this manner display spontaneous catalepsy [32]. In addition, dopamine turnover and release are reduced [37]. On the other hand, with chronic treatment with haloperidol, the opposite response is obtained. The sensitivity for dopaminergic agonists is increased, whereas the effectiveness of haloperidol is reduced [34]. This is accompanied by an increase in ^{3}H-haloperidol binding [7].

Changes in dopaminergic sensitivity after chronic ethanol administration do not follow these patterns. The development of subsensitivity to both agonists and antagonists suggest that more than one class of dopaminergic receptor mechanism may be involved. Several lines of evidence suggest that presynaptic dopaminergic receptors may also be subsensitive. Not only are the behavioral and physiological effects of dopaminergic agonists reduced [17,43], a number of biochemical alterations occur that resemble those obtained after chronic apomorphine treatment. Acute apomorphine treatment normally reduces dopamine synthesis. However, after chronic ethanol treatment, this effect is abolished [5]. Consistent with depressed dopaminergic activity is the finding that dopamine turnover and release are decreased [11,20], similar to that obtained after chronic apomorphine treatment. A mechanism for the chronic stimulation of presynaptic dopaminergic receptors could come from the elevated dopamine release prevalent during the induction of physical dependence on ethanol when blood ethanol concentrations are constantly elevated [11].

One difference between chronic ethanol and apomorphine treatment is that no change in ^{3}H-apomorphine binding is observed after ethanol treatment (Table 3). The uncoupling of the receptor from an effector which helps modulate dopaminergic activity could account for these effects, analogous to the hypothesis advanced by Tabakoff and coworkers [42]. Although speculative, this hypothesis provides an explanation for a variety of biochemical findings found after chronic ethanol treatment on dopaminergic systems.

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