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Effect of chronic alcohol feeding and withdrawal on rat liver plasma membrane structure and function: a study of binding of [^{3}H]prazosin to the membrane bound α_1-adrenergic receptor*

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The biological basis of the actions of ethanol and the molecular mechanisms responsible for tolerance, physical dependence and withdrawal are not known. The recent hypothesis of Hill and Bangham [1] suggesting membrane lipid adaptation as a mechanism to offset prolonged exposure to anesthetics offers a new approach to these problems.

It is now generally accepted that prolonged administration of alcohol induces alterations in membrane lipid composition and fluidity. In the rat liver, changes in lipid composition have been documented in several interior membrane systems [2], but as yet such changes have not been described specifically for the plasma membrane. Nevertheless, due to its central role in preserving the physiological versatility of the cell [3], chronic perturbation of the plasma membrane can be envisioned to influence many cellular functions.

In the rat liver, the hormonal effects of epinephrine are believed to be mediated by a cAMP-independent α-adrenergic mechanism [4] through α_1 subtype receptors [5–7]. Recent work from this laboratory indicated that chronic alcohol administration led to diminished epinephrine-stimulated L-lactate gluconeogenesis in the isolated perfused rat liver, while the activities of phosphoenolpyruvate carboxykinase (PEPCK) and fructose diphosphatase (FDPase), key hepatic gluconeogenic enzymes, remained unchanged [8]. These findings implicated membrane malfunction as a likely cause of the diminished hormone sensitivity subsequent to chronic alcohol feeding and prompted the present study on the effects of long-term alcohol feeding on the structure and function of the rat liver plasma membrane.

In this work, [^{3}H]prazosin was used to identify the α_1-adrenergic receptor. The receptor density and other parameters were examined for changes in this membrane receptor system during chronic ethanol administration and withdrawal.

Materials and methods

Chronic alcohol administration and its subsequent withdrawal, using a totally liquid low fat Metrecal diet, were performed as previously described [9, 10]. Liver plasma membranes were isolated essentially according to the method of Wolfe *et al.* [11].

Binding studies. The incubation medium used for the binding assay contained [^{3}H]prazosin (New England Nuclear, Canada), incubation buffer (50 mM Tris, pH 7.5, 4 mM $MgSO_4$ and 0.8 mM ascorbate), and 125–250 μg of membrane protein in a final volume of 0.5 ml. The final concentration of [^{3}H]prazosin for saturation assays was 0.04 to 1.0 nM. Tubes were incubated at 31° for 15 min, and the incubation was terminated by adding 3 ml of ice-cold incubation buffer. Samples were rapidly filtered under vacuum through GF/C glass fibre filters, and the filters were washed with two 6-ml rinses of ice-cold incubation buffer. Specific binding was defined as the excess over blanks containing 10 μM phentolamine.

Kinetic studies of specific [^{3}H]prazosin (0.8 nM) binding were carried out by determining the time course of ligand association and its displacement by 10 μM phentolamine. Derivation of the various kinetic constants from these experiments has been described in detail elsewhere [12, 13].

Filters were dried overnight and digested in NCS for 2 hr at room temperature (22°). Liquifluor (10 ml) was added, and the samples were counted in a Packard Tri-Carb liquid scintillation spectrometer with an efficiency of 35–40%.

Protein was determined by the method of Lowry *et al.* [14] using recrystallized bovine serum albumin as standard.

Data analyses. Data were subjected to Student's two-tailed *t*-test for examination of statistical significance between mean values. Scatchard analysis [15] was used to analyze saturable binding data. A correlation coefficient (r) of better than 0.90 was taken as the criterion for linearity in all least square linear regression analyses.

Phentolamine, (−)-phenylephrine HCl, (+)-epinephrine bitartrate and (−)-isoproterenol bitartrate were gifts from Dr. G. Kunos, Department of Pharmacology and Experimental Therapeutics, McGill University. All other compounds were obtained commercially.

Results

The time course of ligand association and its displacement by 10 μM phentolamine were identical in the control and alcoholic membrane preparations. In corroborating results from recent work by others with different rat tissues [7, 16, 17], specific [^{3}H]prazosin binding to rat liver plasma membranes was found to be saturable, of high affinity, and stereospecific. These parameters were not affected by chronic alcohol administration.

The effects of chronic alcohol administration and its subsequent withdrawal on properties of specific [^{3}H]prazosin binding to liver plasma membranes are summarized in Table 1. The conglomerate Scatchard plots are shown in Fig. 1. Chronic alcohol administration did not change significantly the affinity of [^{3}H]prazosin binding to the plasma membranes. However, the B_{max} was significantly decreased to 70% of the control value. During withdrawal from alcohol, the B_{max} of specific [^{3}H]prazosin binding fluctuated with time. At 24, 48 and 72 hr post-alcohol, the B_{max} values were 78, 70 and 64% of the control respectively. Again, the affinity of binding was not altered appreciably during this withdrawal period from alcohol.

Adaptation of membranes to alcohol presumably involves fundamental changes in membrane structure which render the membrane more resistant to further disordering by alcohol [18]. To determine whether such altered sensitivity to the presence of alcohol also exists in our experimental plasma membranes, we studied the effect on [^{3}H]prazosin binding of adding alcohol.

Alcohol (1–9%, v/v), added *in vitro* to the incubation medium, did not alter the non-specific binding of [^{3}H]prazosin to either the control or the alcoholic samples. However, specific binding was diminished in the controls in a dose-dependent manner. With the alcoholic membranes, the pattern of decrease was slightly steeper (Fig. 2). Statistically, a significant difference was observed only at the highest alcohol concentration, 9% (v/v). The fact

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Table 1. Comparison of the effects of chronic alcohol administration and withdrawal on various parameters of specific [^{3}H]prazosin binding to rat hepatic plasma membranes*

Treatment	Scatchard analysis K_d (nM)	Scatchard analysis B_{max} (fmoles/mg protein)	Kinetic analysis K_d (nM)	Hill coefficient n_H
Control	0.15 ± 0.04 (5)	1240 ± 50 (5) 100%	0.11 ± 0.02 (3)	1.01 ± 0.03 (4)
Alcoholic	0.11 ± 0.03 (5)	860 ± 20† (5) 70%	0.10 ± 0.02 (3)	1.00 ± 0.01 (5)
24-hr Withdrawal	0.14 ± 0.08 (3)	970 ± 60‡ (3) 78%		0.96 ± 0.03 (3)
48-hr Withdrawal	0.16 ± 0.04 (4)	860 ± 90§ (4) 70%		0.96 ± 0.03 (3)
72-hr Withdrawal	0.08 ± 0.01 (3)	790 ± 80§ (3) 64%		1.00 ± 0.05 (3)

* Equilibrium and kinetic binding experiments were performed as described in the text. Derivation of the various parameters has been described elsewhere [12, 13]. Values represent the means ± S.E.M. for the number of observations in parentheses.

† Statistically different from control ($P > 0.001$).

‡ Statistically different from control ($0.02 > P > 0.01$).

§ Statistically different from control ($0.01 > P > 0.001$).

that all the points collectively formed two different lines suggests that a differential sensitivity to *in vitro* alcohol might exist between the control and alcoholic membranes.

To understand better the nature of the decreased binding in the presence of *in vitro* alcohol, Scatchard plots were generated. These analyses indicated that alcohol, 4% (v/v) added *in vitro*, resulted in a 2- to 3-fold increase in the K_d of [^{3}H]prazosin binding to both the control (K_d = 0.52 and 0.36 nM in two trials) and the alcoholic (K_d = 0.58 and 0.40 nM in two trials) membranes while having a minimal influence on the B_{max} values. This is suggestive of competitive interaction between alcohol added *in vitro* and [^{3}H]prazosin binding.

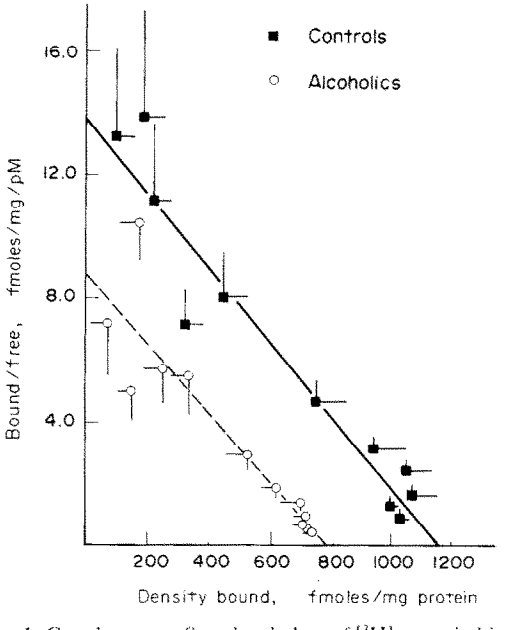

Fig. 1. Conglomerate Scatchard plots of [^{3}H]prazosin binding to hepatic plasma membranes from control and alcoholic rats. The lines were determined by linear regression analysis. Each horizontal or vertical bar represents 1 S.E.M. derived from three to five independent observations.

Discussion

Work presented in this communication showed that chronic alcohol administration to rats led to approximately a 30% decrease in the density (B_{max}) of hepatic α_1-adrenergic

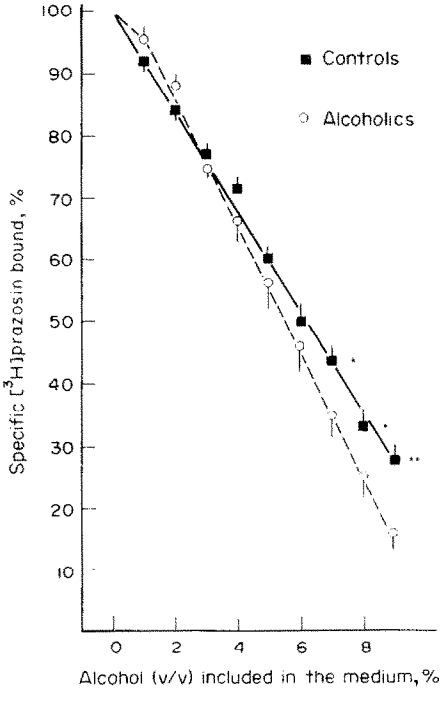

Fig. 2. Inhibition of specific [^{3}H]prazosin binding by alcohol *in vitro*. Hepatic plasma membranes were incubated as described in the text with 0.8 nM [^{3}H]prazosin in the presence of increasing concentrations of alcohol, and specific binding was determined. Each value is the mean of duplicate determinations from three to four separate experiments. The lines drawn from 1–9% alcohol had correlation coefficients of −0.998 and −0.999 for the control and alcoholic samples respectively. Significance level between the preparations at the indicated alcohol concentrations: (*) $0.1 > P > 0.05$; and (**) $0.02 > P > 0.01$.

receptors while having no effect on their affinity (K_d). During withdrawal from alcohol, the α_1-receptor density fluctuated with time but remained steadily below the control value. Interestingly, changes in adrenergic and other receptor systems seen in different tissues during ethanol feeding and withdrawal were also confined to receptor density and not to affinity [19].

Hepatic α_1-adrenergic receptors play a central role in the regulation of carbohydrate metabolism [4, 20]. A persistent alteration in this system following chronic alcohol administration and withdrawal may seriously affect its physiological functions. This has been demonstrated in our laboratory using the isolated perfused rat liver system [8]. These aberrant changes most likely represent some of the biochemical adaptations in the membranes following prolonged alcohol feeding.

The results obtained with the *in vitro* studies (Fig. 2) suggest that a subtle difference may have existed in the patterns of inhibition by alcohol of [^{3}H]prazosin binding to the control and alcoholic membranes. Thus, we may have detected an altered sensitivity of rat hepatic plasma membranes to *in vitro* alcohol subsequent to chronic alcohol administration.

The *in vitro* actions of alcohol on [^{3}H]prazosin binding seem to be competitive in nature since only the K_d rather than the B_{max} was affected. After chronic alcohol administration, however, K_d was normal while B_{max} was decreased (Table 1 and Fig. 1). These results suggest that, following the chronic alcohol feeding, adaptive changes in the plasma membrane had taken place. These changes appeared to have effectively returned the receptors to their proper functional state (normal K_d) at the expense of diminished receptor density.

We [9, 21] and others [22, 23] have shown that the activities of many membrane-bound enzymes are highly sensitive to changes in the lipid microenvironment. Hepatic membrane-bound adrenergic receptor proteins may well be regulated in a similar fashion. Accordingly, an ethanol-adapted plasma membrane may alter the function of its contained membrane components, such as the adrenergic receptors, by modulating the signal transfer following receptor binding, coupling, etc., which is eventually reflected in the responses in the cytoplasm.

In conclusion, the rat liver plasma membrane displayed long-lasting adaptive changes following chronic alcohol feeding and withdrawal. This was reflected in differences in the effects of *in vitro* addition and *in vivo* chronic actions of alcohol on specific [^{3}H]prazosin binding to these membranes. Moreover, the sensitivities of the control and alcohol treated membranes to the *in vitro* effects of alcohol also appear to have been different. These persistent changes represent some of the biochemical adaptations in the plasma membranes to the continuous presence of alcohol.

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REFERENCES

1. M. W. Hill and A. D. Bangham, *Adv. exp. Med. Biol.* **59**, 1 (1975).
2. S. W. French, T. J. Ihrig, S. P. Shaw, T. T. Tanaka and M. L. Norum, *Res. Commun. Chem. Path. Pharmac.* **2**, 567 (1971).
3. W. H. Evans, *Biochim. biophys. Acta* **604**, 27 (1980).
4. J. H. Exton, *Am. J. Physiol.* **238**, E3 (1980).
5. B. B. Hoffman, T. Michel, D. Mullikin-Kilpatrick, R. J. Lefkowitz, M. E. M. Tolbert, H. Gilman and J. N. Fain, *Proc. natn. Acad. Sci. U.S.A.* **77**, 4569 (1980).
6. D. Butlen, G. Guillon, B. Cantau and S. Jard, *Molec. cell. Endocr.* **19**, 275 (1980).
7. B. B. Hoffman, D. F. Dukes and R. J. Lefkowitz, *Life Sci.* **28**, 265 (1981).
8. H. Lee and E. A. Hosein, *Life Sci.* **29**, 135 (1981).
9. E. A. Hosein, H. Lee and I. Hofmann, *Can. J. Biochem.* **58**, 1147 (1980).
10. I. Hofmann and E. A. Hosein, *Biochem. Pharmac.* **27**, 457 (1978).
11. B. B. Wolfe, T. K. Harden and P. B. Molinoff, *Proc. natn. Acad. Sci. U.S.A.* **73**, 1343 (1976).
12. K. P. Minneman, G. A. Weiland and P. B. Molinoff, *Molec. Pharmac.* **17**, 1 (1980).
13. L. T. Williams and R. J. Lefkowitz, in *Receptor Binding Studies in Adrenergic Pharmacology*, Raven Press, New York (1978).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
16. P. Greengrass and R. Bremner, *Eur. J. Pharmac.* **55**, 323 (1979).
17. P. Guicheney and P. Meyer, *Br. J. Pharmac.* **73**, 33 (1981).
18. D. B. Goldstein, in *Membrane Mechanisms of Drugs of Abuse* (Eds. L. G. Abood and C. W. Sharp), p. 151. Alan R. Liss, New York (1979).
19. F. R. Ciofalo, *Proc. west. Pharmac. Soc.* **23**, 441 (1980).
20. D. A. Hems, *Clin. Sci.* **56**, 197 (1979).
21. E. A. Hosein, I. Hofmann and E. Linder, *Archs Biochem. Biophys.* **183**, 64 (1977).
22. M. D. Houslay, T. R. Hesketh, G. A. Smith, G. B. Warren and J. C. Metcalfe, *Biochim. biophys. Acta* **436**, 495 (1975).
23. L. M. Gordon, R. D. Sauerheber, J. A. Esgate, I. Dipple, R. J. Marchmont and M. D. Houslay, *J. biol. Chem.* **255**, 4519 (1980).