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MODULATION OF PROLACTIN BINDING SITES *IN VITRO* BY MEMBRANE FLUIDIZERS I. EFFECTS ON ADULT RAT VENTRAL PROSTATIC MEMBRANES

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SUMMARY: The objective of this study was to determine if aliphatic alcohols, known fluidizers of certain membranes, could increase *in vitro* the apparent fluidity and prolactin binding capacity of membrane preparations obtained from ventral prostate glands of adult male rats. The degree of fluidization was monitored by a fluorescence polarization method using 1,6-diphenylhexatriene. Membrane preparations were either incubated with varying concentrations of ethanol, 1-propanol or 1-butanol and ^{125}I-oPRL overnight at room temperature or were exposed to the alcohols for 15 min at room temperature and washed prior to the overnight incubation with ligand. Regardless of the conditions of incubation, alcohol exposure produced the same effects, a dose-dependent elevation and then decline in specific prolactin binding. Butanol produced a maximal 37-42% increase in prolactin binding at a concentration of 1.0%, propano produced a maximal 40-56% increase in prolactin binding at a concentration of 3.8%, and ethanol produced a maximal 54-77% increase in prolactin binding at concentration of 4.8%. Scatchard analysis of the oPRL binding of ventral prostatic membranes indicated that the *in vitro* treatment of these membrane fluidizers increased the number of oPRL binding sites rather than the apparent affinity constant. The value of the microviscosity parameter decreased by 10-13%, 13-15% and 21-25%, after a 15 min exposure of prostatic membranes to 1.0% butanol, 3.8% propanol and 4.8% ethanol, respectively. These data suggest that *in vitro* fluidization of prostatic membrane modifies prolactin binding capacity and are consistent with *in vivo* prostatic prolactin receptor level-membrane fluidity relationships observed in earlier studies.

The existence of specific prolactin receptors in prostate is well documented (1,2) and is consistent with its physiological effects (3,4). Modulation prolactin and other peptide hormone receptor levels within the cell membranes by various agents is well known (5-8). Recent studies on murine hepatic and rat prostatic membranes reported that the factors known to modulate prolactin receptor levels *in vivo* and *in vitro* also altered membrane lipid fluidity (9-12). These and other studies have indicated that changes in membrane lipid fluidity, possibly by modifying the physical characteristics of the surrounding lipid matrix, may alter the receptor levels for prolactin and other peptide hormones (13-15). To test the above hypothesis from

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another angle, the studies reported herein were undertaken to determine if aliphatic alcohols, known to fluidize certain membranes and to alter a variety of membrane-associated phenomena (16, 17), could increase *in vitro* the apparent fluidity of prostatic membranes and concomitantly alter their prolactin binding capacity.

MATERIALS AND METHODS

Animals: Sprague-Dawley male rats (80-100 days old), obtained from Charles River Breeding Laboratories, were maintained in a 12 hr light-dark cycle with water and Purina rat chow available *ad lib.*

Reagents: Ovine prolactin (oPRL, NIH-P-S-13, 35 IU/mg), obtained from the National Pituitary Agency, was dissolved in 0.01 M NH_4OH in an ice bath and then diluted in 25 mM Tris/0.9% NaCl at pH 7.4.

Tissue Preparation: After decapitation, the ventral prostate glands were excised, frozen in liquid nitrogen, and pulverized. The tissues were homogenized for 1 min using a Brinkmann Polytron set at 6 in approximately 10 vol of 0.3 M sucrose buffered to pH 7.6 with 25 mM Tris-HCl. The supernatant from a 10 min preliminary 125 x *g* centrifugation was recentrifuged at 15,000 x *g* for 20 min to obtain a membrane pellet. This membrane fraction exhibits the highest specific prolactin binding in rat prostate (12, 18).

Prolactin Binding Assay: Ovine prolactin was iodinated with ^{125}I (Amersham) by a modification of the lactoperoxidase method of Thorell and Johansson (12, 19) The membrane pellet was resuspended in 10 mM $MgCl_2$/25 mM Tris at pH 7.6 to provide approximately 3 mg protein/ml (20). Membranes were exposed to aliphatic alcohols in two ways, either by coincubation with ligand or preincubation prior to the exposure of ligand. One hundred µl of membrane suspension was incubated overnight at room temperature with varying concentrations of ethanol, 1-propanol or 1-butanol and 75,000 to 85,000 cpm of ^{125}I-labeled oPRL with and without unlabeled oPRL in a final volume 0.5 ml of buffer (10 mM $MgCl_2$/0.1% BSA/25 mM Tris-HCl, pH 7.6). In other experiments 100 µl of membrane suspension was exposed to the varying concentrations of alcohols for 15 min at room temperature in 0.5 ml of buffer (25 mM Tris-HCl, pH 7.6) and washed free of alcohols (by spinning the tubes at 2500 RPM for 10 min and washing the pellets twice with 1.0 ml buffer) prior to the addition of ligand. Binding assay was terminated by adding 1 ml of chilled buffer. The tubes were centrifuged at 2500 RPM for 20 min. The pellets were washed with an additional 1 ml of buffer and counted in a Nuclear-Chicago gamma counter. Each sample was assayed in triplicate. The diminution of radioactivity produced by coincubation with 1 µg unlabeled oPRL represented specifically bound hormone. Scatchard analysis was performed by incubating the iodinated oPRL with varying amounts of unlabeled hormone (0 to 1000 ng) (21).

Membrane Microviscosity: The fluorescence polarization technique, with 1,6-diphenylhexatriene (DPH) was used for derivation of the steady state polarization constant, P, and microviscosity parameter (22, 23). Membrane suspensions were incubated at 24°C with an equal volume of dispersion of 2 x 10^{-6}M DPH in phosphate-buffered saline at pH 7.1 for 45 min. Alcohols were added for a further 15 min of incubation. The unincorporated DPH was removed by two washes with PBS. This treatment also removed the alcohols whose presence otherwise might contribute some probe partitioning effects on the steady state polarization measurements. Steady state polarization measurements were made at 25°C on a SLM Subnanosecond fluorometer.(4800 series). The ratio output ($I_{\parallel}/I_{\perp}$) and $I_{\perp}$ from the photomultiplier tubes were entered

directly into a 9815 Hewlett-Packard calculator for programming. $I_{\perp}$ and $I_{\parallel}$ are the fluorescence intensities polarized perpendicular and parallel to the polarization of the excitation beam (23). Appropriate measures were taken to avoid light scattering and signal-to-noise effects on the polarization measurements. The values of steady state polarization were used to determine the microviscosity parameter defined as $(r_o/r - 1)^{-1}$, where r is the value of the anisotropy calculated from the measured polarization constant, P, according to the equation $r = 2P/3 - P$ and r_o is the limiting value of the anisotropy of DPH (23). Data presented in the text are expressed both as P values and as 'microviscosity parameter' $(r_o/r - 1)^{-1}$, for comparison with other systems.

RESULTS

Figures 1A-C show that overnight incubation of prostatic membrane preparations with varying concentrations of alcohols and ^{125}I-oPRL resulted in a dose-dependent increase and decline in specific prolactin binding. Ethanol consistently produced a maximal increase in prolactin binding at a concentration of 4.8% that varied in seven different experiments from 154% to 177% of control (Fig. 1A). A concentration of 3.8% 1-propanol consistently produced a maximal increase in prolactin binding that varied in four different experiments from 140% to 156% of control (Fig. 1B). One percent 1-butanol consistently produced a maximal increase in prolactin binding of prostatic membranes that varied in four different experiments from 137% to 142% of control (Fig. 1C). Pre-exposure of prostatic membranes at room temperature to ethanol (4.8%) followed by a wash and subsequent addition of radioactive ligand resulted in a time dependent increase in prolactin binding (Fig. 2). A maximal increase of 61% in prolactin binding was observed after 15 min of preincubation which was consistent with that observed by overnight incubation of membrane preparations with 4.8% ethanol and ^{125}I-oPRL. In other experiments 15 min of membrane preincubation with either 3.8% 1-propanol or 1.0% 1-butanol prior to addition of ligand increased prolactin binding to a value which was consistent in magnitude with that observed by overnight incubation of membranes with the respective alcohol and ^{125}I-oPRL (data not presented).

Figure 3 presents Scatchard analysis of ^{125}I-oPRL binding data of untreated prostatic membranes or membranes treated with 4.8% ethanol, 3.8% 1-propanol or 1.0% 1-butanol. The total number of prolactin binding sites increased from 55 fmol/mg protein in the control to 73 fmol/mg, 83 fmol/mg and 98 fmol/mg

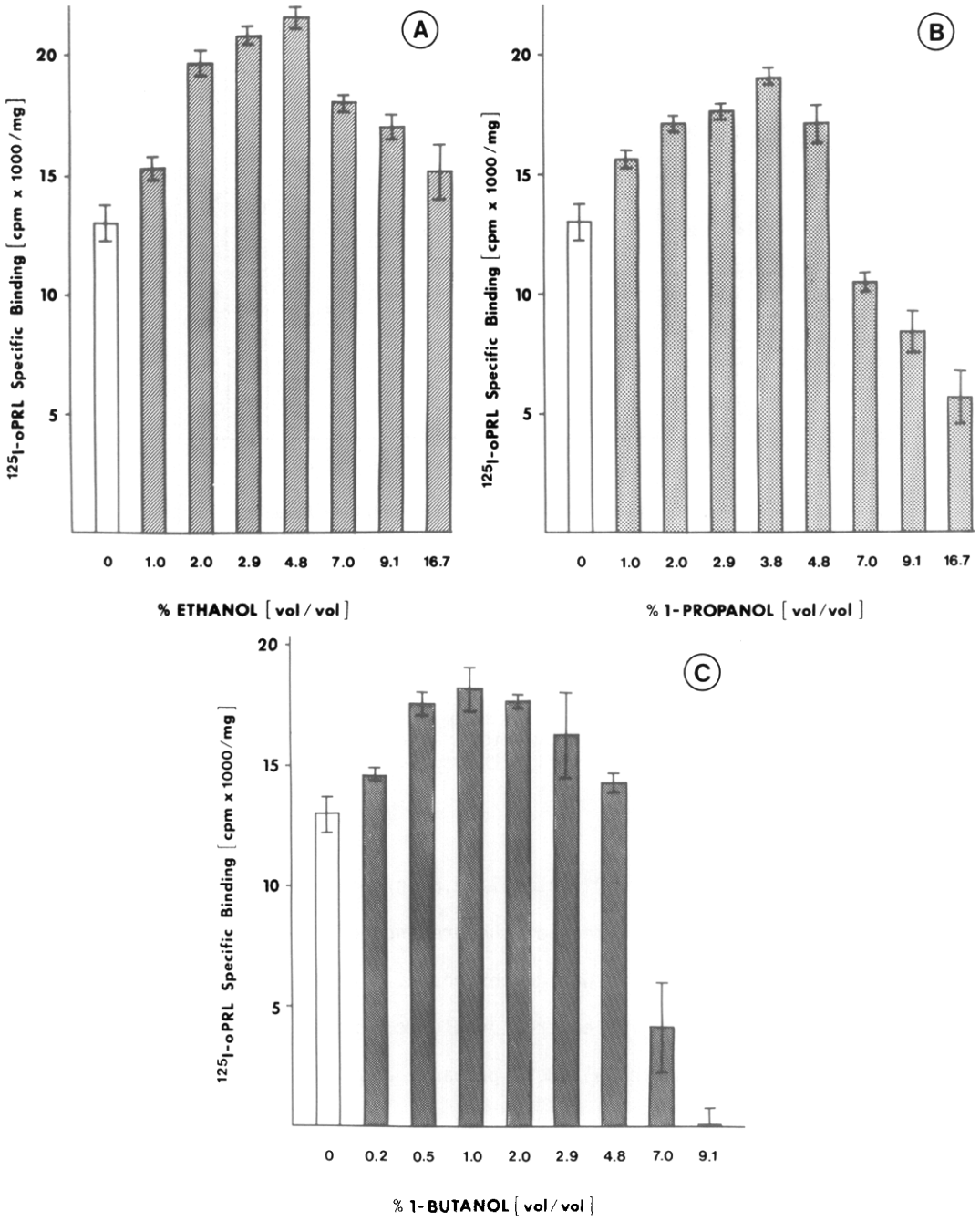

Figure 1 A representative experiment showing that membrane fluidizers *in vitro* increased specific prolactin binding in a dose-dependent fashion.
A: Prolactin binding of ventral prostate membranes coincubated with ^{125}I-oPRL and various concentrations of ethanol.
B: Prolactin binding of ventral prostate membranes coincubated with ^{125}I-oPRL and various concentrations of 1-propanol.
C: Prolactin binding of ventral prostate membranes coincubated with ^{125}I-oPRL and various concentrations of 1-butanol.
Alcohols were coincubated overnight with membranes and ^{125}I-oPRL as described in the text. Each column represents means from membranes assayed in triplicate. Vertical bars = 1 S.E.M.

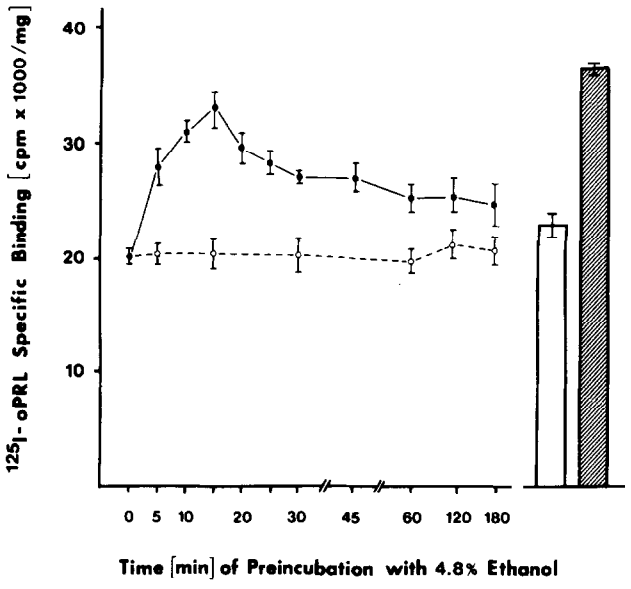

Figure 2

Demonstration that 4.8% ethanol exposure of ventral prostatic membranes at room temperature prior to addition of the ligand increased prolactin binding in a time-dependent fashion. Solid line represents alcohol treated membranes and dotted line represents control membranes left at room temperature for the respective time points. Open (control) and cross-hatched (4.8% ethanol) column represent means from membranes coincubated with ligand in the presence or absence of ethanol overnight. Each point and column represent means from membranes assayed in triplicate. Vertical bars = 1 S.E.M.

protein in the presence of 1.0% butanol, 3.8% propanol and 4.8% ethanol, respectively. No apparent effect of the presence of alcohols on the affinity constant, K_a, of oPRL binding was demonstrable. The K_a values were 7.8 nM, 9.6 nM, 9.6 nM and 8.2 nM for control prostatic membranes and for membranes in the presence of 4.8% ethanol, 3.8% propanol and 1.0% butanol, respectively.

The physical effects of the alcohols on prostatic membrane preparations were quantitated by fluorescence polarization measurements. Table 1 shows the effect of *in vitro* treatment of alcohols on apparent microviscosity (which is inversely related to membrane fluidity) expressed both as the measured values of steady state polarization and calculated values of microviscosity parameter $(r_o/r - 1)^{-1}$. Exposure of alcohols decreased the values of microviscosity parameter in a dose-dependent fashion paralleling the effects of these alcohols on prolactin binding, as described above. The relative

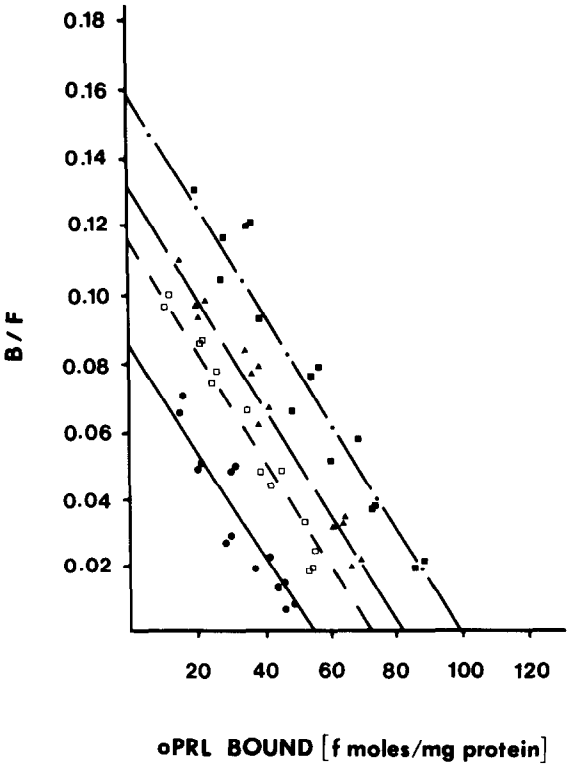

Figure 3

Scatchard plot of oPRL binding data showing that the presence of membrane fluidizers *in vitro* increased the number of prolactin binding sites without affecting their apparent affinity.
Control membranes, ● ; 1.0% butanol treated, □ ; 3.8% propanol treated, ▲ ; and 4.8% ethanol treated membranes, ■ .

degree of fluidization by the alcohols in different experiments varied from 21-25%, 13-15% and 10-13%, after 4.8% ethanol, 3.8% propanol and 1.0% butanol exposure, respectively. At higher alcohol concentrations the value of microviscosity parameter was consistently increased.

DISCUSSION

The non-specific fluidizing effects of aliphatic alcohols on a variety of membrane systems have been documented (15-17). The effects of alcohols on prolactin binding reported in this study suggest a direct relationship between prostatic prolactin receptors and membrane fluidity. This is consistent with a series of previous observations made in rodent prolactin target organs which indicate an interrelation between hormone receptor levels and membrane fluidity

Table 1: Effects of *in vitro* exposure of aliphatic alcohols on steady state polarization and microviscosity parameters of prostatic membranes.

Treatment (*in vitro*)		Polarization constant, P	Microviscosity parameter $(r_o/r - 1)^{-1}$
0		0.155 ± 0.0008	0.431 ± 0.0015
Ethanol	2.0%	0.139 ± 0.0012	0.367 ± 0.0022
	4.8%	0.132 ± 0.0011	0.341 ± 0.0020
	16.7%	0.154 ± 0.0016	0.426 ± 0.0030
Propanol	2.0%	0.149 ± 0.0007	0.406 ± 0.0013
	3.8%	0.142 ± 0.0007	0.376 ± 0.0013
	9.1%	0.163 ± 0.0009	0.465 ± 0.0017
Butanol	0.5%	0.152 ± 0.0009	0.418 ± 0.0017
	1.0%	0.145 ± 0.0006	0.390 ± 0.0011
	7.0%	0.170 ± 0.0006	0.390 ± 0.0011

Steady state polarization, proportional to the apparent microviscosity, was measured at 25°C using DPH. Microviscosity parameter was calculated according to (23), as described in text. Each value represents mean from two experiments assayed individually in triplicate ± S.D.

and tends to support a common mode for the modulation of the prolactin receptors in target organs. For example, *in vivo* administration of indomethacin, a prostaglandin synthesis inhibitor, has recently been reported to simultaneously decrease both prolactin binding capacity and membrane fluidity of mouse hepatic and rat prostatic membranes (12, 24). The same treatment has also been reported in rat testicular membranes to simultaneously decrease both LH binding and membrane fluidity (25).

The present observations in rat prostate and those cited above in other prolactin target organs support the hypothesis that an alteration in the fluidity of the membrane lipid bilayer affects the accessibility of the receptor for its hormone. Changes in the functionality of the receptor are presumably due to changes in the mobility or orientation of proteins which are considered to float within matrix of the lipid bilayer. Increased lipid fluidity would tend to increase the detectability of cryptic prolactin receptors. Such effects of increased membrane lipid fluidity leading to exposure of a larger proportion of the cryptic prolactin and other peptide hormone receptors have been reported by other investigators (26, 27).

The data presented herein suggest that alcohols increase prostatic prolactin binding according to their capability to induce membrane fluidization. It appears that the longer the carbon chain length of alcohol, the lower the concentration required to produce a maximal increase in prolactin binding, whereas the magnitude of the peak response is inversely related to length of the alcohol. The differences in the magnitude of the peak response and in the concentration of alcohols required to produce a maximal increase in prolactin binding in the present study may be related to the differences in stability of prolactin receptors to various alcohols. A similar enhancement of chemoattractant binding to rabbit (29) and human polymorphonuclear leukocytes (28, 30), gonadotropin binding to primate luteal membranes (26) and α-adrenergic binding in brain (15) following *in vitro* exposure to a variety of membrane fluidizers has been documented.

In a recent study, the enhancement of chemoattractant binding to human polymorphonuclear leukocytes following *in vitro* exposure to various aliphatic alcohols was found to be due to increased receptor affinity (28). On the other hand, the Scatchard analysis data in the present study and in several previous reports (26, 30, 31) have shown that changes in membrane fluidity are associated with alterations in hormone, neurotransmitter or chemoattractant receptor number rather than apparent receptor affinity, which is consistent with the concept that membrane fluidity regulates the relative proportions of accessible and cryptic receptors in the membrane.

In conclusion, the present study suggests that *in vitro* fluidization of prostatic membrane modifies prolactin binding capacity and further suggests that modification of the physical characteristics of the membrane lipid matrix may modify the functionality of prostatic prolactin receptors *in vivo*.

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