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EFFECT OF MATERNAL ETHANOL INGESTION DURING PREGNANCY AND LACTATION ON THE STRUCTURE AND FUNCTION OF THE POSTNATAL RAT LIVER PLASMA MEMBRANE

ASSESSMENT WITH [^{3}H]PRAZOSIN BINDING TO THE HEPATIC α_1-ADRENERGIC RECEPTORS*

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Abstract—A liquid low-fat nutritionally adequate Metrecal diet in which alcohol contributed 37% of the total calories was given to pregnant rats and maintained during lactation. Control rats were pair-fed with an isocaloric sucrose–Metrecal diet. After birth, litters were killed at different ages (days 1–30), and the results showed that growth and survival of progeny from the alcohol-treated rats were adversely affected. Likewise, the wet weights of livers from such pups were consistently less than from the pair-fed controls. The yield of hepatic plasma membrane protein per wet liver weight was constant and independent of either age or diet. Using [^{3}H]prazosin as radioligand, equilibrium binding studies were carried out to monitor changes in the structure and function of the plasma membrane in the new-born pups concomitant with the development of α_1-adrenergic receptors. Results obtained with the alcohol-fed pups showed that the binding affinity (K_D) was not altered throughout. However, the receptor density (B_{max}) was decreased significantly. This decrease ranged from 60 to 70% in pups 6- to 15-days-old; 45% at 20 days; and 30% in pups at 25 and 30 days of age. These observations suggest that maternal ethanol ingestion affected the postnatal development of rat liver plasma membranes. Furthermore, by using the hepatic α_1-adrenergic receptor as a metabolic probe, we deduce that a possible impairment exists in the capacity of the alcoholic progeny to respond to the hormonal action of epinephrine. Such a defect may contribute to impaired growth and metabolism in these young animals.

A pattern of multiple congenital abnormalities associated with chronic maternal alcohol ingestion during pregnancy in humans was described by Jones and Smith [1] who collectively named them the "Fetal Alcohol Syndrome" (FAS). Animal models of FAS have since been developed in various laboratories [2–6], and most of these studies have implicated alcohol as the teratogen. However, there is little direct information on the mechanism(s) of the deleterious action(s) of alcohol at the molecular level.

Since alcohol readily diffuses across the placenta, the fetus will be exposed constantly to any unmetabolized maternal alcohol present in the amniotic fluid. The fact that liver alcohol dehydrogenase activity in the young rat only appears a few days prior to birth [7, 8] suggests that fetal development in a dilute solution of alcohol is possible and dependent on the amount and duration of alcohol consumed by the mother.

When micro-organisms or mammalian cells are grown in the presence of alcohol, persistent changes in their plasma membrane lipid composition take place [9, 10]. With the pregnant rat fed alcohol chronically, the fetus, if continuously bathed in a dilute solution of alcohol, might conceivably develop changes in membrane lipid composition analogous to those found in the micro-organisms. Since the plasma membrane performs a wide range of important physiological functions, any induced alteration in its structure as a result of chronic alcohol consumption may, in turn, cause serious changes in those functions.

Many hormones regulate cellular metabolism by acting through plasma membrane-bound receptors. In the rat liver, epinephrine plays an important role in the regulation of carbohydrate metabolism, and its actions are believed to be mediated through α_1-adrenergic receptors [11]. Hepatic membrane hormone receptors are intrinsic membrane-bound proteins. Previous work in this and other laboratories [12–14] has shown that membrane-bound proteins are highly sensitive to fluidity changes in their lipid microenvironment. The hepatic α_1-adrenergic receptors presumably are regulated in the same manner. Chronic perturbation of the plasma membrane through prolonged alcohol exposure may, therefore, be envisioned to affect the function of these receptor proteins.

Recent work in our laboratory with adult rats indicated that chronic alcohol feeding resulted in alteration of the liver plasma membrane as reflected

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by a significant diminution of α_1-adrenergic receptor density, without affecting its binding affinity [15]. In the present work, a similar series of experiments was performed to look for possible adaptive modifications in the liver plasma membrane of pups born of alcoholic mothers.

MATERIALS AND METHODS

Chemicals. Phentolamine HCl was a gift from Dr. G. Kunos (Department of Pharmacology and Experimental Therapeutics, McGill University). [^{3}H]Prazosin (17.1 Ci/mmole) and liquifluor were obtained from New England Nuclear, Canada. Recrystallized bovine serum albumin was purchased from the Sigma Chemical Co. Sucrose, glacial acetic acid, toluene, and phenol reagent were obtained from Fisher Scientific Inc. NCS was purchased from the Amersham/Searle Corp. Vitamin Diet Fortification Mixture was obtained from ICN Nutritional Biochemicals, Canada. Metrecal was prepared by Mead Johnson, Canada. The ethyl alcohol (100%) used in feeding experiments was purchased from Consolidated Alcohol Co. Ltd., Toronto, Ontario.

Animal treatment. Virgin female Sprague–Dawley rats weighing 200–225 g were purchased from Canadian Breeding Farms (St. Constance, Quebec) and housed in polycarbonate shoe-box cages. They were maintained under constant temperature (22 ± 1°) and humidity (31–40%) conditions with a 12 hr light cycle (7:00 a.m. to 7:00 p.m.), and were given water and Purina Rat Chow *ad lib.* After an adjustment period of 1 week they were mated overnight with male Sprague–Dawley rats on a one to one ratio. The appearance of sperm in the vaginal washings the following morning established day 1 of pregnancy. The pregnant rats were randomly divided into three groups. Two groups had their diet changed to a totally liquid low-fat Metrecal–sucrose diet providing 1 kcal/ml, which was supplemented with 3 g/l Vitamin Diet Fortification Mixture. The designated experimental alcohol group was switched to a new diet containing ethanol. This was introduced gradually so that ethanol first provided 10% of the total calories in that diet. After every 2 days, calories derived from ethanol were increased to 20, then 30 and finally 37% of the diet. By the end of the first week of pregnancy, animals in this group were consuming a Metrecal–ethanol liquid diet in which proteins contributed 16%, fat 5%, carbohydrate 42%, and ethanol 37% of the total calories. The pair-fed control animals were maintained on the Metrecal–sucrose diet such that the total calories consumed equalled that of the alcoholic animals (≈85–95 kcal/day; 1 cal = 4.184 J), and this was achieved by matching ethanol-derived calories with sucrose. Both groups of rats were maintained on their respective diets throughout pregnancy and lactation. The third group of pregnant females was kept as an additional set of controls on Purina Rat Chow and water *ad lib.* throughout pregnancy and lactation. All animals were weighed every 2–3 days throughout. The new-born pups were weaned at 21 days and maintained on the liquid diets fed to their respective mothers, until they were 30 days old.

Tissue preparation. New-born pups from the alcoholic and pair-fed control groups were killed at specific ages up to 30 days. The livers were rapidly excised and hepatic plasma membranes isolated essentially by the method of Neville [16] as described by Wolfe *et al.* [17]. Briefly, the livers were minced in 20 vol. of ice-cold 1.0 mM $NaHCO_3$ and homogenized with six up-and-down strokes in a Potter–Elvehjem homogenizer. Homogenates were filtered through four layers of cheesecloth and centrifuged for 10 min at 4000 *g*. The pellet was resuspended in 1.0 mM $NaHCO_3$ and 69% (w/w) sucrose was added to produce a final concentration of 47.5% (w/w). Sucrose, 42.3% (w/w), was then layered over the resuspended pellet, and the samples were centrifuged at 100,000 *g* for 2 hr. The partially purified membranes which floated on top of the 42.3% sucrose were removed, washed, and resuspended twice with 50 mM Tris buffer, pH 7.5. The membranes in the final resuspension were used for binding assay.

Equilibrium binding studies. α_1-Adrenergic receptor binding was determined using [^{3}H]prazosin as the radioligand. Unless otherwise specified, all assays were conducted in triplicate. A typical binding assay contained 125 μl of membrane suspension (in 50 mM Tris buffer, pH 7.5), assay buffer (4 mM $MgSO_4$, 0.8 mM ascorbate, and 50 mM Tris, pH 7.5), and radiolabeled ligand in a final volume of 250 μl. Specific binding was defined as the difference between binding of the radioligand in the absence and in the presence of 10 μM phentolamine. Membrane (0.075 to 0.125 mg protein) suspensions were incubated for 15 min at 31°, and the reaction was terminated by addition of 3 ml of ice-cold assay buffer. The incubation mixtures were rapidly filtered under vacuum through Whatman GF/C glass fiber filters. The filters were washed with two 6-ml rinses of ice-cold buffer, transferred to plastic vials, and dried overnight. Radioactive ligand was extracted from the filters by digestion with 300 μl NCS for 2–4 hr at room temperature (22 ± 1°). Liquifluor (10 ml) and glacial acetic acid (15 μl) were added to the vials which were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer with an efficiency of 35%. Non-specific binding was usually 10–15% of the total radioactivity bound.

Protein determination. Protein was determined by the method of Lowry *et al.* [18] using recrystallized bovine serum albumin as standard.

Statistical analysis. Statistical significance between mean values was examined using Student's two-tailed *t*-test. Least squares linear regression analysis was used to derive the Scatchard plots [19].

RESULTS

Nutritional and reproductive characteristics. The amount of food and ethanol consumed by animals in the maternal alcoholic group is shown in Table 1. The average food intake for animals in this group ranged from 180 to 300 kcal per kg body weight per day, and the average ethanol intake ranged from 10 to 15.7 g per kg body weight per day. The intake of total nutrients and calories for both the control and alcoholic maternal groups fulfilled the nutritional requirements of laboratory rats [20].

Table 1. Total food and ethanol intake during gestation*

Gestation day	Energy intake (kcal/kg/day)	ETOH intake (g/kg/day)	Protein intake (g/kg/day)
1–6	180 ± 10	10 ± 1	6.4 ± 0.3
7–14	300 ± 20	15.7 ± 0.9	11 ± 1
15–22	260 ± 20	13.8 ± 0.7	9 ± 1

* Values represent the means ± S.E.M. of five animals and they are expressed as daily intake per kg body weight.

At the time of sacrifice, blood samples from suckling pups were collected from the neck and ethanol concentration was measured enzymically [21]. Blood ethanol levels varied between 4 and 10 mM during the last 3 days of gestation, increased to 20 mM at the end of the first postnatal week, and reached values between 35 and 40 mM at 18–30 days of age.

As indicated in Table 2, no significant differences were found in maternal weight gain, litter size, and average litter weight at birth between animals in the alcoholic and pair-fed sucrose control groups. However, as shown in Fig. 1 and Table 2, animals fed water and Purina Rat Chow *ad lib.* during pregnancy gained more weight than those in the pair-fed groups.

Postnatal physical performance. As shown in Table 2 and Fig. 2, new-born pups of alcohol-fed mothers gained significantly less weight than pups from either pair-fed sucrose or Rat Chow-fed control mothers. Pups belonging to the pair-fed sucrose group did not gain as much weight as those from the Rat Chow-fed control group, but this may be a consequence of the restriction in daily caloric intake due to pair-feeding.

General characteristics of livers from new-born rats. The wet weight of livers from pups born of mothers pair-fed sucrose was consistently greater than those fed alcohol (Fig. 3). However, regardless of diet treatment and age, the liver wet weight to body weight ratio of pups from both pair-fed groups varied between 30 and 40 mg/g during the first month after birth. The yield of hepatic plasma membrane protein per wet liver weight was constant, independent of either age or diet, and close to 2.5 mg/g (Fig. 3).

[^{3}H]*Prazosin binding to liver plasma membranes.* The experimental conditions described for equilibrium binding studies were based on previous work done in our laboratory [15] where it was demonstrated that [^{3}H]prazosin binds with high affinity, in a saturable manner and stereospecifically to the α_1-receptors in the rat liver plasma membrane. The same conditions were found to characterize α_1-adrenergic binding in the hepatic plasma membrane of the new-born rat. Figure 4 shows a typical saturation curve and a Scatchard analysis of [^{3}H]prazosin binding to liver plasma membrane from 30-day-old control and alcoholic pups. Figure 5 represents the first characterization of the postnatal development of the

Table 2. Reproductive and postnatal physical characteristics

Physical parameters	Treatment conditions		
	Ethanol	Pair-fed	Water and Rat Chow *ad lib.*
N*	11	10	5
Viable offspring	140	121	62
Number of stillbirths	6	2	0
Average offspring per litter (± S.E.M.)	12.7 ± 0.4	12.1 ± 0.8	12.4 ± 0.8
Average weight (g) per pup at birth (± S.E.M.)	5.7 ± 0.1	5.8 ± 0.3	6.5 ± 0.1
Average maternal weight gain (g) during pregnancy (± S.E.M.)	123 ± 5†	122 ± 5†	150 ± 10†
Average body weight gain (g) per pup up to 30 days after birth (± S.E.M.)	38 ± 1‡	60 ± 1	71 ± 4
% Survival	70	94	95

* Number of litters.

† $N = 5$ for all treatment conditions.

‡ Significantly different ($P < 0.05$) from control values as determined by Student's two-tailed t-test.

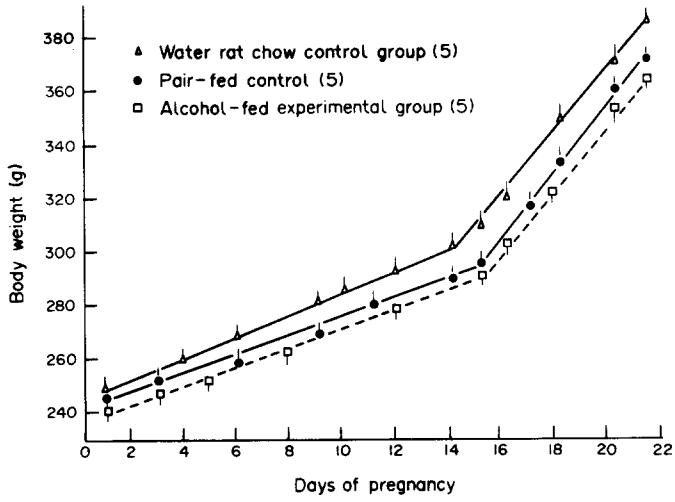

Fig. 1. Body weight gain profile of pregnant rats. Pregnant rats were given either alcohol (□), sucrose (●) or water and Purina Rat Chow *ad lib.* (△) throughout pregnancy and lactation as described under Materials and Methods. The body weight gain was followed until time of delivery. Each value is the mean ± S.E.M. from five different animals kept under the corresponding diet.

physiologically relevant α_1-adrenergic receptors in rat liver using [^{3}H]prazosin as the radioligand. No detectable binding was observed at day 1 in both animal groups. Specific binding was detected at day 6, and this increased gradually in a sigmoidal fashion. This increase was relatively slow up to day 20 at which time the receptor density was approximately 20% of adult values for both the control and alcoholic progenies. After day 20, receptor number increased dramatically and at day 30 adult values [15] were obtained for both groups.

Although the postnatal developmental pattern of hepatic α_1-adrenergic receptors in new-born rats from the alcoholic group did not appear to differ from the pair-fed sucrose controls, there was consistently a significant decrease in the total number of receptors (B_{max}) in the plasma membrane of pups in this group (Table 3 and Fig. 4). This decrease in receptor density was 60–70% in pups 6–15 days of age; 45% at 20 days; and approximately 30% in pups at 25 and 30 days. The equilibrium dissociation constant (K_D) did not change with postnatal age and was similar for both groups studied (Table 3 and Fig. 4). Chronic maternal ethanol administration during pregnancy and lactation has, therefore, a marked detrimental effect on the postnatal hepatic plasma membranes as reflected by a dysfunction in the development of the α_1-adrenergic receptors.

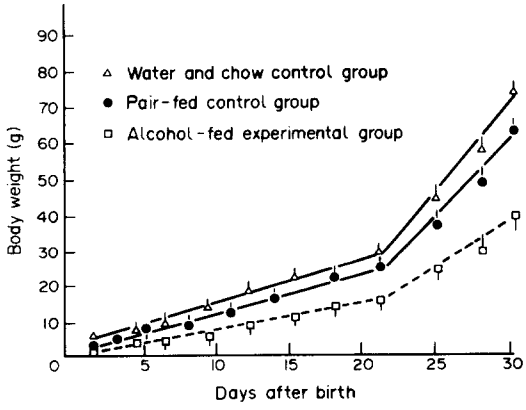

Fig. 2. Body weight gain profile of new-born rats. Maternal feeding of their respective diets continued throughout lactation and beginning of weaning period up to day 30. All pups were weighed up to 30 days postnatally. Each value represents the mean ± S.E.M. from five different litters for each group.

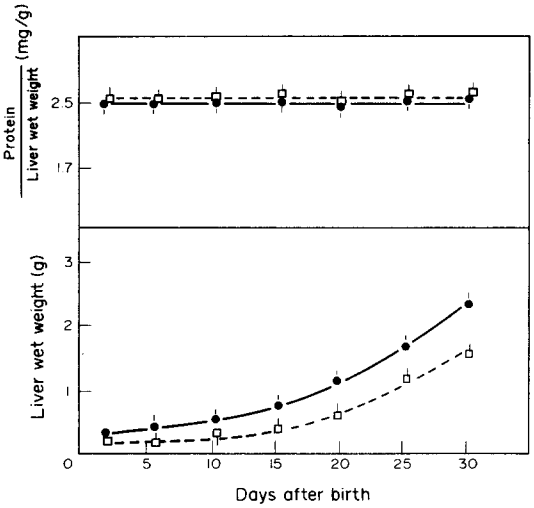

Fig. 3. General developmental characteristics of livers from new-born rats belonging to maternal alcoholic and pair-fed sucrose groups. New-born rats were weighed and decapitated at specific postnatal ages. The livers were rapidly excised and placed in 20 vol. of ice-cold 1.0 mM $NaHCO_3$. The wet weights were recorded before proceeding with the purification of hepatic plasma membranes. Total protein in the plasma membrane fractions was determined as described in the text. Each value is the mean of three to six experiments. Key: (□ - - - □) alcoholic; and (●—●) pair-fed.

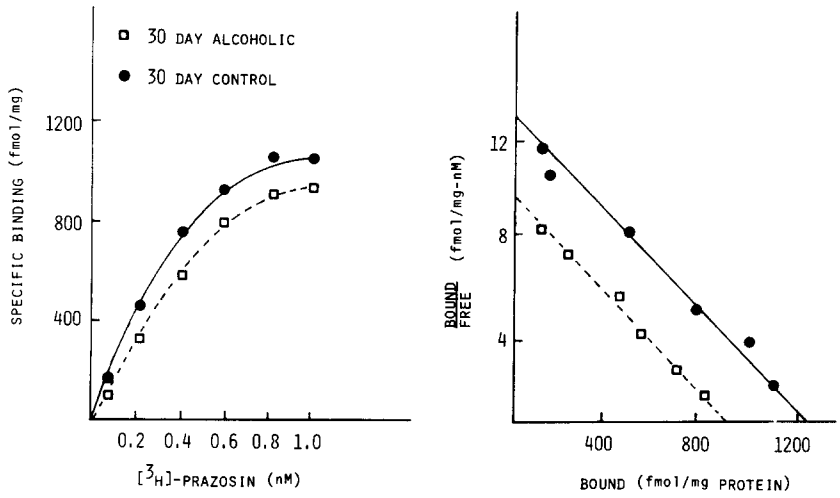

Fig. 4. Typical saturation curves of specific [3H]prazosin binding to hepatic plasma membranes. The left panel (A) illustrates the dose-dependent binding of [3H]prazosin to liver plasma membranes from 30-day-old rats born of alcoholic (□ - - - □) and pair-fed sucrose control (●—●) mothers. The right panel (B) shows the corresponding Scatchard plots for the saturation curves. B/F ratios for the [3H]prazosin bound by membrane protein to free labeled ligand were plotted as a function of bound [3H]prazosin (B). The slopes of the plots ($-1/K_D$) were determined by linear regression analysis, and the number of binding sites (B_{max}) was computed from the X intercepts of the plots. Values are the means of duplicate determinations from four experiments.

DISCUSSION

In the first part of this study, the effect of chronic ethanol feeding during pregnancy and lactation on maternal reproductive performance, as well as litter growth, was investigated. Maternal weight gain during pregnancy was greatest in the Rat Chow control group compared with the alcoholic and pair-fed sucrose control groups. Similar observations have been reported previously by other investigators [22, 23]. Since in those as well as our present study the pair-fed sucrose controls had the same caloric intake as the alcoholic group, the lower maternal weight gain during pregnancy may be attributed to a restrictive effect of alcohol administration on daily caloric intake. Litter size was the same in all groups studied, in agreement with other authors [24–26]. No difference was found in the body weight of pups born of mothers kept on the different diets. This is in agreement with the recent work of Borges and Lewis [27] but contrasts with others [28, 29] who reported decreased body weight of alcoholic pups at birth. Such discrepancies may be due to different methods of ethanol feeding, dosage, and duration of ethanol consumption.

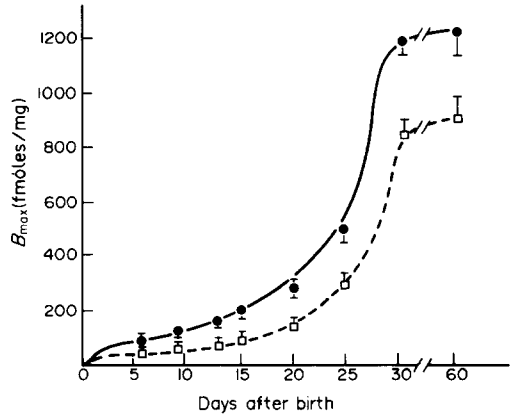

Fig. 5. Postnatal development of α_1-adrenergic receptors characterized by [3H]prazosin. Hepatic plasma membranes from pups born of either alcoholic (□ - - - □) or pair-fed sucrose control (●—●) mothers were isolated at the ages indicated and incubated with [3H]prazosin for 15 min at 31° as described in the text. Scatchard plots were derived from specific equilibrium binding data to determine the total amount of binding sites (B_{max}) and the dissociation constants (K_D). Values are the means of triplicate determinations from the number of experiments indicated in Table 3.

Earlier studies with the rat demonstrated that offspring development and survival were impaired subsequent to maternal ethanol ingestion [22]. Our results showed that the number of stillbirths was considerably higher in the ethanol-fed compared to the pair-fed group. Furthermore, pups from alcoholic mothers exhibited a lower percentage survival and gained weight at a significantly lower rate than pups from pair-fed sucrose control and Rat Chow control mothers. A recent report [30] suggests that prenatal alcohol exposure can interfere with the development of normal suckling behavior. In addition, ethanol-consuming lactating mothers may display significant deficits in maternal behavior towards alcoholic pups [31]. Both of these factors can contribute to a lowered survival rate, food intake, and thus decreased weight gain in our experimental pups.

In the second part of this work, the effect of chronic maternal ethanol feeding on the postnatal hepatic plasma membrane, as assessed with the

Table 3. Comparison of the effects of chronic alcohol ingestion during pregnancy and lactation on different characteristics of specific [^{3}H]prazosin binding to rat hepatic plasma membranes during postnatal development*

Days after birth	Treatment condition: Control N†	Control K_D (nM)	Control B_{max} (fmoles/mg)	Alcoholic N†	Alcoholic K_D (nM)	Alcoholic B_{max} (fmoles/mg)
1	4			4		
6	3	0.21 ± 0.01	100 ± 20	3	0.23 ± 0.04	40 ± 10‡
9	3	0.25 ± 0.05	140 ± 20	3	0.08 ± 0.01	50 ± 10‡
13	4	0.15 ± 0.02	190 ± 20	5	0.11 ± 0.05	60 ± 20‡
15	2	0.30 ± 0.05	210 ± 20	4	0.33 ± 0.02	90 ± 10‡
20	2	0.17 ± 0.02	270 ± 20	2	0.12 ± 0.02	150 ± 20‡
25	3	0.15 ± 0.01	480 ± 50	3	0.10 ± 0.03	320 ± 20‡
30	3	0.19 ± 0.01	1200 ± 100	3	0.16 ± 0.05	880 ± 10‡

* Equilibrium binding experiments were done as described under Materials and Methods. Scatchard analysis [19] was performed to determine the various binding characteristics. Values are given as the means ± S.E.M. for the number of experiments indicated.

† Number of experiments.

‡ Significantly different from pair-fed controls at $P < 0.05$ by Student's two-tailed t-test.

development of α_1-adrenergic receptors, was investigated. Alpha-adrenergic receptors play an important role in the regulation of hepatic carbohydrate metabolism [32]. In adult rats, α_1-receptors comprise approximately 80% of the total rat hepatic α-adrenergic receptors, and they have been identified as the physiologically-relevant receptors which mediate the activation of glycogen phosphorylase [11]. [^{3}H]Prazosin, an α_1-adrenergic antagonist, has been shown recently in this and other laboratories to be a highly suitable and selective radioligand for identifying these receptors [11, 15].

The results shown in Fig. 5 indicate that, in general, the postnatal development of the hepatic α_1-adrenergic receptors was quite similar for the pair-fed sucrose control and alcoholic pups, both following a sigmoidal pattern. Adult values for the total number of receptors (B_{max}) were found in the 30-day-old pups in the pair-fed sucrose control and alcohol-fed groups ($B_{max} \simeq 1200 \pm 100$ and 860 ± 20 fmoles/mg protein respectively) [15]. Butlen *et al.* [33] have described a biphasic pattern for the development of hepatic adrenergic receptors in rat pups fed water and Rat Chow *ad lib.* In that study, the maximal binding capacity (B_{max}) was observed in the 19-day-old fetal liver. A progressive decrease in B_{max} ensued thereafter until the second week after birth. B_{max} then increased again between 18 and 30 days after birth until the adult value was reached. In this study we were unable to detect any significant binding with [^{3}H]prazosin 1 day after birth. This difference may be due to the fact that [^{3}H]prazosin binds specifically to α_1-receptors while [^{3}H]dihydroergocryptine used by Butlen *et al.* [33] binds to both α_1 and α_2 receptors [11]. Therefore, during the perinatal period most of the rat hepatic α-adrenergic receptors present may not be of the α_1 subtype.

Data presented in Table 3 and Fig. 5 show that, between 6 and 30 days, receptor density (B_{max}) for pups in the alcoholic group was decreased significantly compared with the pair-fed sucrose controls. No difference was observed in the binding affinity (K_D) during the entire postnatal period for both groups. This suggests that the fundamental functional characteristics of these receptors were not altered.

These results indicate that chronic maternal ethanol ingestion during pregnancy and lactation has, in some way, a detrimental effect on the postnatal rat liver plasma membrane, as reflected by a diminished density of α_1-adrenergic receptors. In the rat liver, α_1-receptors mediate the regulatory actions of epinephrine on glucose homeostasis [11]. In both humans and rats, glucose is the main oxidative fuel for the fetus and the new-born [34, 35]. Most of the key enzymes regulating hepatic carbohydrate metabolism are known to be activated postnatally, and selective developmental changes in the control of carbohydrate metabolism have been shown to occur after birth [36–39]. Accordingly, a persistent reduction in the postnatal development of hepatic α_1-adrenergic receptors subsequent to maternal ethanol feeding may seriously affect the capacity of the liver to respond to the regulatory actions of epinephrine in those pups.

The precise mechanism(s) responsible for the diminished hepatic α_1-adrenergic receptor density from the experimental pups is not known. One likely explanation is that this anomaly represents the manifestation of some fundamental intrinsic difference between the control and alcoholic plasma membranes as was observed earlier in similar studies with adult male rats [15]. There is some experimental evidence to support this view. Cellular membranes are known to adapt to prolonged alcohol exposure by altering their lipid composition [9, 10, 40]. Lipid compositional changes could conceivably modify membrane lipid fluidity as well as the dynamics of lipid–protein interaction [14]. These may, in turn, alter the accessibility of the receptor to the ligand [41], i.e. some functional receptors may be masked. Thus, the decreased receptor binding to the experi-

mental plasma membrane could represent another dimension of membrane adaptation to ethanol.

Alternatively, the decreased receptor density may represent insufficient assembly or insertion of these receptors into the outer surface of the experimental plasma membrane. Such a flaw could be the result of improper membrane biogenesis during fetal or postnatal development. Defective maturation of structural membrane components in the central nervous system has been demonstrated recently in neonatal rats suffering from hypothyroidism and undernutrition [42]. Similar defects might have occurred in the postnatal rat liver plasma membrane following prolonged alcohol exposure.

Regardless of the mechanisms involved, the enduring anomaly in the experimental plasma membranes must be presumed to have originated from chronic *in utero* ethanol exposure. Long-lasting membrane defects of this nature would ultimately contribute to some of the abnormalities associated with chronic maternal alcohol ingestion.

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