Pharmacology Biochemistry & Behavior, Vol. 19, pp. 567–570, 1983. Ankho International Inc. Printed in the U.S.A.

Effects of Ethanol, Given During Pregnancy, on the Offspring Dopaminergic System

LAURA LUCCHI,[1] VITO COVELLI,[2] VESSELIN V. PETKOV,[3] PIER-FRANCO SPANO AND MARCO TRABUCCHI

Department of Pharmacology and Pharmacognosy, University of Milan, Milan, Italy

Received 23 June 1983

LUCCHI, L., V. COVELLI, V. V. PETKOV, P.-F. SPANO AND M. TRABUCCHI. *Effects of ethanol, given during pregnancy, on the offspring dopaminergic system.* PHARMACOL BIOCHEM BEHAV 19(4) 567–570, 1983.—The fetal alcohol syndrome is characterized by a number of abnormalities consisting of a pre- and post-natal growth deficiency, microcephaly, areas of abnormal nerve cell migration in the brain, mental and psychomotor retardation in children of alcoholic women. These findings may be referred as a teratogenic effect of ethanol on the central nervous system. In order to investigate the above ethanol-neurotoxic effect the striatal dopaminergic transmission was studied. The dopaminergic turnover was measured by 3,4-dihydroxyphenilacetic acid content and ^{3}H-Spiperone binding has been carried out to determine dopaminergic receptor alterations induced by chronic ethanol consumption during pregnancy. Our work demonstrates long-lasting modifications of dopaminergic neuronal function after exposure of the experimental animal to ethanol during fetal life. In particular, a decreased receptor function has been observed in rats exposed to ethanol only during the perinatal period. In the same group of rats, diminished receptor activity leads to an enhancement in DOPAC content still detectable after a long period from cessation of ethanol treatment. Neurochemical data are reinforced by behavioral observations. In fact, a significant decrease of spontaneous locomotor activity in the rats chronically treated with ethanol during fetal life was observed. In addition, the altered response of locomotor activity after drug administration may be ascribed to the modified dopaminergic function. With this experimental approach we assume that the action of ethanol on the central nervous system may be a marker of its teratogenic effect.

Fetal alcohol syndrome Behavior Rat brain Dopaminergic transmission

THE teratogenicity of alcohol has been demonstrated in humans by clinical observations and behavioral studies [21, 24, 29]. On the other hand, several experimental studies on animal models have examined the potential hazards for the fetus induced by maternal ingestion of alcohol during pregnancy [1, 3, 9, 12, 15, 18, 19]. Moreover, during lactation the pups suckled milk containing ethanol at the same concentration as that of maternal blood [14]. Experimental data indicate that ethanol moves freely across the placental barrier whereas acetaldehyde does not [3,13]. In fact, biochemical observations show a direct action of ethanol on the developing nervous system causing an impairment of embryonic cellular proliferation [3]. In relation with these outcomes, several studies have been demonstrated that ethanol consumption during pregnancy affects various neurochemical parameters in offspring brain [4, 10, 20, 28].

The purpose of our work is to provide further data on the long-lasting alterations induced by ethanol administered during pregnancy at neuronal level, in particular on brain dopaminergic transmission. In the light of these results, it may be assumed that certain neurochemical and behavioral events are possible markers of the teratogenic effect of ethanol.

METHOD

Pregnant Sprague Dawley rats were supplied from Charles River, Calco Italy. Rats were individually caged and ethanol was administered as the only available liquid in a 6% aqueous solution (v/v), using Richter tubes. The 40 pregnant rats used were separated in four groups (10 animals per group). The first group received ethanol from the fourth day of pregnancy to delivery; the pups were not treated with ethanol. Another group of dams received the same solution from the fourth day of pregnancy to delivery, and subsequently for three weeks during lactation. A third group of dams was not treated with ethanol during pregnancy but ethanol was administered to the mothers during lactation for three weeks after birth. The body weight of dams from the groups receiving ethanol remained unaltered during the treatment with respect to control dams receiving an equicaloric sucrose-solution (230±20, 235±24, 238±18, 229±20 g, respectively).

[1]Requests for reprints should be addressed to Laura Lucchi, Via A. Del Sarto, 21, 20129 Milan, Italy.
[2]IIa Clinica Neurologica, University of Bari, Bari, Italy.
[3]Institute of Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

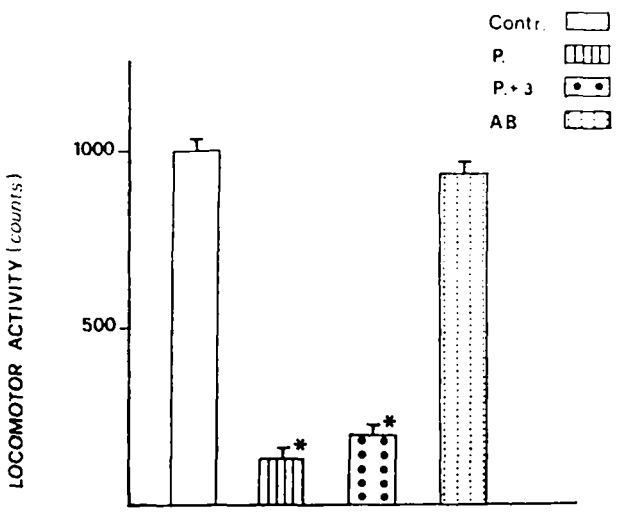

FIG. 1. Rats locomotor activity (counts/30 min) after exposure to ethanol during pregnancy (P), during pregnancy and 3 weeks after birth (P+3), and 3 weeks after birth (AB). The bars represent the ±S.E.M. of 3 determinations each of which was carried out at the basis of 5 animals which were individually tested. Statistical significance was calculated by one way ANOVA followed by the two-tailed Student's *t*-test. $^{*}p<0.01$ in relation to control values.

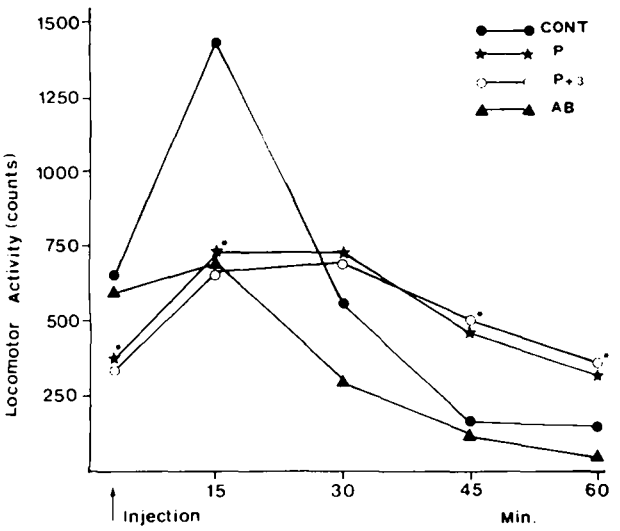

FIG. 2. Effect of d-amphetamine (3 mg/kg, IP) on the locomotor activity (counts/15 min) of rats exposed to ethanol during fetal life (P), during pregnancy plus 3 weeks after birth (P+3) and only after birth (AB). Each point represents the mean ± S.E.M. of 3 determinations run with 5 animals which were individually tested. One way ANOVA was used for statistical significance then followed by the two-tailed Student's *t*-test. $^{*}p<0.01$ in respect to controls.

The daily intake of liquid was measured and the quantity of ethanol consumed was in the range of 9 g kg $^{-1}$ per day for each dam belonged to the three ethanol groups. No weight changes were observed in the treated offspring groups in respect to control pups receiving equicaloric sucrose.

At eight weeks of age the spontaneous and drug induced locomotor activities of male rats in electronic activity cages were tested. Only the male rats from each treatment group (6 animals) were included in the behavioral studies. An electronic activity cage (ANIMEX, LKB FARAD) has been used. The ANIMEX sensing unit consists of an oscillator operating at a frequency of 1.2 MHz and a resonance circuit tuned to this frequency. Each rat movement providing a signal large enough to exceed the Schmitt-trigger threshold (10 μA), produces one count. The total counts derived from the rat movements done have been considered.

After adaptation to the cages, (30 min) the spontaneous activities of the rats were recorded every 15 minutes for a period of two hours. In order to determine the locomotor activity response to drugs, the first group of rats were removed from the cages and treated acutely with d-amphetamine (3 mg/kg, IP) and the locomotor activity was recorded again for one hour after drug administration. A second rat group was treated with haloperidol (0.05 mg/kg IP) acutely after basal locomotor activity recording. The durg-induced motility was recorded again for about two hours. Each locomotor activity determination was carried out for the four rat groups with the same experimental protocol.

The offspring were killed by decapitation at the end of the 9th week of age (63rd day). Brains were rapidly removed and striata dissected as indicated by Glowinski and Iversen [11]. In order to investigate the biochemical modifications, we measured dopamine (DA) metabolism in the striata derived from control and ethanol treated rats. The striatal 3,4-dihydroxyphenlyacetic acid (DOPAC) concentration was assayed according to the radioenzymatic micromethod described by Argiolas *et al.* [2]. ^{3}H-Spiroperidol specific binding was carried out following the method of Burt *et al.* [6] with minor modifications. A tissue suspension corresponding to 200 μg/protein was put in each incubation tube containing various concentrations of radioligand. Specific binding was measured as the difference in binding obtained after incubation in presence or absence of 10^{-6} M haloperidol. ^{3}H(−)-Sulpiride specific binding, as indicated by Spano *et al.* [23], was determined in crude synaptic membrane fractions corresponding to 15 mg of original tissue per incubation tube (400 μg/protein). Stereospecific binding was measured as the difference in binding obtained in presence or in absence of 10^{-6} M(−) sulpiride. K_d and B_{max} values are expressed as nM and fmol/mg protein, respectively. The protein content was determined according to Lowry *et al.* [16]. The statistical significance was calculated by one way ANOVA followed by Student's *t*-test.

RESULTS

Results on rat spontaneous locomotor activity are summarized in Fig. 1 showing a significant decrease in the basal locomotor activity only in rats which had received ethanol during fetal life.

In an attempt to understand the neuronal mechanism inducing the decrease in locomotor activity, each group of rats was treated with d-amphetamine. In Fig. 2 it is shown that d-amphetamine increased locomotor activity by 140% in controls, 85% in prenatal period, 87% in prenatal period plus 3 weeks after birth and 15% in after birth.

On the other hand, haloperidol (Fig. 3) induced a more pronounced decrease in locomotor activity in the prenatal period and the prenatal period plus 3 weeks groups (90%), while the effect in the after birth group is not significantly different from that of controls.

In order to investigate the biochemical basis of these be-

TABLE 1

STRIATAL DOPAC LEVELS (ng/mg TISSUE) IN RATS EXPOSED TO ETHANOL DURING PREGNANCY, DURING PREGNANCY AND THREE WEEKS AFTER BIRTH, AND THREE WEEKS AFTER BIRTH

	ng/mg Tissue	Percent
Control	1.92 ± 0.02	—
Pregnancy	2.93 ± 0.09*	+52
Pregnancy + 3 weeks	3.01 ± 0.1*	+56
After birth (3 weeks)	2.09 ± 0.03	+ 9

Each value is the mean ± S.E.M. of three experiments in which 4 animals per group were used.

*One way ANOVA indicated a significant effect for the factor of treatment group. Post hoc comparison was made using the two tailed Student's *t*-test.

*$p < 0.05$ in respect with control value.

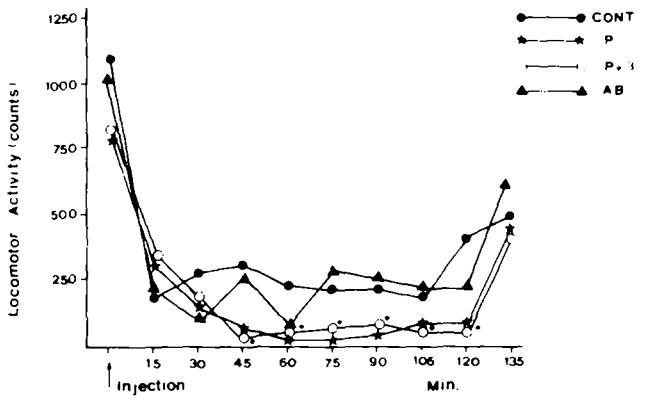

FIG. 3. Effect of haloperidol (0.05 mg/kg, IP) on the locomotor activity (counts/15 min) of rats exposed to ethanol during pregnancy (P), during pregnancy plus 3 weeks after birth (P+3) and after birth (AB). Each point represents the mean ±S.E.M. of 3 determinations run with 5 animals which were individually tested. Statistical significance was performed by one way ANOVA followed by the two-tailed Student's *t*-test. *$p < 0.01$ in respect to controls.

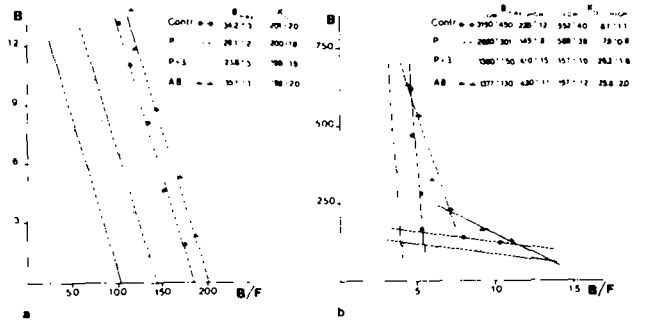

FIG. 4. Scatchard analysis of (a) ^{3}H-Spiroperidol and (b) ^{3}H(−) Sulpiride specific bindings in striatal membranes obtained from control rats (●—●) and ethanol exposed rats during pregnancy (△—△), during pregnancy and three weeks after birth (▲—▲) or three weeks after birth (▲—▲). Points are the mean ± S.E.M. of three experiments run in triplicate in which 5 animals per group were used.

havioral responses, we measured DA metabolism in the striata of rats receiving ethanol. Table 1 demonstrates that an impairment of dopaminergic activity only occurs after exposure to ethanol during the fetal period. In fact, the DOPAC levels, which are a marker of DA turnover, are increased in relation to the values of the control group and to animals receiving ethanol only during lactation. Further research was developed on the mechanisms involved in dopaminergic transmission by studying dopamine receptor function by means of various radioligands, such as ^{3}H-Spiroperidol and ^{3}H(−)Sulpiride. The results reported in Fig. 4 show that receptor impairment caused by ethanol only develops during the fetal period. In particular, Scatchard's analysis demonstrates a decreased receptor population of the binding sites characterized by ^{3}H-Spiroperidol. In addition, the dopaminergic recognition sites, labelled by ^{3}H(−) Sulpiride, seem to be modified by ethanol exposure. In fact, the B_{max} values decreased in rats exposed to ethanol in prenatal period, whereas the K_d values increased in the case of high affinity and decreased for the low affinity components only after treatment during the first three weeks of age.

DISCUSSION

The consumption of alcohol by pregnant mothers is known to impair fetal development. The molecular mechanisms by which alcohol causes fetal dysmorphogenesis are not yet understood. One of the consequent pathological conditions is the Fetal Alcohol Syndrome which is distinct from other genetic or nutritionally related fetal defects, as demonstrated in animal studies [7]. In fact, we have shown that the derangement of dopaminergic transmission in rats is still evident when the food intake is normal.

Our results clearly reinforce the hypothesis that ethanol has teratogenic effects during the development of the central nervous system. Previous studies on the organogenesis of the central nervous system [8,22] lead us to the conclusion that ethanol exerts its toxic action on brain dopaminergic neurons during this time. For example, the rat embryos exposed to ethanol during organogensis have shown retardation of growth *in vitro* [5] and behavioral impairment [17].

The decreased spontaneous locomotor activity in the rats and the altered response after drug administration may be correlated with the biochemical observations on the modified dopaminergic function. In particular, the effect of the increased release of dopamine induced by d-amphetamine detected in control rats, is lower in rats treated during fetal life with ethanol, suggesting that ethanol may reduce the availability of specific receptor proteins. The same conclusions may also be reached in the case of haloperidol. It has been shown that when the number of dopaminergic receptors is reduced, low doses of haloperidol are sufficient to block locomotor activity.

The changes of receptor function after ethanol consumption may be due to a derangement in the biosynthesis of the receptors. Ethanol administration in the post-natal period induces an alteration in the affinity of the dopaminergic receptor recognition sites, labelled by ^{3}H(−)Sulpiride, which may be due to a modification in receptor protein conformation. Various studies have led to the conclusion that ethanol interferes with the genetic code transcription and protein synthesis [25, 26, 27]. Receptor units might be impaired and/or modified in their own protein structure. The decreased receptor activity leads to an increase in DOPAC content that is still detectable after a long period from treatment suspension as a compensatory response to the loss of receptor function.

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