Stage-Specific Sensitivity of Rat Embryogenesis to Ethanol Injected Into the Amnion and via Other Routes

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Although ethanol easily crosses the placenta [7], rat embryos are virtually insensitive to its injurious effect [3-5]. On the other hand, ethanol has a marked dose-dependent embryolytic effect *in vitro* in a culture of postimplantation embryos [26,8]. This discrepancy prompted us to compare the embryolytic effects of ethanol after its injection directly into the amniotic cavity, bypassing the placenta, and after administration by other routes.

MATERIALS AND METHODS

Experiments were caried out with white outbred rats weighing 180 to 200 g. The day on which spermatozoa were first detected in vaginal smears was considered the first day of pregnancy. On the 14th day of pregnancy one group of females received intraperitoneal injections of a 20% ethanol solution three times at 3-h intervals, another group was administered a 40% solution intragastrically, and in group 3 on the same day the abdominal cavity was opened under ether anestesia in the first or second half of the day and 3 μl of 96% ethanol were injected intraamniotically into each fetal sac. The total dose was 4.8 g/kg in group 1, 9.7 g/kg in group 2, and 52 mmol in group 3 females. On the 20th day of pregnancy the animals were killed by cervical dislocation, the uterus was opened and the number of corpora lutea in the ovaries and number of dead, live, and abnormal fetuses counted. The significance of differences was estimated using the Wilcoxson-Mann-Whitney test.

RESULTS

No embryolytic effect was induced by intraamniotic injection of ethanol at 11:00-12:00 h of day 14 (stage XVI [1]) (Table 1). A similar exposure at 16:00-17:00 h on the same day (stage XVI-XVII) resulted, in contrast, in a sharp increase of embryonal mortality and in a marked teratogenic ef-

TABLE 1. Embryotoxic Effect of Ethanol (52 mmol) for Intraamniotic Injection on the 14th Day of Pregnancy ($M \pm m$)

Agent	Time of day, h	Number of fetuses in experiment	Number of fetuses on day 20					Mean fetal mass, g
			dead		*n*	live		
			n	%		*n*	%	
Ethanol	11:00–12:00	32	4	12.5±5.6	28	0	0	2.13
Ethanol	16:00–17:00	85	47	55.3±5.4	25	13	34.2±7.7	2.06
Nornal saline	16:00–17:00	76	8	10.5±3.5	68	0	0	2.24

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TABLE 2. Embryotoxic Effect of Ethanol for Para and Enteral Administration on the 14th Day of Pregnancy ($M \pm m$)

Agent	Total dose, g/kg	Hours of exposure	Route of Administration	Number of Pregnant females	Number of corpora lutea	Number of embryos implanted	Number of fetuses on day 20					Mean fetal mass, g
							dead		live			
							n	%	total	abnormal		
										n	%	
20% ethanol	4.8	10, 13, 10:00	i.p.	12	147	142	24	16.9±3.1	118	0	0	2.09
20% ethanol	4.8	15, 18, 21:00	i.p.	18	214	204	23	11.3±2.2	181	3	1.7±0.9	2.26
40% ethanol	9.7	15, 18, 21:00	i.g.	17	195	190	23	12.1±2.4	167	0	0	2.25
Normal saline	6.0 ml	15, 18, 21:00	i.p.	29	345	326	12	3.7±1.0	314	0	0	2.27

Note. i.p. – intraperitoneal, i.g. – intragastric administration.

fect. Intraamniotic injection of isotonic NaCl solution at the same stage of embryogenesis led to the death of approximately 11% of the embryos but no malformations, whereas ethanol induced the death of 55% of the embryos ($p<0.05$) and makformations were found in 13 out of 38 fetuses still alive at the moment of examination (34%). The detected malformations involved mainly the front and hind paws with the left side predominantly affected: phocomelia, adactyly, syndactyly, absence of distal phalanges.

The embryolethal effect showed virtually no increase after intragastric administration of ethanol at 15:00, 18:00, and 21:00 h on day 14 (stage XVII) in a total dose of 9.7 g/kg (being 11% vs. 8.7% in controls), and no malformations were found in the fetuses (Table 2). Intraperitoneal injection of ethanol in a total dose of 4.8 g/kg at 10:00, 13:00, and 16:00 h did not induce a teratogenic effect either; however, embryonal mortality in this group was significantly higher than in the controls (17%, $p<0.05$). Similar ethanol injections in the second half of day 14 increased embryonal mortality vis-a-vis control, and malformations were found in three out of 181 fetuses surviving till the moment of examination. The detected abnormalities were left-side phocomelia of the front and hind paws, adactyly, and cleft palate.

Hence, ethanol induced developmental abnormalities in rat embryos on the 14th day of pregnancy only when administrated in the second half of the day. A marked teratogenic effect was observed after intraamniotic and, in some embryos, after intraperitoneal injection, that is, rat embryo sensitivity to ethanol was strictly stage-specific. The increased sensitivity of the paw blastema to the teratogenic effect of ethanol in the second half of day 14 seems to be expected by the fact that this stage represents a critical period of paw development or by the increased activity at this time of embryonal alcohol dehydogenases, which metabolize ethanol into a more toxic secondary product, acetaldehyde, producing a potent teratogenic effect on rat embryos [2].

Our finding permit the conclusion that ethanol administrated via certain routes and at specific stages of embryogenesis may induce malformations even in a sspecies biologically resistant to such an effect. It is thus possible that even a single alcohol intake by a pregnant woman at certain times during pregnancy in hte presence of specific shifts in her homeostasis (placental dysfunction, impaired ethanol elimination processes, etc.) may result in the birth of infants with fetal alcohol syndrome or with signs of this symdrome.

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