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Report

Kinetics of Drug Action in Disease States. XXII. Effects of Contraceptive Steroids on the Pharmacodynamics of Ethanol in Rats

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This investigation was designed to determine the effect of treatment with contraceptive steroids on the central nervous system depressant activity of ethanol. Adult female rats received oral doses of ethynyl estradiol (0.1 mg kg^{-1} day^{-1}), ethynyl estradiol and norethindrone (0.1 and 10 mg kg^{-1} day^{-1}), or vehicle only for 14 days. Ethanol was then infused slowly iv until the animals lost their righting reflex. The concentrations of ethanol at that time in serum and cerebrospinal fluid were statistically significantly higher in rats treated with the estrogen–progestin combination than in control animals. Ethanol concentrations in rats treated only with the estrogen were intermediate and did not differ significantly from control values. These results indicate that treatment with an estrogen–progestin combination is associated with a decreased sensitivity of the central nervous system to the hypnotic activity of ethanol. This evidence of a pharmacodynamic interaction between contraceptive steroids and ethanol in rats is consistent with a recent clinical report of significant contraceptive steroid-related improvement in tolerance to ethanol with no apparent effect on the pharmacokinetics of ethanol.

KEY WORDS: ethanol; oral contraceptive steroids; ethynyl estradiol; norethindrone; drug interaction.

INTRODUCTION

Oral contraceptive steroids are known to alter the pharmacokinetics of many drugs. For example, they impair the oxidative biotransformation of antipyrine (1), enhance the conjugation of acetaminophen with glucuronic acid (2), and reduce the clearance of theophylline in women (3). Less is known about the effect of contraceptive steroids on the pharmacodynamics (concentration–effect relationship) of drugs. Several groups of investigators have found that estrogens and progestins have no apparent effect on the sleeping time of mice or rats after the administration of barbital, a hypnotic drug that is not eliminated by biotransformation (4,5). On the other hand, prolonged estrogen treatment induces changes in opiate, benzodiazepine, and β-adrenergic binding sites in the hypothalamus of female rats (6). Estrogens also affect serotonergic and dopaminergic receptor systems in female rats (7,8).

Women taking oral contraceptives are apparently more sensitive to the psychomotor effects of certain benzodiazepines; differences in pharmacokinetics do not explain this interaction (9). A recent study has shown that women taking various oral contraceptives are more tolerant to ethanol, as reflected by the degree of impairment of motor function, despite a lack of effect on the pharmacokinetics of the alcohol (10). The investigation reported here was instituted to determine and compare the effects of an estrogen and an estrogen–progestin combination on the central nervous system depressant activity of ethanol in rats. The study served also to evaluate further the suitability of our previously developed methodology of pharmacodynamic testing in animals (11,12) for assessing variables that are of clinical interest and potential significance.

MATERIALS AND METHODS

Female Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.), weighing 280–300 g and being 15 weeks old upon arrival, were maintained for 7 days on Charles River Rat-Mouse-Hamster Formula and water in university animal facilities. They were then divided randomly into three groups.

Pretreatment of Animals

Pretreatment was for 14 days by once-daily gavage in the morning between 9 and 11 AM. One group of rats received ethynyl estradiol (Sigma Chemical Co., St. Louis, Mo.), 0.1 mg kg^{-1} day^{-1}, in a 50% polyethylene glycol 400–normal saline solution (2 ml kg^{-1} day^{-1}). Another group received the same ethynyl estradiol solution but also containing norethindrone (Sigma), 10 mg kg^{-1} day^{-1}. A third

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group of rats served as controls and received only the vehicle.

Pharmacodynamic Study

The rats had an indwelling cannula implanted in the right jugular vein under light ether anesthesia on the 14th pretreatment day. Food, but not water, was then withdrawn overnight. The next morning, the animals were placed in individual plastic metabolic cages, their rectal temperature was determined, and a small blood sample was withdrawn via the cannula for determination of hematocrit and for checking possible interference with the ethanol assay. An ethanol solution (36.5%, v/v, in normal saline) was then infused iv at a rate of 29.7 mg/min (~100 mg kg^{-1} min^{-1}) while the rats were on a heating pad to maintain the body temperature. The infusion was stopped when the rats lost their righting reflex (determined without the stimulus of tail pinch, used in some of our other studies) and samples of cerebrospinal fluid (CSF) and blood (for serum) were obtained immediately. The onset of loss of the righting reflex was defined as the inability of the rat to right itself within 6 sec after being placed on its back. The blood sample at the onset of action was taken from the abdominal artery. More detailed descriptions of the procedures have been published previously (11–13). The liver was removed and weighed.

Drug and Biochemical Analyses

Ethanol concentrations were determined enzymatically (12), with a commercial kit (No. 332-UV, Sigma). Various biochemical assays were performed by standard procedures, as previously described (14).

Statistical Analysis

The experimental results were examined by one-way analysis of variance, and when differences between groups were noted, the means were compared by the Newman–Keuls test (15). Bartlett's test was used to assess the homogeneity of variance and a nonparametric analysis of variance (Kruskal–Wallis test) was applied in the one case (body weight) where variances were not homogeneous between groups.

RESULTS

The rats used in this investigation are described in Table I. The three groups had similar body weights before the start of the pretreatment period. On the day of the pharmacodynamic study, control animals weighed about 4% less than before pretreatment, probably due largely to the overnight fast. The average weight loss in the two groups of treated rats was considerably more pronounced, about 10% ($P < 0.05$ by Tukey test). Rectal temperature was normal except for one animal in the ethynyl estradiol group, which had a temperature of 40.6°C but otherwise yielded results close to the average of the group. Serum urea nitrogen was slightly higher in the estrogen–progestin-pretreated group than in the other two groups but all values were within normal limits. Other biochemical indices were normal and did not differ significantly between groups. The relative liver weight was significantly higher in the estrogen-pretreated rats than in controls and significantly higher in estrogen and progestin-pretreated animals than in either estrogen-pretreated rats or controls. All animals appeared to be in good health. No animals died during the study and none were eliminated from the study for any reason.

The results of the pharmacodynamic study are summarized in Table II. Ethanol concentrations in serum and CSF of estrogen and progestin-pretreated rats at the onset of loss of the righting reflex were slightly but statistically significantly higher than in the control animals. The ethanol concentrations in the estrogen-pretreated group were interme-

Table I. Description of Female Sprague–Dawley Rats Used in the Study of the Effect of Contraceptive Steroids Treatment on the Hypnotic Response to Ethanol[a]

Variable	Controls	E	E + N	Analysis-of-variance *P* value
No. of animals	9	9	10	—
Body weight, g				
14 days before the study	313 ± 21	320 ± 8	312 ± 15	NS[b]
On the study day[c]	299 ± 19	288 ± 8	279 ± 27	NS
Rectal temperature, °C	38.3 ± 0.4	38.6 ± 0.8	38.4 ± 0.7	NS
Hematocrit, %	41 ± 3	42 ± 2	42 ± 3	NS
Serum urea nitrogen, mg/100 ml	10 ± 3	11 ± 3	14 ± 3*	<0.05
Serum alanine aminotransferase, IU/liter	9 ± 3	11 ± 4	11 ± 6	NS
Serum total protein, g/100 ml	7.3 ± 0.4	7.4 ± 0.5	7.0 ± 0.6	NS
CSF total protein, mg/100 ml	11 ± 4	11 ± 5	8 ± 2	NS
Liver weight, g/100 g body weight	2.92 ± 0.25	3.54 ± 0.19**	3.94 ± 0.39**,***	<0.001

[a] Rats were treated with oral contraceptive steroids for 14 days before the pharmacodynamic experiment. One experimental group received ethynyl estradiol (E), 0.1 mg kg^{-1} day^{-1}. Another group received E, 0.1 mg kg^{-1} day^{-1}, and norethindrone (N), 10 mg kg^{-1} day^{-1}. The control animals received only the comparable volume of vehicle, i.e., 50% polyethylene glycol 400 in normal saline solution. Results are reported as means ± SD.

[b] No significant difference.

[c] After an overnight fast.

* Significantly different from controls and group E, $P < 0.05$.

** Significantly different from controls, $P < 0.001$.

*** Significantly different from group E, $P < 0.01$.

Table II. Effect of Contraceptive Steroid Treatment on Concentrations of Ethanol at the Onset of Loss of the Righting Reflex in Rats Receiving an Infusion of the Drug[a]

Variable	Controls	Treatment		Analysis-of-variance *P* value
		E	E + N	
Infusion time, min	26 ± 2	24 ± 2	26 ± 4	NS[b]
Total dose, g/kg	2.53 ± 0.20	2.48 ± 0.17	2.73 ± 0.29	NS
Serum concentration, mg/ml	3.38 ± 0.34	3.53 ± 0.30	3.84 ± 0.32*	<0.05
CSF concentration, mg/ml	3.84 ± 0.37	3.99 ± 0.32	4.30 ± 0.35*,**	~0.05

[a] The rats received an iv infusion of ethanol, 29.7 mg/min, until they lost their righting reflex. Results are reported as means ± SD. Number of animals as in Table I.

[b] No significant difference.

* Significantly different from controls, $P < 0.05$.

** Significantly different from group E, $P < 0.05$.

diate between the corresponding concentrations in the other two groups. Serum and CSF taken from pretreated animals before ethanol administration and polyethylene glycol 400 itself had no apparent effect on the ethanol assay.

DISCUSSION

The physiologic changes associated with contraceptive steroid administration in this investigation are consistent with previously reported observations. Thus, a reduced rate of gain or a loss of body weight during treatment of rats with an estrogen (16,17), a progestin (5,17), or an estrogen–progestin combination (18) have been previously documented. Similarly, an increase in relative liver weight in rats and mice treated with ethynyl estradiol (16,19–22) and in rats treated with medroxyprogesterone (23) has been reported.

The ability of ethynyl estradiol to cause intrahepatic cholestasis in rats (21), mice (19,22), and humans (24) is well established but this is usually associated with elevated serum transaminase and alkaline phosphatase activities (19). Elevated activities of transaminases and alkaline phosphatase in serum occur also in rats with ligated bile duct, i.e., extrahepatic cholestasis (25). Interestingly, an earlier study in this laboratory showed that ethanol concentrations at the onset of loss of the righting reflex were slightly but statistically significantly *lower* in rats with this type of *extra*hepatic cholestasis than in normal controls (25). Considering the lack of elevated transaminase activity in the present study, it is likely that the hepatomegaly in the contraceptive steroid-treated rats was due to increased water (and perhaps protein and "lipid materials") accumulation in the liver (19,22,23) rather than being a concomitant of cholestasis.

In view of the rank-order correlation of average relative liver weight and average ethanol concentration at the pharmacologic end point for the three experimental groups, the correlation between these values for all 28 individual rats used in this investigation was determined. A weak ($r = 0.331$) but statistically significant ($P < 0.05$) positive correlation was found but this does not necessarily imply a cause–effect relationship.

The body-weight loss experienced by rats treated with contraceptive steroids is dose dependent (18). It is apparently due to a decreased appetite for food; water consumption remains normal and the animals appear healthy (18). Studies performed in our laboratory have shown that total food (but not water) deprivation for 3 days appreciably increases the concentration of phenobarbital in the cerebrospinal fluid of rats at the onset of loss of the righting reflex, indicative of a decreased sensitivity of the central nervous system to the hypnotic effect of the barbiturate (26). Starvation had no such effect on the pharmacodynamics of ethanol (26) and it is therefore unlikely that the pharmacodynamic alterations observed in the present investigation are secondary effects referable to decreased food intake. However, the possibility of an interaction between decreased body weight or food intake and steroid administration cannot be excluded.

Pretreatment of the rats with a combination of estrogen and progestin was associated with a relatively small but statistically significant increase in the concentrations of ethanol in serum and cerebrospinal fluid at the onset of loss of the righting reflex, indicating a decrease in the sensitivity of the central nervous system to the depressant effects of ethanol. Due to its very rapid distribution in the body, concentrations of ethanol in both plasma and cerebrospinal fluid reflect biophasic concentrations under the experimental conditions (12). The coefficient-of-variation values of the ethanol concentrations at the pharmacologic end point in the female rats used in this study are comparable to those observed in male rats (26). Thus, differences in the stage of the estrous cycle among the control rats apparently did not contribute appreciably to the variability of the ethanol concentration data. This is consistent with a report that the amnesic effects of ethanol in women are not affected by the menstrual-cycle phase (27).

The lack of a statistically significant effect of ethynyl estradiol alone on the pharmacodynamics of ethanol does not necessarily mean that the effect of the estrogen–progestin combination is due only to the progestin. For one, pretreatment with the estrogen alone yielded intermediate results (although the very small differences in ethanol concentration among the three groups limit definitive comparisons). Further, the progestin norethindrone has some estrogenic activity, possibly due to partial biotransformation into an estrogen (24). The estrogenic effect of the ethynyl estradiol–progestin combination may therefore be greater than that of ethynyl estradiol alone.

The results of this investigation are consistent with and support clinical observations that the use of oral contraceptive steroids enhances functional "tolerance" to ethanol in

healthy women (10). This is an additional example of qualitative agreement between our animal model and clinical findings with respect to the influence of certain physiologic and pathologic variables on the kinetics of drug action (14,28,29). Suzdak *et al.* (30) have recently suggested that many of the behavioral and biochemical actions of ethanol may be mediated by central γ-aminobutyric acid (GABA)–benzodiazepine receptors. Since treatment with estrogen alters benzodiazepine binding to receptors in the hypothalamus of female rats (6), it may be that oral contraceptives change the central nervous system response to ethanol by their effect on the GABA–benzodiazepine receptor–ionophore complex.

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