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Fetal testosterone surge: specific modulations induced in male rats by maternal stress and/or alcohol consumption

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Abstract

Plasma testosterone (T) was measured in control male and female rats on gestational days 16, 17, 18, 19, and 20 and on days 17–20 in males from dams who were fed ethanol and/or were stressed during pregnancy. Circulating T in control males showed an earlier rise, yielding a longer period of prenatal T elevation, than was reported previously (Endocrinology 106 (1980)306). Compared to control males, exposure to alcohol-alone augmented T on days 18 and 19, stress-alone attenuated prenatal T, and the combination of stress and alcohol completely blocked the normal rise in T between days 17 and 18. When these prenatal alterations in T are viewed along with effects these same treatments have on the postparturient T surge (Horm. Behav. 41 (2002) 229), a possible explanatory mechanism emerges for the uniquely different behavioral patterns of sexual behavior differentiation induced in males by prenatal exposure to alcohol, stress, or both factors. Whereas the potential for feminine behavior is retained to the extent that either the prenatal or the neonatal T surge is attenuated, the male potential is more sensitive to reductions in the fetal surge and is maximally disrupted if both the prenatal and the postparturitional T surges are suppressed.

Keywords: Prenatal stress; Sexual differentiation; Fetal alcohol; Androgen; Masculinization; Defeminization

Male offspring of rats stressed or fed ethanol during late pregnancy display atypical sexual behaviors in adulthood. An increased potential for female lordotic behavior, i.e., incomplete defeminization, is found after early exposure to ethanol (Broida et al., 1988, as cited in Ward, 1992; Hård et al., 1984; Ward et al., 1994) and/or to stress (Ward, 1972, 1977; Ward and Reed, 1985; Ward et al., 1994). Effects of these conditions of pregnancy on behavioral masculinization are more variable. The probability that a male will initiate copulation with a receptive female is largely unaffected by prenatal exposure to alcohol alone (Chen and Smith, 1979; Dahlgren et al., 1989; Hård et al., 1984; Ward et al., 1996; Ward et al., 1999) and moderately attenuated by prenatal stress (Dunlap et al., 1978; Ward, 1972, 1977; Ward and Reed, 1985; Ward et al., 1999), but quite severely suppressed when alcohol and stress are combined (Ward et al., 1994, 1996, 1999).

Differentiation of adult sexual behavior potentials in rats is driven by the relative exposure to testosterone (T) or T metabolites incurred during critical stages of fetal and neonatal development (see review by Ward and Ward, 1985). In male rats, T surges markedly during days 18–19 of gestation (Weisz and Ward, 1980; Ward and Weisz, 1984) and again during the first few hours following parturition (Baum et al., 1988; Corbier et al., 1978; Lalau et al., 1990; Slob et al., 1980). Both the prenatal (Hoepfner and Ward, 1988; Huffman and Hendricks, 1981; Nadler, 1969; Rhees et al., 1997) and the neonatal (Corbier et al., 1983; Roffi et al., 1987; Thomas and Gerall, 1969) surges have been linked to the process of sexual behavior differentiation.

We have previously investigated the impact of fetal exposure to stress and/or ethanol on the postparturitional surge in plasma T (Ward et al., 2002). The changes noted in the

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neonatal surge did not provide a complete explanation for the unique pattern of sexual behaviors associated with each treatment. Specifically, while alcohol alone induced as much suppression of the neonatal T surge as did the combination of alcohol plus stress, only the latter group showed a failure in behavioral masculinization. Further, the neonatal surge in stress-alone males did not differ from that in control males. Thus, it appeared that an understanding of the hormonal mechanisms underlying the diverse patterns of reproductive behaviors shown by male rats prenatally exposed to ethanol and/or to stress would require the entire perinatal T pattern to be characterized. We hypothesized that similar variations in T shared by all treatments could constitute an explanation for the increase in lordosis, while variations in T unique to individual treatments might account for the varying effects these same treatments have on ejaculatory potential. If the etiology of the abnormal sexual behaviors resulting from these treatments is to be linked to androgenic mechanisms, then the fetal, as well as the neonatal, T surge must be described. Such data also might provide insight into the temporal pattern in perinatal T that underlies normal formation of male and female sexual behavioral potentials in the rat. Finally, this study provided an opportunity to revisit the question of the timing of the prenatal T surge in control males. A limited number of control samples were gathered 1 day earlier (day 16 of gestation) than in our previous work (Weisz and Ward, 1980).

Methods

Subjects

Sprague–Dawley rats (Colony 205; Harlan Sprague–Dawley, Inc., Madison, WI) were allowed to adapt for 3 weeks in a temperature (21°C) and humidity (50 ± 10% RH) regulated vivarium, maintained on a reversed light cycle (lights on 2000–800 h). Ad libitum access to water and Purina Lab Chow (No. 5012) was given, except when animals were maintained on a liquid diet. The study was approved by the Villanova University Institutional Animal Care and Use Committee and adhered to standards on animal care and treatment specified by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHEW Publication No. 80-23).

Prenatal treatments

Estrous rats were placed with males between 1200 and 1430 h until two ejaculatory sequences were observed (day 0 of pregnancy). Impregnated animals were singly housed in wire-mesh cages (24.5 × 17.5 × 17.5 cm). Each was randomly assigned to one of the gestational treatment groups (ad libitum control, yoked control, stress, alcohol, stress plus alcohol) detailed below that then were divided into subgroups killed on days 17, 18, 19, or 20 of gestation. In addition, four ad libitum control litters were sacrificed on day 16 of gestation.

Liquid diets

Solid food and water were removed at 1230 h on day 10 of pregnancy and replaced with a liquid diet presented in a drinking tube (Bio-Serv, Inc., Frenchtown, NJ; No. 9007). Two of the groups were given ad libitum access to a diet in which 36% of the calories were derived from ethanol (Diet No. 1265SP; Bio-Serv). These groups were housed in a separate vivarium to prevent contamination of the other treatment groups with alcohol fumes. Three other groups were given a control diet in which the alcohol was replaced by an isocaloric amount of maltose–dextrin (Diet No. 1264SP; Bio-Serv). The drinking tubes were filled and weighed at the same time every day. The tubes were reweighed 24 h later, allowing daily intake to be measured.

Stress

Beginning on day 14 of pregnancy, half of the dams placed on the alcohol diet and half of those given ad libitum access to the control diet were stressed for 45 min daily at 930, 1330, and 1730 h. Each dam was placed into a 13 × 5 × 8.3 cm Plexiglas restrainer (A. H. Thomas; No. 1123-C30) positioned under two 150-W floodlights delivering about 2150 $1m/m^2$. Animals that were not being stressed, i.e., control and alcohol-alone groups, were not handled.

Control groups

One group of dams was given ad libitum access to control diet. Rats dislike the taste of alcohol and thus consume fewer calories than those offered the control diet (Ward, et al., 1994, 1996). To control for possible consequences on fetal development of reduced caloric intake by mothers fed the alcohol diet, a yoked control group was included in the design. Amounts of control diet given these dams were restricted to the number of calories spontaneously consumed by randomly chosen females in the alcohol and the combination alcohol and stress groups.

Sampling procedure

Beginning at 1500 h on days 17, 18, 19, and 20 of pregnancy, dams from each treatment group were taken individually from the vivarium, stunned by a sharp blow to the head, and decapitated using a guillotine. Animals in the stress groups were killed 75–135 min after the second stress session of the day (1530–1630 h). The uteri were excised within 30 s and placed on crushed ice. Fetuses were removed individually, the jugular veins and carotid arteries were cut, and the blood was collected in heparinized capillary tubes. The gonads of 16-, 17-, and 18-day-old fetuses were visualized under a dissecting microscope following blood collection. On days 19 and 20, fetal sex was clearly discernible from the anogenital distance. The capillary tubes were centrifuged (IEC MB Micro Hematocrit Centrifuge) at 11,500 RPM for 5 min. Pooled plasma samples of at least 60

μl each were harvested from the males in each litter. In addition, samples were gathered from female fetuses in the yoked control group. When possible, two 60-μl male samples were obtained from a single litter. With one exception, each sample contained plasma from at least two fetuses. No litter contributed blood toward more than two same-sex samples. Plasma samples were stored at −60°C until assay.

In order to obtain 18–23 usable plasma samples per treatment group on each of the 4 days of gestation, 254 litters were harvested. The number of litters per treatment used on each day of gestation was as follows: 16–18 on day 17; 11–14 on day 18; 10–13 on day 19; 10–11 on day 20. The larger number of litters was required on day 17 due to the smaller volume of plasma extracted from the younger fetuses. Each of the four day-16 ad libitum control litters yielded only a single sample for each sex.

Assay procedure

Plasma testosterone was measured in duplicate (25 μl each) in all 500 usable samples using radioimmunoassay. Six samples with a high coefficient of variation and 6 samples not run in duplicate were eliminated from the data analysis. A T antibody (ICN Diagnostics, Costa Mesa, CA) with a low cross-reactivity with other androgens (5α-dihydrotestosterone, 7.8%; 11-oxotestosterone, 2.0%; all other steroids <1.0%) was used. Although chromatographic separation was not performed, the data are referred to as "plasma T," because of the high specificity of the antibody for T. Serial dilutions of plasma samples from fetal male rats yielded displacement curves that were parallel to the displacement curve produced by the T standards. Samples that yielded T values below the standard curve were assigned a value of 0.20 ng/ml, the lower limit of sensitivity of the assay. All male samples were within the detectable range. Only 4 of the 84 female samples attained this criterion.

The samples were assessed in six assay runs. Variation among assays was determined by measuring, in duplicate, a sample of pooled fetal rat plasma in each assay run. The interassay coefficient of variation, based on the pool concentrations, was 6.1%. Within-assay variation was determined with reference to the variation between the duplicate pool determinations in each assay. The average intra-assay coefficient of variation for these duplicate pools was 3.8%.

Results

Diet consumption

The mean daily consumption of liquid diet by the dams killed at various stages of pregnancy can be seen in Fig. 1. The intake of the two groups fed the alcohol diet and that of the yoked control group were virtually identical throughout the treatment period. Dams given ad libitum access to the control diet consumed considerably more calories than

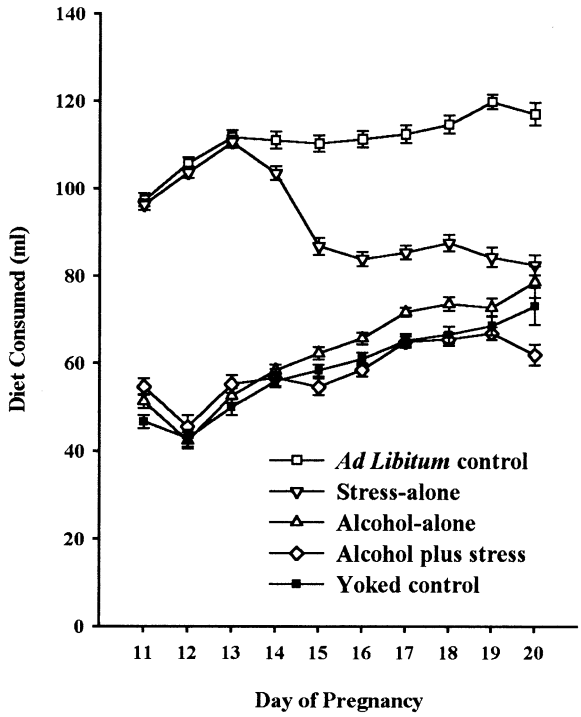

Fig. 1. Mean daily intake (g ± SE) of rats given access to a liquid control diet or a diet containing ethanol beginning on day 10 of gestation. Half of each group was exposed to light/restraint stress beginning on day 14 of pregnancy. Diet made available to animals in one control group was yoked to amounts spontaneously consumed by dams in the alcohol groups. The second control group had ad libitum access to control diet. Intake was measured in the following number of dams/treatment group from days 11–16 of gestation: ad libitum control = 48; stress alone = 56; alcohol alone = 51; alcohol plus stress = 48; yoked control = 52. Thereafter, the number of dams/treatment group was reduced by the number of litters sampled on each day of gestation.

those maintained on the alcohol diet. However, once the stress treatment began, stressed dams gradually reduced their intake of control diet. A 2 (time periods) by 5 (treatments) mixed design analysis of variance (ANOVA) was used to compare the mean intake on the 3 days preceding the imposition of stress (intake on days 11, 12, and 13) with that during the 3 days following the initiation of stress (days 15, 16, and 17). There were significant effects for treatment ($F(4, 250) = 573.72$, $P < 0.001$), prestress vs stress time period ($F(1, 250) = 88.83$, $P < 0.001$), and the interaction ($F(4, 250) = 103.52$, $P < 0.001$). The consumption of ad libitum control and stress-alone dams did not differ during the prestress period, but was significantly greater than that of the two alcohol-consuming groups and the yoked controls ($P < 0.01$). The latter three groups did not differ from one another. Intake fell significantly during the stress period (18%) in the stressed animals given the control diet, but increased in all other groups, including the stressed animals that were fed an alcohol diet. The intake of stress-alone dams during the 15- 17-today period was significantly below ($P < 0.01$) that of ad libitum controls, but exceeded ($P < 0.01$) that of the other groups.

Plasma T

Plasma T levels obtained in the ad libitum and yoked control male groups are presented in Fig. 2A. For days 17, 18, 19, and 20, these two groups were compared using a 2 (treatments) × 4 (days) ANOVA. There was a significant days effect ($F(3, 157) = 45.2$, $P < 0.001$), but no significant treatment effect or interaction between treatment and days. The difference in amount of diet consumed by dams in the two control groups clearly had no effect on fetal T levels during this period of gestation. Therefore, the data from the *ad libitum* and the yoked intake groups were collapsed on each of the 4 days to yield a single control group against which all other treatments were compared in subsequent data analyses.

Fig. 2A also presents the mean plasma T of the four male ad libitum control samples collected on day 16 of gestation. An ANOVA revealed blood levels of T to be markedly lower ($F(1, 45) = 25.82$, $P < 0.001$) on day 16 than day 17 of fetal development.

Plasma T titers found in fetal male rats exposed to the various treatment conditions and in the yoked control female littermates are shown in Fig. 2B. A 4 treatments × 4 days ANOVA applied to the male data disclosed significant effects for treatments ($F(3, 392) = 18.68$, $P < 0.001$), for days ($F(3, 392) = 77.05$, $P < 0.001$), and for the interaction ($F(9, 392) = 3.05$, $P = 0.002$). Newman–Keuls tests on the main effect of treatments revealed that over these 4 days of gestation, the males exposed only to stress and those exposed to both stress and alcohol had significantly lower T levels than the control group ($P < 0.01$). Paradoxically, T in males exposed to alcohol alone exceeded that in all other treatment groups ($P < 0.001$).

Treatment effects on T levels on each day of gestation were probed using pairwise comparisons, and planned comparisons were used to evaluate T changes across days of gestation within each treatment group. On day 17, T in control males was higher than in alcohol-plus-stress (combination treatment) males ($P = 0.008$) and marginally higher than in stress-alone males ($P = 0.055$). Males exposed only to alcohol had higher T levels than those exposed either to stress alone ($P = 0.011$) or to both alcohol and stress ($P = 0.002$), but did not differ from control males.

Plasma T levels increased significantly between days 17 and 18 in the control ($P = 0.025$), the alcohol-alone ($P = 0.002$), and the stress-alone ($P = 0.04$) groups. However, T in the combination alcohol-plus-stress males did not differ between days 17 and 18. As a consequence, plasma T on day 18 was considerably lower in the combination treatment animals than in the control ($P < 0.001$) or stress-alone ($P = 0.017$) groups. The latter two groups did not differ from one another on day 18, but alcohol-alone males had considerably higher levels of T than any other treatment group ($P < 0.004$).

Between days 18 and 19, T fell significantly in the control ($P = 0.021$) and stress-alone ($P = 0.024$) groups, but not in the alcohol-alone males. In contrast, the combination treatment males now experienced the surge in T ($P =$

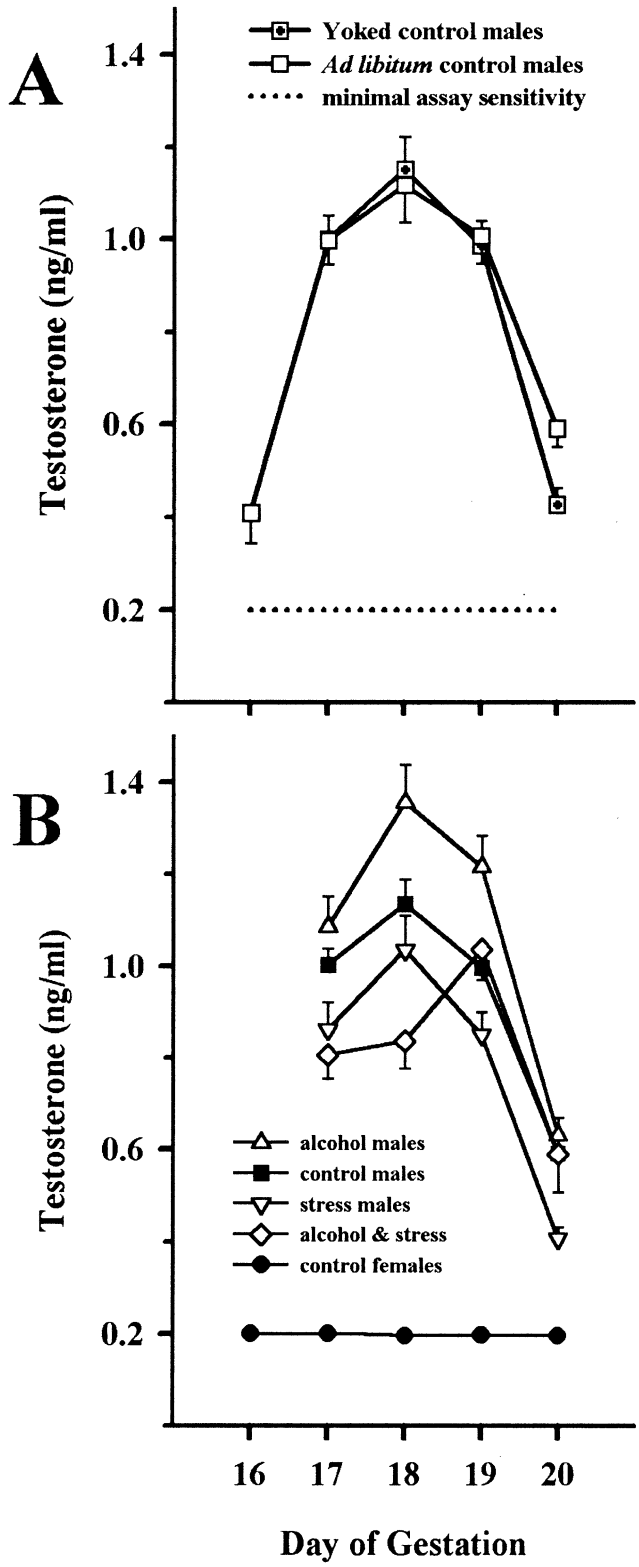

Fig. 2. Mean plasma testosterone levels (ng/ml ± SE) of (A) the male fetuses of rats given ad libitum access to control diet or access yoked to the intake of dams fed alcohol. From 19 to 23 samples were obtained for each control group for each data point on days 17–20, and 4 samples were obtained on day 16 for the ad libitum group. (B) Male fetuses of dams exposed to stress and/or alcohol. Each data point for the alcohol-alone, stress-alone, and alcohol-plus-stress males is the mean of 18–23 samples, and the combined control group had 39–44 samples per data point. Both male and female pups were sampled in the ad libitum control group.

0.018) seen 24 h earlier in the other groups. In fact, T in the combination treatment males had increased to such an extent on day 19 that this group no longer differed from the control animals. T in stress-alone males was significantly lower than in the control group on day 19 ($P = 0.042$). T continued to be higher on day 19 in alcohol-alone males than in any other group ($P < 0.02$).

From days 19 to 20, all treatment groups experienced a sharp decline ($P < 0.001$) in T. On day 20, no group differed significantly from the control males, but T levels were higher in both alcohol groups ($P < 0.05$) than in the stress-alone group.

Plasma T was sufficiently high to reach the level of detectability of the assay in only one of each of the control female samples gathered on day 17, 18, 19, and 20 of gestation and in none of the day-16 female samples. Clearly, plasma T in the females was markedly below that of any of the male groups on each of the days of gestation measured.

Discussion

The fetal pattern of plasma T in the control males was similar to that already described by our laboratory (Weisz and Ward, 1980; Ward and Weisz, 1984). However, the limited data gathered on day 16 of gestation suggest that our previous portrayal needs to be revised and extended. As before, we found a significant rise in plasma T between days 17 and 18 of gestation. A small decline by day 19 and a much sharper drop by day 20 followed the day-18 peak in T. However, it is now clear that control males experience an even earlier surge in fetal T than was identified in our previous work. The rise in T between days 17 and 18 of gestation is preceded by an even more pronounced surge between days 16 and 17. Plasma T rises steadily beginning on day 16, reaching a peak on day 18. The major difference in the prenatal T pattern found by Weisz and Ward (1980) and in the present study occurred on day 17. In the current study, T on day 17 was already at the same relatively high level that still prevailed on day 19. In the previous study, T on day 17 was still at the same low level as was seen later on day 20. Clearly, the prenatal T surge has an earlier onset, i.e., day 16 rather than day 17, and persists longer, i.e., for 3 rather than 2 days, than was previously thought. A nonsignificant upward trend between days 16 and 17 of gestation has been observed previously in the whole body androgen content of male and female rats (Baum et al., 1991).

The three treatment groups deviated from the control males, but each displayed a different atypical pattern. While the pattern of T in males from dams stressed and fed a control diet simulated that of the control group, there were considerable differences in the magnitude of the T titers. Plasma T in the stress-alone group was lower than control levels on day 19 of gestation and marginally lower on day 17, leading to significantly less T exposure during the entire period of the prenatal surge. In a previous study we found stressed males to have abnormally low T on days 18 and 19 of gestation (Ward and Weisz, 1980). The diminished fetal surge in plasma T characteristic of prenatally stressed males seems to be the result of a depression in gonadal steroidogenesis. Exposure to stress results in a trough in the activity of the key steroidogenic enzyme Δ5-3β-hydroxysteroid dehydrogenase (3β-HSD) in the testes of 18 and 19-day-old fetuses (Orth et al., 1983; Pollard and Dyer, 1985; Ward et al., 1990), the days that testicular 3β-HSD activity peaks in control males. As a consequence of lower circulating T, aromatase activity is diminished in hypothalamic–amygdaloid tissue of stressed males on days 18 and 19 of gestation (Weisz et al., 1982). Estrogenic metabolites of T have been implicated in the behavioral masculinization of male rats (see review by Meisel and Sachs, 1994).

Males exposed only to alcohol had T levels on days 18 and 19 of gestation that substantially exceeded control levels. Previous studies on the effects of ethanol on fetal T have yielded equivocal results. Blood levels of T in alcohol-exposed males were reported not to differ from control males on day 18 of gestation (Udani et al., 1985), to be higher than control levels (Dahlgren et al., 1989), and to be lower than in controls on day 19, but not 21, of gestation (Revskoy et al., 1997). One other study found plasma T titers in the male offspring of dams exposed to alcohol during pregnancy to be lower than in controls on days 18 and 19 of gestation (Sinha et al., 1997). However, both the control and the ethanol-fed mothers in the study had been surgically manipulated during pregnancy, i.e., were either adrenalectomized or sham-adrenalectomized. If anesthesia and surgery acted as a stressor then the low T levels found in the alcohol-exposed fetuses could reflect the same phenomenon uncovered in the present study, i.e., when alcohol-exposed fetuses are stressed the fetal T surge is even more severely suppressed than occurs following exposure to stress alone. T levels measured in trunk tissue taken from alcohol-exposed males failed to exhibit the normal upsurge seen from day 17 to days 18 and 19 in control fetuses (McGivern et al., 1988a; McGivern et al., 1998). In our studies of hormonal changes during the perinatal period we have utilized plasma T because T reaches the brain through the circulatory system to influence behavioral masculinization. The temporal relationship between fluctuations in plasma T and trunk T is unclear.

It is a well-established phenomenon that chronic exposure of adult males to ethanol suppresses blood levels of T (Adams et al., 1997; Cicero, 1981; Little et al., 1992). However, alcohol markedly elevates (by 200–300%) plasma T in prepubescent (25 and 30 day old) rats (Little et al., 1992) and adolescent (35 day old) hamsters (Ferris et al., 1998). While the mechanism underlying this elevation has not been fully established, the effect occurs only with high doses and is believed to involve a direct stimulatory effect of alcohol on T biosynthesis in the testes (Little et al., 1992). Thus, the effects of alcohol on circulating T vary profoundly at different life stages. Our data would agree with those of Dahlgren et al. (1989) that alcohol exposure of male fetuses raises plasma T above control levels, just as it does in

prepubertal males. Abnormally high plasma T would also explain why alcohol exposure leads to increased aromatase activity in the hypothalamus of 18- and 19-day-old male fetuses (McGivern et al., 1988b). T increases fetal levels of brain aromatase in a dose-dependent manner (Beyer et al., 1994; Paden and Roselli, 1987).

The pattern of T in males from dams both exposed to stress and fed an alcohol diet differed markedly from that in control males. Plasma T was significantly lower on day 17 of gestation in alcohol-plus-stress animals than in controls. An even more striking alteration was the absence of any surge on day 18, when T levels peaked in all other groups. The delayed surge that did occur on day 19 was immediately followed by the same abrupt decline in T on day 20 that characterized the other groups. Thus, alcohol-plus-stress male fetuses were exposed not only to suppressed levels of T, but also to an abbreviated period of T elevation that temporally did not coincide with the day-18 peak experienced by control males. These data are the first report of the effects of simultaneous exposure of males to a combination of stress and alcohol on the prenatal T pattern.

Several mechanisms may interact to produce the unique plasma T pattern characteristic of combination treatment fetuses, compared to those exposed only to stress or to alcohol alone. We have previously demonstrated that blood alcohol levels (BAL) are markedly lower on days 16–20 of pregnancy in dams stressed and fed alcohol in relation to those not exposed to stress, although the same amounts of ethanol were consumed (Ward et al., 1996). BAL in the alcohol-plus stress dams may have been too low to trigger the mechanism responsible for the stimulatory effect on T biosynthesis in the gonads of alcohol-alone fetuses. However, in adult males, alcohol exposure also is known to increase hepatic aromatase activity, thereby increasing the peripheral conversion of testosterone to estradiol (Chung, 1990; Gordon et al., 1979). Possibly, the BAL in combination treated fetuses was sufficient to trigger this mechanism to some degree. If so, the suppression in T caused by prenatal stress would combine with the alcohol-induced loss in circulating T resulting from increased hepatic aromatization. The overall effect would be to exaggerate the degree to which the prenatal T surge is blocked.

The demonstrated alterations in prenatal plasma T alone are not sufficient to explain the particular variations in sexual behaviors shown by males exposed to stress, to ethanol, or to both factors. However, if the prenatal T pattern is combined with what is already known about the effects these treatments have on the postparturient T surge, a more instructive picture emerges. It is known that, regardless of genetic sex, the substrate controlling the expression of dimorphic behaviors in mammals is fundamentally female. Exposure to various androgenic metabolites at critical stages of perinatal ontogeny is required to suppress the innate potential of the CNS to trigger lordosis behavior in adulthood and, instead, to actively masculinize areas able to promote male-typical copulatory patterns (see review by Ward and Ward, 1985). Normal behavioral defeminization and masculinization in the rat are both probably dependent on exposure of the developing CNS to the bursts in T that occur during late gestation and the first few hours after birth. These surges can differ not only in timing, but also in duration and magnitude. Normally the prenatal T surge lasts up to 3 days. The postparturitional surge continues only for a few hours, but is more than double in magnitude compared to the fetal surge (Lalau et al., 1990; Slob et al., 1980). Experimental manipulation of either the fetal (Hoepfner and Ward, 1988; Huffman and Hendricks, 1981; Nadler, 1969; Rhees et al., 1997) or the neonatal T surge (Corbier et al., 1983; Thomas and Gerall, 1969; Roffi et al., 1987) alters the sexual behavior patterns exhibited in adulthood.

The idiosynchratic perinatal T patterns that occur as a result of fetal exposure to alcohol, to stress, or to both factors provide some interesting insights regarding the optimal hormonal pattern underlying full behavioral masculinization and defeminization of male rats. The female sexual behavior potential appears to be retained in males to the extent that nervous system development occurs while either T surge is attenuated. This would explain why all three treatment groups retained an elevated potential for female behavior (Ward et al., 1994). Despite different patterns of T exposure, each group experienced subnormal T during at least one of the critical perinatal stages of sexual behavior differentiation. Stress alone suppresses only fetal T. Alcohol alone does not suppress fetal T, but does attenuate the postparturitional surge by approximately 50% (Ward et al., 2002). It should be noted that while the degree of behavioral feminization exhibited by all three groups is markedly higher than that shown by control males, it is clearly distinguishable from that of normal females (Ward et al., 1994). Females do not experience a T surge during either the critical fetal or the neonatal stage of perinatal ontogeny (Weisz and Ward, 1980; Ward et al., 2002). The hormone-sensitive period during which behavioral defeminization occurs in the rat has been shown to begin on day 18 of gestation (Rhees et al., 1997) and to extend through the neonatal period (Grady et al., 1965; Diaz et al., 1995). Research on the possible feminization of dimorphic CNS structures in stress- and/or alcohol-exposed males is limited. One study has shown that the volume of the dimorphic anteroventral periventricular nucleus (AVPV) of the rat hypothalamus is increased (feminized) in prenatally stressed males (Rhees et al., 1999).

The processes through which the developing nervous system actively acquires the potential to execute male copulatory behavior overlap but may be different and independent from defeminization. This conclusion is suggested by comparing the differential behavioral and perinatal T patterns characterizing males prenatally exposed to alcohol, to stress, or to both treatments. Copulatory potentials are normal in males prenatally exposed only to ethanol (Ward et al., 1996, 1999). Fetal exposure to ethanol alone leads to subnormal T levels at delivery on day 21 of gestation and severely suppresses (up to 50%) the postparturitional T surge (McGivern et al., 1993; Rudeen and Kappel, 1985;

Ward et al., 2002). However, the current study suggests that on days 18 and 19 of gestation, the CNS of alcohol-alone males develops under levels of T that exceed all other groups, including the control animals. It is possible that the supranormal T environment that alcohol-alone males experience during fetal development sensitizes the developing nervous system to androgenic stimulation to such an extent that even an attenuated neonatal T pulse is sufficient to yield an essentially normal male copulatory potential.

Males exposed to both stress and alcohol stand out for the extraordinarily low incidence of animals that copulate, despite repeated access to estrous females (Ward et al., 1994, 1996, 1999). The timing and magnitude of their perinatal T pattern are also unique. On day 18 fetal T was 26% below control levels and significantly lower than in males exposed to just one of the two treatments. There was a delay of at least 24 h in the onset of the T surge in the alcohol-plus-stress fetuses. Most importantly, the prenatal alterations are coupled with the same diminished (by 40%) surge in postparturient T seen in males exposed to alcohol alone (Ward, et al. 2002). When T is low during both perinatal stages, minimal masculinization of the CNS takes place. While both alcohol-exposed groups experienced the same depression in the T surge associated with birth, what sets the two groups apart is the prenatal T pattern. The data emphasize that certain pharmacological substances (e.g., alcohol) experienced during pregnancy in combination with certain environmental conditions (e.g., stress) can lead to relatively limited reductions in the plasma T of the male fetuses and neonates. These changes are sufficient to prevent full sexual behavior masculinization, but leave adequate amounts of T to ensure normal virilization of gross anatomical structures such as the penis, testicular size, epididymis, and anogenital distance (Ward et al., 1994).

Prenatal stress alone does not alter the magnitude or timing of the postparturitional surge in plasma T from that of controls (Ward et al., 2002). The perinatal T pattern of males exposed only to stress is distinguishable from the two alcohol-exposed groups in that only the fetal, but not the postparturitional, T surge is suppressed. Males exposed to stress alone show a moderately suppressed ejaculatory potential (e.g., Dunlap et al., 1978; Rhees, et al., 1999; Ward, 1972, 1977). In this case the initial process of masculinizing the CNS may have been minimized by a low prenatal T surge, but incomplete masculinization was not extended neonatally because the postparturitional T surge was normal. Thus, development of the male copulatory potential fell intermediate to that in the other two groups.

In summary, while attenuation of either the fetal or the neonatal T surge disrupts defeminization of male rats, behavioral masculinization seems to be particularly sensitive to reductions in the prenatal surge. Males exposed to alcohol alone have deficits only in the postparturitional surge and show a normal male copulatory potential. Males exposed to stress alone experienced a reduction in T only during fetal development, which led to a moderately attenuated potential for male copulatory behavior. However, when a prenatal reduction in T is combined with a suppression of the neonatal T surge, as occurred in the combination stress-plus-alcohol males, a particularly severe failure in behavioral masculinization results. Thus, normal male sexual behavior potentials probably require that the various developmental events that underlie sexual differentiation of the CNS be tightly synchronized with the normal onset of repeated spikes in plasma T that occur at several critical perinatal stages. These hormonal bursts must be sufficient in volume and duration to sustain neuronal masculinization. Desynchronization or changes in the intensity of T stimulation would be reflected in abnormal differentiation of the CNS and its ability to activate male-typical sexual patterns when stimulated by gonadal hormones in adulthood.

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