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Endocrine Control of Ethanol Intake by Rats or Hamsters: Relative Contributions of the Ovaries, Adrenals and Steroids

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MORIN, L. P. AND N. G. FORGER. *Endocrine control of ethanol intake by rats or hamsters: Relative contributions of the ovaries, adrenals and steroids.* PHARMAC. BIOCHEM. BEHAV. **17**(3) 529–537, 1982.—Alcohol intake and preference by female rats decreases during pregnancy or following ovariectomy (OVX) in accordance with a model derived from the saccharine preference literature. However, the present Experiment 1 revealed that ovariectomized rats only modestly increased 4% ethanol preference following subcutaneous implantation of Silastic implants containing estradiol. In Experiment 2, OVX female hamsters actually had greater alcohol preference compared to controls. This information led to the suggestion that ovarian estradiol in rats might be effecting alcohol intake by influencing adrenal corticosteroid release, something that does not occur in female hamsters. Adrenal glucocorticoids are known to profoundly alter sensitivity to taste, olfactory and auditory stimuli. Therefore, groups of rats were sham operated, OVX, adrenalectomized (ADX) or both OVX and ADX to test for the relative endocrine effects on alcohol preference. The rank ordering of preference deficits produced was SH < OVX < ADX < OV-ADX. Reductions in alcohol preference relative to the SH group were about 34%, 58% and 75% for the OVX, ADX, and OV-ADX groups, respectively. Daily corticosterone injections increased alcohol preference of the ADX and OVX groups, but not of the OV-ADX animals. The results support the hypothesis of adrenal-ovarian interactive control of ethanol intake, but provide no satisfactory explanation for the diminished alcohol preference during pregnancy.

Rat Hamster Ethanol Estradiol Adrenal Ovary Corticosterone

WADE and Zucker [29, 30, 31] developed a model for rats in which the ovary significantly modulated saccharine preference. Data from our initial experiment [7] demonstrated, for the most part, that female rats treat 4% ethanol in much the same manner as saccharine. As predicted from the saccharine preference data, (1) ovariectomized rats drank, and preferred, a 4% ethanol concentration less than did intact rats and (2) pregnant rats markedly diminished ethanol preference. Moreover, individual ovariectomized female rats show a significant positive correlation between saccharine preference and 4% ethanol preference [7]. During the estrous cycle, however, absolute ethanol intake and preference for ethanol were both minimal during the estrous cycle day associated with greatest estradiol availability [7]. Thus, ovary removal and maximal estradiol were both correlated with diminished ethanol preference, depending upon the circumstances. According to the saccharine preference model, elevated exogenously provided estradiol leads to increased preference; unfortunately, estrous cycle data were complicated by experience with saccharine [30,31].

The following series of experiments was designed to further evaluate the hypothesis that gonadal estradiol is responsible for the enhanced ethanol intake seen in intact compared to ovariectomized animals. Toward this end, we conducted a hormone replacement study in which exogenous estradiol was continuously provided to ovariectomized rats. The results were equivocal and forced a reassessment of the ovary-based saccharine preference model as an appropriate explanation for endocrine control of ethanol ingestion. Many of our findings might be caused by adrenal steroid activity, possibly acting in conjunction with ovarian hormones. With respect to this view, the reports from women with diminished alcohol intake during pregnancy were considered significant. They suggest that cognitive factors pertinent to taste and taste memory (e.g., alcohol was "repugnant" in early pregnancy) contribute strongly to the ingestion of alcoholic beverages [15,16]. Adrenal glucocorticoids have been widely implicated as mediators of perceptual sensitivity (audition, [5,11]; taste, [19, 22, 24]; olfaction, [11,23]). In fact, adrenalectomy greatly reduces rat saccharine preference while a glucocorticoid restores it [25]. Generally, when adrenal corticoids are minimally available, sensitivity is substantially greater (up to 10,000 times; [20]). Furthermore, in female rats, estradiol greatly facilitates adrenal corticosterone synthesis and release [13]. The results that we present here demonstrate major adrenal involvement as a regulator of female rat ethanol intake and preference.

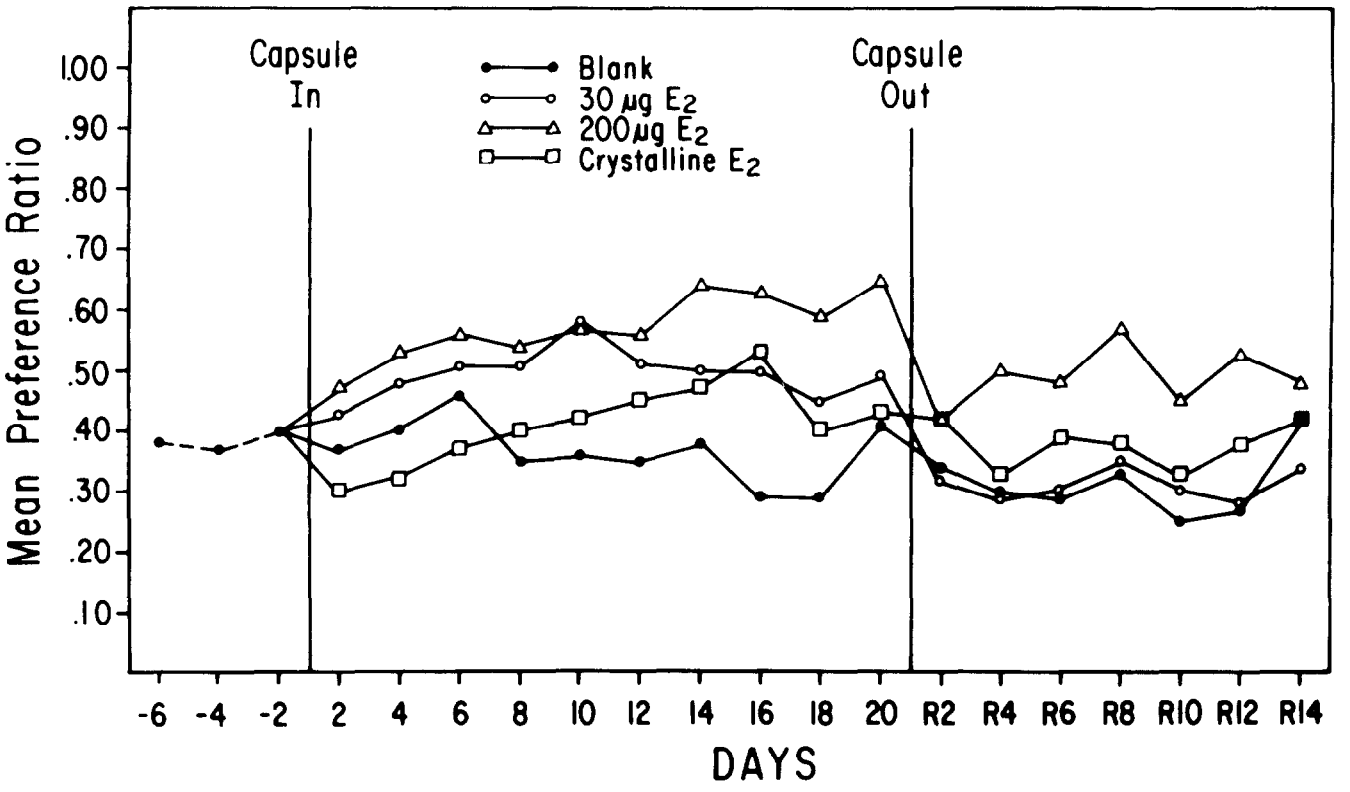

FIG. 1. Effect of various estradiol treatments on APR of ovariectomized rats. Capsules that were blank (BK) or contained 30 μg/ml, 200 μg/ml or crystalline estradiol were implanted subcutaneously on Day 1 and removed on Day 21.

GENERAL METHOD

Female Sprague-Dawley rats weighing about 235–350 g or golden hamsters (about 95–105 g) were individually housed in a 14 light:10 dark photoperiod (lights off at 2000) and provided with ad lib Charles River rat, mouse and hamster formula and tap water. Both species were purchased (except as in Experiment 3) from Charles River-Lakeview. Estrous smears were obtained prior to surgery for all hamsters. Appearance of the sticky, postovulatory vaginal discharge indicated estrous cycle Day 1. Each hamster showed at least two consecutive estrous cycles before inclusion in the experiment.

Unless specifically state otherwise, the statistical probabilities reported here in this and subsequent experiments are 2-tailed and were derived from analyses of variance with repeated measures and t-tests. In general, $p<0.05$ was accepted as statistically significant.

EXPERIMENT 1

Zucker [31] proposed that saccharine preference and quinine aversion were regulated by ovarian hormones with progesterone (P) able to facilitate or inhibit the action of estradiol benzoate (EB). Preliminary work utilizing EB, P or EB + P given as daily injections (using the doses and procedure of Wade and Zucker [29,31]) failed to provide any useful indication that the hormones affected ethanol intake. Inasmuch as estradiol in large doses can mimic many of the combined effects of EB and P [3] we used chronic estrogen-containing implants to minimize animal handling, provide a more stable level of hormone and obtain a dose-response to estradiol.

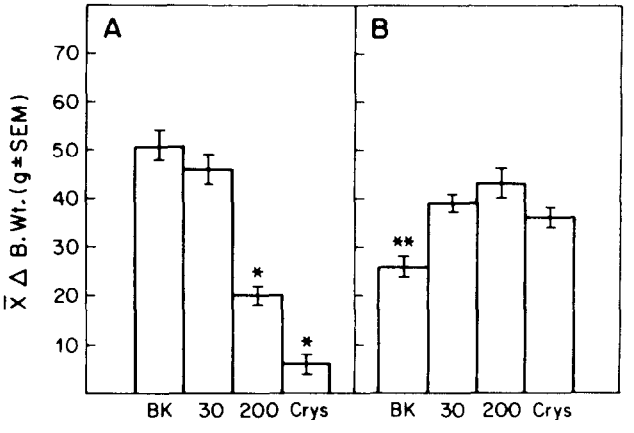

FIG. 2. Mean change in body weights of ovariectomized rats while (A) implanted for 3 wks with a blank (BK), 30 μg/ml, 200 μg/ml or crystalline estradiol-containing Silastic capsule or (B) during 2 weeks after capsule removal. *=$p<0.007$ vs each other group; **=$p<0.005$ vs each other group.

METHOD

Female rats adapted to the conditions for at least three weeks, then were bilaterally ovariectomized under Nembutal (sodium pentobarbital; Abbot, 50 mg/kg). One week after surgery each animal had its water bottle and feed holder removed. Food was then available inside each animal's cage and two Richter drinking tubes were attached to the cage's front. The level of fluid in each tube was recorded at 1000 hr daily during the experiment. During Phase I, both tubes (one of which was designated the "Marked" tube) contained tapwater. During Phases II and III, one tube contained tapwater and the Marked tube contained a 4% (v/v) mixture of ethanol and tapwater. An alcohol preference ration (APR) was calculated by the formula: APR=ethanol fluid consumed/total fluid consumed. Fluid was replaced as required. Tubes from six different animals were progressively rinsed every second day and right or left location of the Marked tube varied according to a random number table.

After seven days in Phase I, each animal was anesthetized with ether and given a subcutaneous Silastic (Dow-Corning dimethysiloxane tubing, 1.56 mm i.d. × 3.15 mm o.d.) capsule implant. Four groups were treated as follows: (1) blank capsules (N=10) which were empty; (2) capsules which contained 30 μg 17-β-estradiol (E_2; N=11) per ml sesame oil; (3) capsules which contained 200 μg E_2/ml oil (N=11); and capsules stuffed with crystalline E_2 (N=10). All hormone capsules were sealed by tying nylon monofilament line around each end. Length was made proportional to each individual's body weight (0.1 mm/g) taken two days before implantation. This method of capsule construction results in serum E_2 levels of about 12, 50, and 115 pg/ml for the 30 μg, 200 μg and crystalline E_2 groups, respectively. The 200 μg groups most closely resembles the proestrus physiological level of 40–70 pg/ml (J. Moreines and J. B. Powers, in preparation). The capsule was removed from each animal, under ether anesthesia, 21 days after implantation and this condition was considered Phase III.

RESULTS

Blank animals demonstrated an approximately 35% preference for 4% ethanol and this did not change after capsule removal (Fig. 1). This group did not differ from any group receiving E_2 according to a repeated measures analysis of variance for ethanol intake or APR during Phase II. Both the 200 μg and crystalline E_2 groups showed significant increases in APR ($p<0.08$ and 0.002, respectively) and ethanol consumption ($p<0.007$ and 0.001, respectively) across days in Phase II. Following implant removal, there was a general decline to lower ethanol preference and consumption. This change approached statistical significance only for the APR of the crystalline E_2 ($p<0.065$).

The capsules had a parametric effect on body weight (Fig. 2; cf., [28]). The 200 μg and crystalline E_2 implanted animals gained less weight (mean increase=10±2 and 6±2 g, respectively; $p<0.001$) when the hormone was available than did the 30 μg or Blank implanted animals (mean increase=46±3 and 51±3 g, respectively; not significantly different). The weight gain shown by each of the latter two groups differed from each of the former groups ($p<0.007$ in each case). When the capsules were removed, the Blank group gained the least weight (26±2 g) and this increase differed from the increases shown by the crystalline, 200 μg or 30 μg groups (mean increases=36±2, 43±2 and 39±29, respectively; Blank vs each other group, $p<0.005$ in each case). The weight increases of the three hormone groups did not significantly differ.

DISCUSSION

At best, normal to large doses of E_2 were only able to induce a gradual increase in APR across a three week period. The results do not offer strong support for the hypothesis that estradiol is the primary steroid facilitating ethanol consumption. Nevertheless, consumption did increase at a time when a caloric regulation hypothesis [21,31] would predict a decrease. Estradiol is well-known as an inhibitor of caloric consumption and body weight during the estrous cycle and during exogenous replacement in ovariectomized animals [28]. That the two highest E_2 concentrations did have a suppressing effect on body weight is evident in Fig. 2.

EXPERIMENT 2

The results of Experiment 1 were not particularly robust, but in part because of this, they provided the basis for an alternative to the saccharine intake model as an explanation for hormonal modulation of alcohol consumption. During the estrous cycle and after castration or hormone replacement, the effects of E_2 on food intake and body weight are large and rapid. (In this sense, the estrous cycling rat regulated ethanol as if it is a source of calories by suppressing APR during proestrus, the day of maximal estradiol [7]). In Experiment I, the changes in ethanol consumption were relatively slow to develop under the influence of E_2. It was conceivable that the observed effects were secondary to the actions of adrenal corticosterone which was released under the influence of the exogenous E_2 [13]. This ovarian-adrenal relationship, so evident in the female rat, does not exist in the female hamster [8]. Therefore, this species was utilized for comparison of its ethanol intake patterns during the estrous cycle and after ovariectomy with those of the rat [7].

METHOD

Female hamsters were used and daily examination of the vaginal discharge continued following a sham operation (SH; N=9) or ovariectomy (OVX; N=12) under Nembutal (96mg/kg) anesthesia. Food (Charles River mouse and hamster formula) and water were always available. Two Richter tubes were used to deliver and measure tapwater or 10% (v/v) ethanol in tapwater. This has been shown to be the maximally preferred concentration for female hamsters [14]. The ethanol tube was considered the Marked tube and had a small piece of tape at its apex.

Phase I began about 6 days after surgery with both 10% ethanol and tapwater available in separate tubes; left or right tube location was randomly varied. During Phase II, the Marked tube was always located on the animal's left side of the cage front. During Phase III, the Marked tube remained to the animal's left, but both tubes contained only tapwater.

RESULTS

Ovariectomy

During Phase I, hamsters showed a remarkable position preference. In 150/167 (90%) of animal-days across the combined OVX and SH groups, there was more drinking from the tube at the animal's left regardless of the tube contents. Two animals accounted for 9 of the 17 exceptions; no other animal had more than two exceptions to the "left preference" rule.

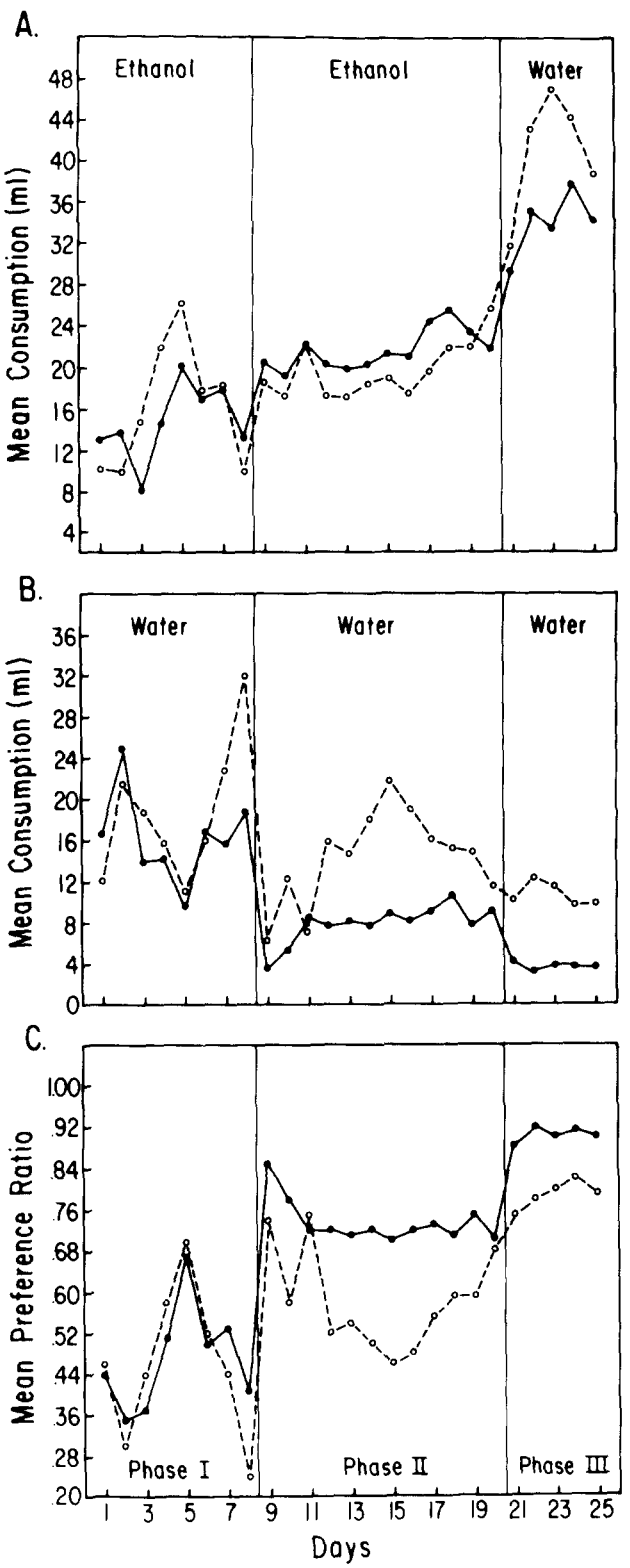

FIG. 3. Comparison of fluid consumption measures in ovariectomized (●—●) or sham-operated (○---○) hamsters. Tube location varied randomly in Phase I and was held constant in Phases II and III. (A) compares consumption from the Marked tube; (B) compares consumption from the Unmarked tube and (C) compares the APR.

Neither did OVX and SH groups differ on this measure or on measures related to water or alcohol intake (Fig. 3).

In Phase II, the marked tube containing ethanol was always on the animal's left. By removing the factor of tube position, comparison of APRs between groups was facilitated. Analysis of variance applied to the last nine days of this phase showed that the two groups did not differ in ethanol consumption (Fig. 3A), but there was a significant intake increase across days, $F(1,8)=4.19$, $p<0.001$. The OVX animals tended to drink less water (right tube) than the SH animals, Fig. 3B, $F(1,19)=4.00$, $p<0.06$, but there were no differences in total fluid intake. The resulting APR also tended to differ for the two groups, $F(1,19)=3.64$, $p<0.07$, the OVX animals having a stronger ethanol preference than SH animals (Fig. 3C).

During Phase III, with water in both tubes, there was no difference between groups in intake from the Marked tube (on the animal's left), but there was an effect of days, $F(1,4)=3.41$, $p<0.02$). There was a large difference in consumption from the tube on the animal's right $F(1,19)=17.5$, $p<0.001$, the OVX group again drinking less than the SH group (Fig. 3B). This group difference translated into a marginal difference between groups in total fluid consumed, $F(1,19)=2.94$, $p<0.10$, and a significant effect across days, $F(1,4)=3.0$, $p<0.03$. With respect to preference for the Marked solution, OVX animals were significantly greater than SH animals, $F(1,19)=9.47$, $p<0.01$ (Fig. 3C).

These results show that OVX and SH animals did not differ in consumption from the left tube regardless of whether it contained 10% ethanol or water. However, because OVX animals drank less water from the right tube when 10% ethanol was available in the left (Phase II), this group had a significantly greater APR than SH animals. In transition to Phase III, both groups significantly increased left tube preference when the alcohol was replaced by water ($p<0.02$ in each case). The amount of increase was not different between the groups. Therefore while OVX animals preferred alcohol more during Phase II than did SH animals, the presence of alcohol tended to inhibit consumption from the left tube by both groups.

Estrous Cycle

Intake across an average estrous cycle was obtained from two consecutive estrous cycles for each intact animal during Phase II. Analysis of variance showed that ethanol consumption (Marked tube, Fig. 4A) varied according to day of the estrous cycle, $F(3,24)=7.80$, $p<0.002$, as did total fluid consumed, $F(3,24)=3.27$, $p<0.04$. Neither the water intake from the tube on the animal's right nor the APR varied with the estrous cycle.

Across a single estrous cycle during Phase III, there also tended to be systematic variation in consumption of fluid from the Marked tube, still on the animal's left, but now containing water; $F(3,21)=2.44$, $p<0.095$, and again a systematic change in daily total fluid consumed during the cycle $F(3,21)=3.07$, $p<0.05$. Neither consumption of water from the unmarked (right side) tube nor left-right preference ratio varied significantly across the estrous cycle (Fig. 5).

DISCUSSION

The very strong position preference exhibited by the hamsters was unanticipated because similar methods have previously been used with hamsters [14] and rats [7] with no apparent problems. However, hamsters in a T-maze do not

show spontaneous alternation strategies similar to rats [26] so perhaps our surprise was unwarranted.

During the estrous cycle, consumption from the preferred (left) side varied systematically, regardless of whether the solution was ethanol or water. Lowest consumption was on Cycle Day 4. Preference for the solution in the Marked tube was not affected by the estrous cycle either during Phase II or III. It appears, therefore, that the hamsters were not especially varying ethanol consumption across the estrous cycle. Rather, when endocrine factors favored decreased consumption, it was effected by decreasing consumption from the left (preferred) tube, regardless of content. Consumption from the right tube remained minimal and unchanged.

The comparison of intact and OVX animals suggests ovarian involvement in ethanol intake regulation. The major effect of ovariectomy was not on ethanol consumption directly, but on water intake which was significantly less than that of intact animals (Fig. 3B). This reduction in water intake was compensated by a mild increase in ethanol consumption providing no between-group difference in overall fluid ingestion, but a group of OVX animals which showed significantly greater preference for the ethanol solution compared to intact animals (Fig. 3C). These data markedly contrast with results from the rat in which ovariectomy results in significantly lower ethanol preference and consumption (experiment 3 [7]) and with data from hamsters in which ovariectomy apparently reduces saccharine preference [32].

EXPERIMENT 3

In the previous paper we demonstrated a response difference between intact and OVX rats to varying concentrations of ethanol [7]. That difference can be interpreted as a sensitivity and/or responsiveness change induced by ovariectomy. Ovariectomized animals may have found the ethanol solutions more noxious than did the intact animals.

Normal women who report decreases in alcoholic beverage consumption during pregnancy also tend to report that the taste, or mere thought, of such drink is repugnant [16]. This verbal response may be analogous to our report that ovariectomy increases sensitivity and/or responsivity to varying concentrations of ethanol [7]. Taste sensitivity and/or responsivity is strongly modulated by the presence or absence of adrenal glucocorticoids [19, 20, 22, 23]. Saccharine preference is also diminished by adrenalectomy in male rats and it restored by glucocorticoid treatment [25]. This fact, in conjunction with the results from Experiment 2 with a species in which estradiol fails to facilitate glucocorticoid synthesis (unlike rats) and in which OVX fails to reduce alcohol intake preference (also unlike rats), suggested direct adrenal involvement in alcohol ingestion and preference. The present experiment tested this hypothesis by utilizing OVX, adrenalectomized (ADX) or OV-ADX treatment groups. The effects of surgical treatment were then further tested by providing certain groups with glucocorticoid replacement therapy.

METHOD

About half of the rats in this experiment were born in the laboratory (litters resulting from Experiment 3 [7]. The remainder were purchased directly from the Charles River animal colony and housed in the same room for about one month prior to the experiment. From age 30 days until one month prior to the experiment, the laboratory-reared animals

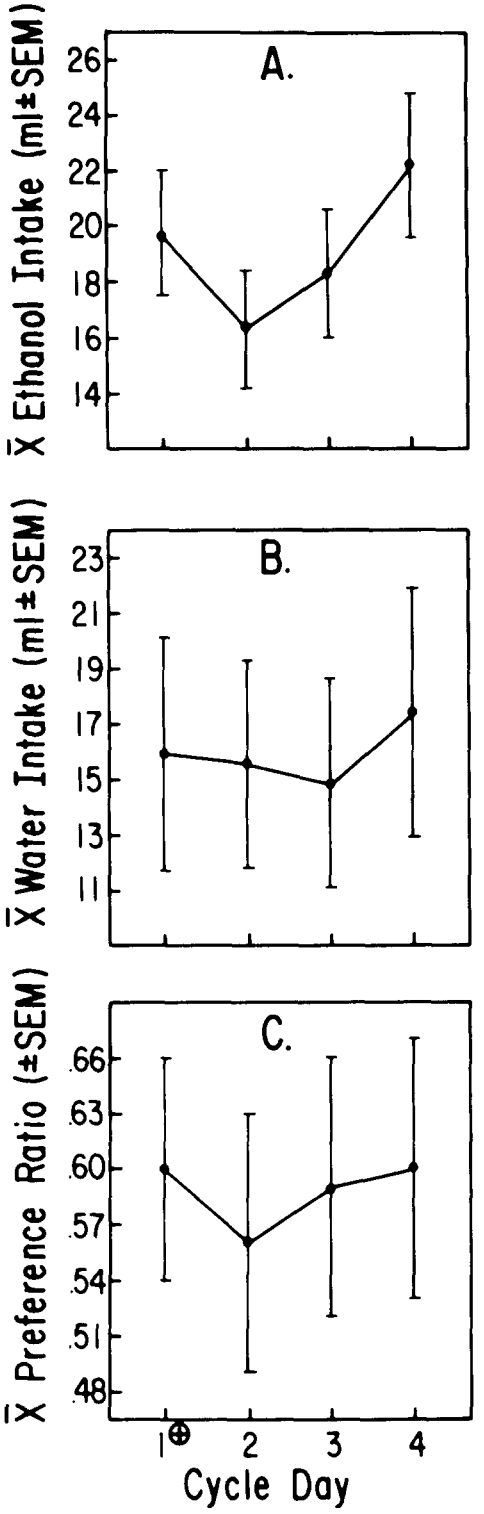

FIG. 4. Consumption of fluid by hamsters relative to the estrou cycle day during Phase II. An ethanol solution is in the left tube ⊕=day of ovulation.

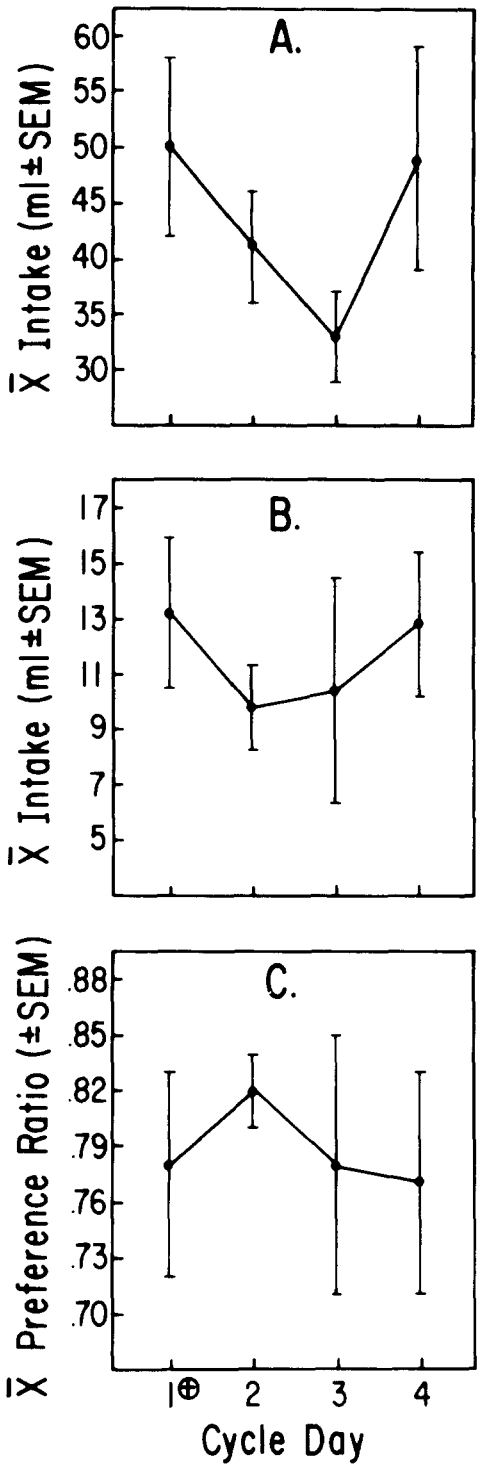

FIG. 5. Consumption of fluid by hamsters relative to the estrous cycle day during Phase III. Both tubes contain water. The left tube is the Marked tube.

TABLE 1

STATISTICAL COMPARISON OF APR BETWEEN OVARIECTOMIZED (OVX), ADRENALECTOMIZED (ADX), OVARIECTOMIZED-ADRENALECTOMIZED (OV-ADX) AND SHAM OPERATED (SH) RATS DURING THE LAST FIVE DAYS OF PHASE II

	OVX*	ADX	OV-ADX
SH	$t(27)=1.80$	$t(28)=2.84$	$t(29)=4.38$
	$p=0.24$†	$p<0.01$	$p<0.001$
OVX	—	$t(37)=0.74$	$t(38)=2.37$
		$p<0.53$	$p<0.025$
ADX	—	—	$t(39)=1.82$
			$p<0.075$

*All probabilities are 2-tailed; parentheses contain degrees of freedom.

†SH and OVX groups differ according to the percent of each group with APRs greater than 0.5 ($p<0.04$); see text.

were housed in sex-segregated groups. Animals were then individually housed throughout the experiment. All animals were at the same age (±5 days) and were roughly the same initial weight. The number of animals of each background was approximately equal in each experimental group and all animals were tested simultaneously.

About one week prior to surgery, each animal to be adrenalectomized had a 0.9% saline solution (NaCl in tapwater) substituted for its drinking water. Through the remainder of the experiment each adrenalectomized animal received 0.9% saline in place of water and the 4% ethanol solution was made with 0.9% saline and ethanol (v/v).

Four surgical groups were created: Sham operated (SH; N=10); ovariectomy (OVX; N=19); adrenalectomy (ADX; N=20) and ovariectomy plus adrenalectomy (OV-ADX; N=21). All operations were performed through bilateral dorsomedial incisions while the animals were anesthetized with ether. Each adrenal was visually located, the accompanying fat grasped (to avoid puncturing the gland) and carefully torn to remove the intact adrenal. Sham removal of either ovaries or adrenals entailed locating and touching the organs with forceps. Beginning 10 days after surgery, the normal drinking solutions (water or 0.9% saline) were placed in two Richter tubes (Phase I). One tube was designated the "Marked" tube. After four days, a 4% ethanol solution was placed in the Marked tube (Phase II). After 10 days in Phase II, each experimental group was divided into two sub-groups and each animal was given a subcutaneous injection daily at 1900 hr of 5 mg corticosterone per 0.4 ml propylene glycol or 0.4 ml propylene glycol vehicle. The SH controls were injected with vehicle only. Throughout the experiment, tubes were read daily and refilled, as necessary, at 1100 hr. Tube location (right or left) was randomly varied and tube cleaning followed the procedure in Experiment 1.

RESULTS

Because of the different surgical conditions and drinking solutions, comparison of results between groups is limited to the APR ratio. During Phase I, there was no difference between groups in preference for the Marked tube. When ethanol became available in Phase II, the groups demonstrated clear differences in the preference for 4% ethanol, for days 10–14, $F(3,66)=7.07$, $p<0.001$; Fig. 6. Comparisons be-

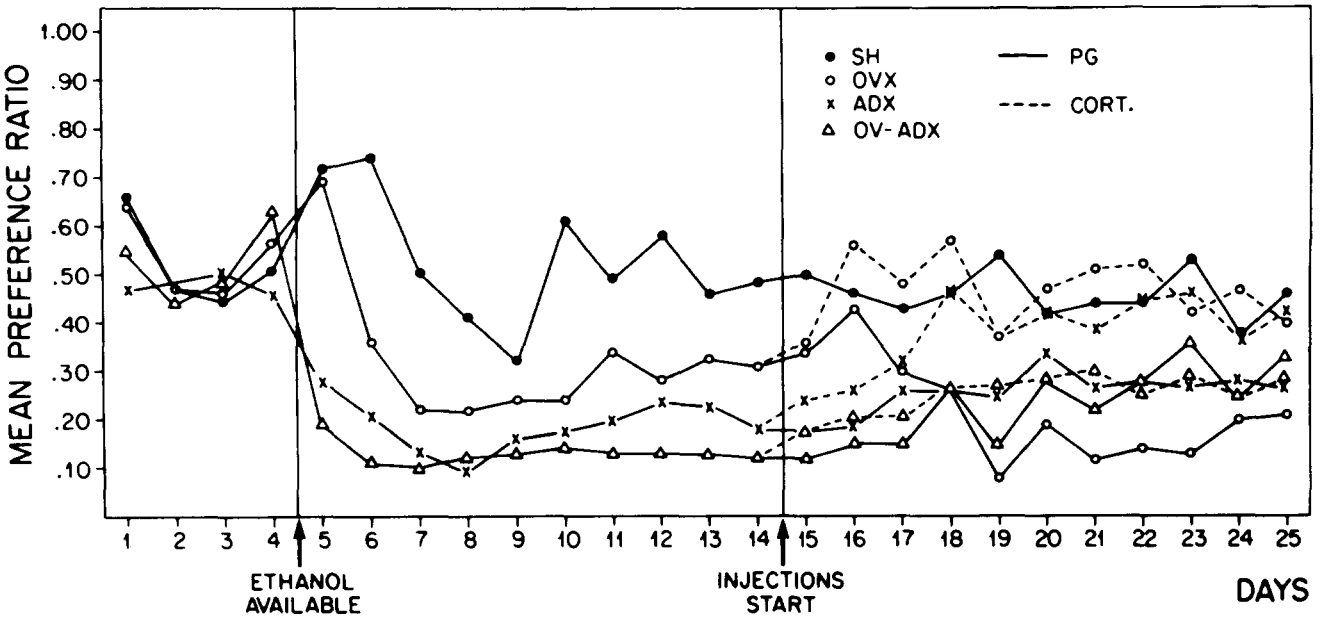

FIG. 6. APR of female rats following sham-operation (SH), ovariectomy (OVX), adrenalectomy (ADX), combined surgery (OV-ADX) and during corticosterone (Cort) or propylene glycol (PG) injection.

TABLE 2

MEAN DIFFERENCE SCORES FOR FLUID INTAKE AND APR OF SURGICALLY OPERATED RATS FOLLOWING CORTICOSTERONE (C) OR PROPYLENE GLYCOL (PG) TREATMENT

	Ethanol Intake Difference		Saline or Water Intake Difference		Preference Ratio Difference	
	C	PG	C	PG	C	PG
SH	—	0 ± 3	—	3 ± 2	—	−0.07 ± 0.06
OVX	6 ± 5*	−4 ± 2	−2 ± 5	1 ± 2	0.15 ± 0.12†	−0.08 ± 0.05
ADX	17 ± 5*	6 ± 3	−12 ± 4§	−1 ± 4	0.21 ± 0.06†	0.05 ± 0.07
OV-ADX	12 ± 4	11 ± 3	5 ± 6‡	−19 ± 4	0.17 ± 0.06	0.15 ± 0.04

vs PG: *$p<0.05$, 1 tail; †$p<0.06$, 1 tail; ‡$p<0.001$, 2 tails; §$p<0.04$, 1 tail.

tween groups are shown in Table 1. During days 10–14, each group differed from each other group with two exceptions: ADX did not differ from OVX and SH did not differ from OVX. In the latter instance, however, the two groups clearly differed with respect to the proportion of animals in each group having preference ratios greater or equal to 0.5, SH = 6/10; OVX = 4/19; $\chi^2(1)= 4.40$, $p<0.04$; cf. [7].

During Phase II, very large within-group variations interfered somewhat with the statistical analysis (e.g. SH vs OVX results) and this problem was exacerbated in Phase III because the number of animals per group was halved. To partially circumvent this problem, each individual's mean performance during the last five days of Phase III was compared with the same subject's mean performance during the last five days of Phase II, thus creating a difference score. Change in performance based on the difference scores for each corticosterone treated group was then compared to that of its appropriate control group, with a one tail $p<0.05$ acceptable as significant.

Propylene glycol treatment of the SH group resulted in a 0±3 ml change in 4% ethanol consumption and a −0.07±0.06 change in the preference ratio from Phase II to III. Table 2 shows the between-group numerical comparisons illustrated in Fig. 6. Corticosterone restored the preference ratios to approximately normal for the OVX and ADX groups (Fig. 6). In both cases, the steroid treated group increased its ethanol fluid consumption compared to the respective vehicle injected group ($p<0.05$, 1 tail in each case). Likewise, corticosterone tended to increase SPR relative to the respective OVX or ADX vehicle groups ($p<0.06$, 1 tail in each case). However, corticosterone had no discernible effect on ethanol consumption by the OV-ADX group regardless of the measure considered (Table 2; Fig. 6). With respect to plain saline consumption, OV-ADX animals given corticosterone increased intake more than those given PG ($p<0.001$), but ADX animals given corticosterone decreased consumption compared to controls ($p<0.04$, 1 tail).

Response to corticosterone was additionally assessed by

correlating individual's mean APR score on ethanol intake during the five days prior to hormone treatment with the same measure obtained during the last five days of the hormone. Because the OVX and ADX groups were positively affected by corticosterone, the two groups were combined for this analysis. A preliminary least squared regression analysis of the APR scores revealed two animals in the OVX group as statistical outliers and atypical of the scatter plot distribution. Their data were thereafter excluded. Correlation analysis revealed a positive relation between performance before and performance during corticosterone treatment, APR: $r(16)=.55$, $p<0.02$; ethanol intake: $r(16)=.63$, $p<0.01$. This supports the concept that corticosterone can modulate consumption amplitudes of individuals in a group without altering the individual differences in response to alcohol-related stimuli.

DISCUSSION

Adrenalectomy greatly reduced the APR relative to SH animals and approximately doubled the reduction caused by ovariectomy. The combined OV-ADX treatment further reduced the APR suggesting that the regulatory roles of ovaries and adrenals may be additive. When corticosterone was provided, the APR of the ADX group was elevated relative to PG treated controls. APR elevation by corticosterone in OVX animals was unexpected, as was the failure of that steroid to affect APR in the OV-ADX animals. Presence of one or the other gland is apparently necessary in order for corticosterone to exert its facilitatory effects on APR. Similarly, normal hippocampal binding of ^{3}H-corticosterone appears contingent upon the availability of circulating estradiol [27].

The large within-group variability in APR which tended to diminish the effects of corticosterone may have been caused by the animals' histories. A ten-day experience with saccharine solutions can prevent the effects of ovariectomy or ovarian hormone treatment on preference in most, but not all rats [30]. Exposure to a saccharine solution for six days blocked the inhibitory effect of adrenalectomy on preference and only two days pre-exposure blocked the facilitating effect of dexamethasone on saccharine preference of adrenalectomized male rats [25]. Clearly, however, the APR is not as drastically affected by experience as is saccharine preference. A second source of variability may have been derived from the fact that all rats which had adrenalectomies, with or without ovariectomy, regardless of hormone treatment, were maintained on the pre-existing 0.9% saline solution. This occurred despite the fact that corticosterone treated animals no longer need saline maintenance.

GENERAL DISCUSSION

According to our original hypothesis [7], 4% ethanol intake should be regulated by ovarian constituents in a manner similar to the regulation of saccharine [29, 30, 31]. Ovariectomy results in decreased saccharine intake. Exogenous estradiol in synergy with progesterone facilitates saccharine preference, and excess progesterone inhibits the facilitatory estrogen effects. The latter hormonal state mimics, to some extent, pregnancy and produces decreased saccharine preference similar to that during pregnancy and lactation [29].

The ethanol consumption model based on the saccharine preference data is valid to the extent that, as predicted [31], ovariectomy diminishes ethanol intake and preference is reduced during pregnancy and lactation. Furthermore, at least for OVX rats, individual consumption of ethanol is positively correlated with intake of a saccharine solution. Ovariectomized rats also appear to be more sensitive and/or responsive to different alcohol concentrations than intact rats [7].

About 40 years ago, Richter [19,20] showed that greatly increased responsivity by rats to weak saline solutions was a consequence of adrenalectomy. More recent research using auditory or olfactory stimuli has demonstrated large changes in sensory processing capabilities following adrenalectomy or adrenal cortical insufficiency in rats, chinchillas or humans [5, 10, 23]. The present experiments support a two-fold approach to the role of adrenal hormones in modulating ethanol consumption. First, as demonstrated by Experiment 3, adrenalectomy alone greatly reduced ethanol preference. However, the combined removal of ovaries and adrenals produces a distinguishable, greater dimunition in the alcohol preference ratio. Therefore, the actions of ovaries and adrenals are, in some fashion, additive with respect to the APR. This additivity may occur through the action of ovarian estradiol on the female rat adrenal. Ovariectomy reduces blood corticosterone levels by about 45% and exogenous estradiol restores them [13]. If the effect of estradiol on alcohol preference is only indirect (i.e., through adrenal hormones), then this may explain why the changes in APR following large estradiol doses (Experiment 2) were slow to appear and not very clear.

Unlike its effect on rats, ovariectomy of hamsters failed to reduce the APR or ethanol intake. Instead, water consumption was lessened and the APR increased (Fig. 2B,C). The failure to observe a response similar to the rat is consistent with earlier observations that hamster adrenal glucocortoid output is not modified by ovarian function [8]. The dual regulation of rat ethanol consumption by adrenal and ovary seems clear. However, the foregoing results remain to be reconciled with several related and seemingly contradictory issues: (1) rats can treat ethanol as a source of calories [21] and this apparently happens during the estrous cycle when ethanol consumption is depressed on cycle days associated with high estradiol levels [7]; (2) removal of any ovarian influence by ovariectomy also reduces ethanol intake (present data and [7]) and (3) estradiol in moderate to heavy doses can increase ethanol intake (Experiment 1). Finally, none of these potential endocrine contributions are as yet sufficient to explain why rats or humans change preference for (but do not necessarily reduce intake of) alcohol during pregnancy. In humans, the free cortisol index and plasma cortisol are at approximately pre-pregnancy levels during the first trimester, but become increasingly elevated thereafter [2, 4, 18]. In the rat, plasma corticosterone levels remain unchanged until 5–7 days before parturition when a rapid increase begins [12]. Therefore, the diminished APR in late rat pregnancy and lessened alcohol consumption in early human pregnancy [16] do not correspond well with the concept that decreased corticoid output causes decreased ethanol preference. As previously suggested [30,31], increased progesterone levels during pregnancy may directly or indirectly (via blockade of estradiol activity (see [17] for a review), inhibit ethanol consumption. Alternatively, inhibition during pregnancy may be achieved by an unidentified antihormone, possibly of placental origin [1,6]. Regardless of source, any such inhibitory compound must be regarded as a significant natural protective agent against the occurrence of the fetal alcohol syndrome [11].

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