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THE EFFECTS OF ALCOHOL ON WEIGHT GAIN AND THE HYPOTHALAMIC–PITUITARY–GONADOTROPHIN AXIS IN THE MATURING MALE RAT

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Abstract—Male rats allowed food *ad lib.* and 15% (v/v) alcohol in place of water *ad lib.* from 3 to 10 weeks of age were found to have a slightly slower growth rate than control animals. This was due to a lower caloric intake. The normal increase in basal luteinizing hormone (LH) levels that takes place during maturation in the male rat was completely abolished by alcohol feeding. That this was not only (or merely) due to impaired weight gain was suggested by the observation that intubation of alcohol (0·28 g) into 150-g male rats established that alcohol was capable of lowering circulating LH levels within 1 hr. The sensitivity of the anterior pituitary to 100 ng exogenous luteinizing hormone-releasing hormone (LH-RH) was the same in alcohol-fed and control rats, suggesting that the alcohol effect on the reproductive endocrine system is at the hypothalamic or higher neural centres.

The effects of alcohol on the various endocrine functions in man and animals, in particular the endocrine system involved with reproduction, has received only moderate attention in the past decade; Stokes [1], and Marks and Chakraborty [2] review the current position on alcohol–endocrine relationships, which primarily include hypothalamic–pituitary–adrenocortical function, sympathetico–adrenal medullary function and thyroid function.

The few reports that have appeared on the effects of alcohol on the reproductive system include those of Chaudhury and Matthews [3] who reported an antifertility effect of ethanol in female rabbits, and Kieffer and Ketchel [4] who showed that administration of ethanol (7·9 g/kg s.c.) to female rats at 13.00 hr on the day of prooestrus inhibited ovulation. Administration of luteinizing hormone (LH) 90 min after ethanol reversed this inhibition however. These authors concluded that ethanol inhibited the preovulatory surge of LH in female rats.

The experiments reported here were designed to investigate whether the basal level of LH in the male rat changed with long-term feeding of alcohol and short-term intubation and whether or not the release of LH from the pituitary after injection of exogenous luteinizing hormone-releasing hormone (LH-RH) was different in alcohol-treated and untreated rats.

MATERIALS AND METHODS

Animals. Male Wistar albino rats bred at the University of Surrey animal house were used. They were caged in groups of ten and kept in a room lighted from 07.00 to 19.00 hr. The temperature was maintained at 22° and the humidity at 40–50 per cent. All animals were fed Spratt's laboratory diet No. 1 and either tap water or 15% v/v alcohol. Intake of food and liquid was monitored for each group and individual weight gains also recorded.

LH-RH. Synthetic LH-RH kindly supplied by Hoescht Pharmaceuticals Ltd. was used for pituitary function tests. The synthesis and characterization of LH-RH have been described by Geiger *et al.* [5] and its action in various species of animals reported by Schally and co-authors [6].

LH assay. A double antibody radioimmunoassay was used with reagents kindly supplied by the National Institutes of Health, Bethesda, Maryland, U.S.A. NIAMD-anti-rat LH serum-1 (raised in rabbits) was used at a final dilution of 1:40,000 in a buffer consisting of 0·01 M phosphate, 0·15 M NaCl, 0·05 M EDTA, 0·3% normal rabbit serum and 0·01% merthiolate, pH 7·6. Purified rat LH (NIAMD-Rat LH-1-3) was used for radioiodination with ^{125}I using a modification of the lactoperoxidase method of Marchalonis [7]. Five micrograms of hormone were used in each iodination and specific activities of approximately 90, 107 and 86 μCi/μg were obtained in three successive iodinations.

NIAMD-Rat LH-RP-1 was used as the standard reference preparation.

The assay was performed in polystyrene LP3 tubes (Luckhams, Burgess Hill, Sussex), each standard, blank or experimental sample being performed in duplicate. Buffer (200 μl 1% BSA in 0·01 M phosphate, 0·15 M NaCl, 0·01% merthiolate, pH 7·6) was added to each tube. One hundred microlitres of the above buffer alone, or containing 1–1000 ng standard LH was then added to either blank or standard tubes and 100 μl of plasma samples under assay to others. One hundred μl of iodinated rat LH in 0·1% BSA phosphosaline buffer, pH 7·6 (20,000 c.p.m., equivalent to approximately 0·5 ng LH) was then added to each tube followed by 200 μl of anti-rat LH serum. The contents of each tube were then agitated on a vortex stirrer and incubated at 20° for 24 hr.

After this time 100 μl of anti-rabbit globulin antiserum (Wellcome Reagents Ltd.) at a dilution of 1:20 in phosphosaline buffer was added, the contents mixed and incubation continued for a further 24 hr. All tubes were then centrifuged at 2500 *g* for 30 min to sediment the precipitated antibody bound labelled rat LH. After decanting the supernatant and drying the tubes, they were counted in a Wallac gamma

counter with optimum settings for ^{125}I. Standard calibration curves were then constructed and the values of unknown samples read from them (see Swerdloff *et al.*) [8].

LH-RH test for pituitary response. Preliminary experiments using 21-day-old weaner male rats were conducted to establish the dose of LH-RH necessary to give a measurable release of LH and to establish experimental procedure. Groups of 24 animals were used and either injected intramuscularly with 0·1 ml of 0·9% saline alone (controls) or containing various amounts of LH-RH. Sub-groups of four animals were then put under light ether anaesthesia at 15, 30, 45, 60 and 90 min after injection, blood removed by cardiac puncture into heparinized tubes, plasma separated by centrifugation and stored at −15° until assayed for LH.

Seven-week alcohol experiment. Sixty 21-day-old weaner male rats were used. They were divided into six cages of 10, one group of three cages fitted with alcohol drinking bottles and the other group of three with water. After 7 weeks, ten rats from each group were used for the measurement of basal LH levels and the remaining 20 in each group for the assessment of responsiveness to injected LH-RH (100 ng i.m.) as for the immature rats previously mentioned.

Intubation experiment. Twelve 150-g male rats were used. Six were intubated with 1 ml of 35% (v/v) alcohol (0·28 g, dose of 1·9 g/kg body wt) and six with 1 ml of 0·9% saline (controls). All animals were killed after 60 min, blood samples taken, plasma separated and stored at −15° until assayed for LH.

RESULTS

A radioimmunoassay for rat LH was successfully developed using reagents supplied by the National Institutes of Health, Bethesda, U.S.A. Sensitivity to LH and cross-reactivity with purified FSH, prolactin and growth hormone were similar to the systems developed by Swerdloff *et al.* [8] and Anderson *et al.* [9].

It was established that 21-day-old male rats had basal plasma LH levels of 75·8 ± 1·8 S.E.M. (4) ng/ml (NIAMD-Rat LH-RP1). Intramuscular injection of 100 ng LH-RH led to maximal plasma LH levels of 337·5 ± 48·7 S.E.M. (4) ng/ml 15 min after injection. The level then rapidly declined and was approaching the pre-injection level by 60 min. Figure 1 shows a graphical representation of the results.

Figure 2 shows the results obtained for liquid and food intake in the 60 male rats used for the 7-week experiment. Both food and liquid intake increased relatively rapidly during the 4th–6th week of age but then slowed down to reach a fairly constant intake from the 8th week onwards. The level of food intake was always lower in the alcohol-fed rats than in the water-fed rats. The volume of 15% (v/v) alcohol consumed per rat was also always lower than the volume of water drunk by the control group (Fig. 2). As a consequence of these lower intakes, the weights of alcohol-fed rats were always lower than the control group (see Fig. 3). After 7 weeks, alcohol-fed rats weighed 240·9 ± 5·1 g S.E.M. (30) compared to controls weighing 295·0 ± 5·0 g S.E.M. (30). Despite their lower weights, the alcohol fed rats appeared perfectly healthy. Table 1 shows the alcohol intake in exper-

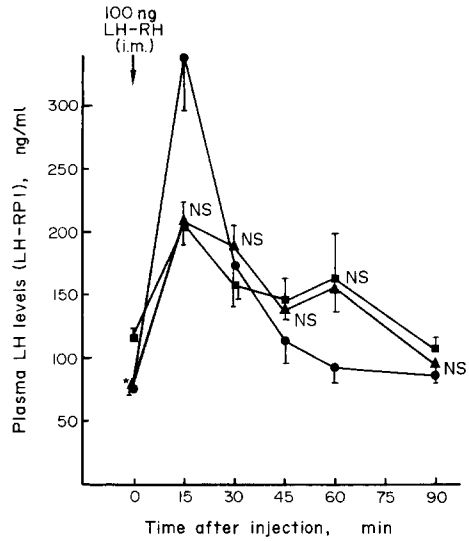

Fig. 1. Plasma LH levels (LH-RP-1) before and after the injection of 100 ng LH-RH i.m. in 0·1 ml 0·9% saline at zero time in: (i) 3-week-old untreated male rats (●——●); (ii) 10-week-old untreated male rats (■——■); and (iii) 10-week-old male rats allowed alcohol for 7 weeks (▲——▲). Results are shown as means ± S.E.M. Where S.E.M.s are not shown they are smaller than the symbols used. $n = 4$ For all points except zero time points for (ii) and (iii) where $n = 10$. Results for groups (ii) and (iii) have been compared statistically (Student's t-test); NS = not significantly different; * P = <0·001.

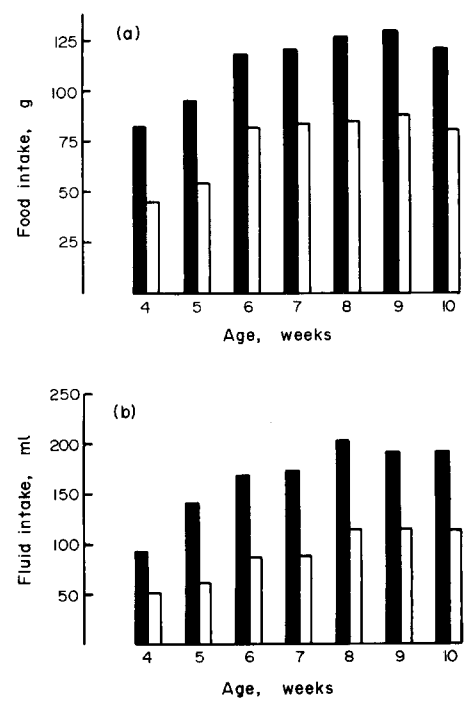

Fig. 2. (a) Intake of solid food (g) given *ad lib.* per rat per week from 4th to 10th week of age. Solid boxes are control rats allowed water, open boxes experimental rats allowed 15% (v/v) alcohol. Each observation is the mean of 30 animals. (b) Intake of fluid (ml) of the same groups of animals as in (a). Solid boxes are control rats drinking water *ad lib.*, open boxes experimental rats drinking 15% (v/v) alcohol *ad lib.* Each observation is the mean of 30 animals.

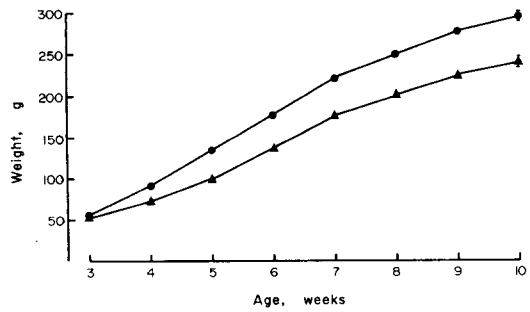

Fig. 3. Weight gain in male Wistar albino rats from 3 to 10 weeks of age given (i) water and food *ad lib.* (●——●) and (ii) 15% (v/v) alcohol and food *ad lib.* (▲——▲). Results are shown as means ± S.E.M. (30 animals). In all but the 10-week results S.E.M.s are smaller than the symbols used.

imental rats related to their body weight. The dose of alcohol received per kg body wt declined from 86·8 g in the 1st week of the experiment to 56·7 g in the 7th and final week of the experiment. Table 2 shows the caloric intake of experimental and control animals per week over the same 7-week period. Caloric intake in the alcohol-fed animals was always lower than in control animals and consequently weight gain also lower. However, the ratio of weight gain to caloric intake between the two groups was very similar except for the 1st week of the experiment when the alcohol-fed animals were probably adjusting to their new food source.

The basal levels of LH and the response of the alcohol and water-fed groups of rats to 100 ng LH-RH after the 7-week experimental period are shown in Fig. 1 with the corresponding LH-RH experiment in 3-week-old rats. An intramuscular injection of 100 ng LH-RH in both water-fed and alcohol fed rats produced a lower maximum level of LH in the circulation than the same dose in younger rats. This is probably merely a reflection of the difference in dose of LH-RH on a body weight basis. The levels of LH at all time points (15, 30, 45, 60 and 90 min) after administration of LH-RH are not significantly different in the alcohol and water-fed rats. Examination of Fig. 1 however, shows that the basal level of LH in the alcohol-fed rats (77·7 ± 5·0 S.E.M. (10) ng/ml), is highly significantly lower ($P = < 0.001$) than in the water-fed rats (114·9 ± 7·6 S.E.M. (10) ng/ml).

Intubation of 0·28 g alcohol into six 150-g male rats showed an even more dramatic reduction in basal LH levels. Control animals intubated with saline had a level of 83·1 ± 6·9 S.E.M. (6) ng/ml compared to a level of < 25 ng/ml (6) in the alcohol treated animals, this being the limit of sensitivity of the assay, under the conditions used.

DISCUSSION

Levels of LH in the plasma of the male rat have been shown to increase progressively with maturity from 3 to 13 weeks of age [10]. There is no sharp increase in LH levels associated with the first signs of spermatogenesis or presence of sperm [10]. In the experiments reported here, LH levels in untreated control rats rose from 75·8 ± 1·8 (4) ng/ml at 3 weeks of age to 114·9 ± 7·6 (10) ng/ml at 10 weeks of age,

Table 1. Intake of 15% (v/v) alcohol in male rats over the 7-week experimental period

Week of experiment	Age of rats (weeks)	Vol. of 15% (v/v) alcohol (ml.) consumed per rat*	Wt of alcohol (g) consumed per rat*	Dose of alcohol received (g) per kg body wt*
1	4	52	6·2	86·8
2	5	62	7·4	74·7
3	6	87	10·4	77·0
4	7	88	10·5	62·0
5	8	115	13·7	68·5
6	9	114	13·6	61·2
7	10	113	13·5	56·7

*Mean of 30 animals.

Table 2. Caloric intake in experimental (alcohol-fed) and controls (water fed) male rats over the 7-week experimental period

Week of experiment	Age of rats (weeks)	Caloric intake* (kcal) Experimental†	Caloric intake* (kcal) Control†	Weight gain (g) Experimental†	Weight gain (g) Control†	Weight gain (g)/ caloric intake (kcal) Experimental	Weight gain (g)/ caloric intake (kcal) Control
1	4	193·0	278·8	16·5	35·5	0·09	0·13
2	5	235·4	326·4	27·2	43·0	0·12	0·13
3	6	351·6	401·2	37·1	42·2	0·11	0·11
4	7	362·5	411·4	39·8	44·5	0·11	0·11
5	8	384·9	428·4	22·9	27·5	0·06	0·06
6	9	394·4	438·6	26·6	27·9	0·07	0·06
7	10	369·9	411·4	16·6	19·0	0·04	0·05

* Calculated using values of 3·4 kcal/g for solid rat food (figure supplied by manufacturer) and 7·0 kcal/g for alcohol.

† Mean of 30 animals.

by which time they were mature animals. Rats given 15% (v/v) alcohol to drink throughout this period of 3–10 weeks of age, however, had basal LH levels of 77·7 ± 5·0 (10) ng/ml at 10 weeks of age, a marked reduction from normal levels. Even allowing for the lighter weight of alcohol-fed rats compared to water-fed controls at 10 weeks of age (241 g against 295 g), the LH level was comparable to normal rats of 3-weeks-old weighing only 55 g. It would appear, therefore, that alcohol treatment during maturation of the male rat leads to impairment of endogenous LH-RH synthesis and/or its release from the hypothalamus, or impairment of LH release from the pituitary into the peripheral circulation. The former seems the more likely explanation as it has been shown that exogenous administration of LH-RH to alcohol-fed rats produces the same magnitude of LH release as in normal untreated animals (Fig. 1). It could be argued, in fact, that the alcoholic rats showed a greater incremental increase in circulating LH after LH-RH treatment than did control animals, because of their much lower basal LH levels. However, it has been shown that the pituitary is at least as sensitive, if not more so, to LH-RH in alcohol-fed than water-fed animals. The site of action of ethanol in reducing basal LH levels is therefore likely to be the hypothalamus, or higher brain centres responsible for the normal production and release of endogenous LH-RH.

Coppola [11] suggested that LH secretion is suppressed by agents that either lower the catecholamine content of the brain or raise the indoleamine content (serotonin) and that a balance between the two types of amine may be important for regulating LH release. Although the effects of alcohol, and its active metabolite, acetaldehyde, on biogenic amine metabolism have been well documented (see review by Davis and Walsh [12]) there still remains much controversy as to the effect of alcohol on brain amines. Much of the contradiction in this field is due to species and concentration differences but alterations in amine content of the brain by alcohol may be responsible for decreased LH release from the pituitary.

Intubation experiments showed that a single dose of alcohol (1·9 g/kg body wt) in 150-g male rats significantly lowered circulating LH levels after only 1 hr. The depression in LH levels was greater than in the 7-week chronic feeding experiment. A single acute dose of alcohol seemed therefore more capable of lowering circulating LH levels after 1 hr than the 7-week chronic dosing. Rowe *et al.* [13] have recently reported that in male subjects the level of circulating LH was reduced in light drinkers during a 6-hr 'drink-in' but returned to normal soon after whereas no such effect was seen in heavy drinkers. The dose of alcohol could therefore be critical in assessing its effect on the reproductive endocrine system. It has been shown to be capable of lowering LH levels, and therefore of affecting the male reproductive processes controlled by LH.

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