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Effect of Castration and Gonadal Hormones on Insulin-Induced Drinking

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Water intake Estrous Testosterone Estradiol Thirst Angiotensin

PERIPHERAL administration of insulin enhances water intake in rats (2,20,26,27) and humans (31,32). This effect is not related to the concomitant enhancement of food intake (26). It has previously been demonstrated that some mechanisms of thirst are influenced by sexual factors. Angiotensin (7,30) or polyethylene-glycol-induced drinking (29) is higher in female than in male rats, and these differences were diminished or even abolished by castration. On the other hand, thirst responses to peripheral or central Angiotensin II (AII) fluctuate with the estrous cycle (7, 8,30) or estrogen administration (12,13,16–18,24,29). Sexual differences in insulin-induced drinking have also been reported (21).

The aim of this set of experiments was to analyze the possible role of sexual hormones and sexual maturation in the onset of sexual differences in insulin-induced drinking.

METHOD

Animals

Wistar rats were obtained from the vivarium of the University of Oviedo and housed individually in a room illuminated from 0900–2100 h, where they had ad lib access to a standard laboratory diet (Panlab A04) and tap water. During experiments food was withdrawn but water remained available from graduated tubes fitted with glass spouts. Behavioral studies were, in all cases, made during the afternoon, starting 1500 h approximately, on rats at least 3 months old. Consecutive insulin injections in the same rat were separated by at least 2 days.

Procedures: Experiment 1. Intact Adult Male and Female Rats

Injection of 5 U/kg b.wt. of commercial human insulin (ACTRAPID HM, Novo; vials of 10 ml containing 400 Units

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of insulin, generously supplied by Novo España S.A.) diluted in physiological saline (up to a volume of 1 ml/kg b.wt.) were administered IP to male (n = 15, 250–350 g b.wt.) and female (n = 16, 225–290 g b.wt.) rats. Females were tested during the four phases of their estrous cycle, monitored by daily microscopic observation of nonstained vaginal smears. Water intake was measured 60 and 120 min after the insulin administration. To accustom the animals to the experimental procedure, every rat received at least two IP injections of insulin before starting the actual experiments. Equivolumetric IP injections of physiological saline (0.9% NaCl), were used as a control.

Experiment 2A: Castrated Adult Male and Female Rats

In males (n = 8, 350–400 g b.wt.) both testicles were removed through a scrotal incision made under ether anesthesia. In females (n = 9, 250–300 g b.wt.) both ovaries were removed through a medial dorsal and two bilateral muscular incisions made under ether anesthesia. After suturing, all rats were placed in individual cages for a 10-day recovery period. Insulin administration and water intake measurement were performed as described in Experiment 1.

Experiment 2B: Testosterone or Estradiol Effects on Insulin-Induced Drinking in Castrated Adult Male and Female Rats

To the animals of Experiment 2A, SC injections of 600 μg of testosterone enantate (TESTOVIRON-DEPOT, Schering España, S.A.) were administered for 4 days. On the fourth day, insulin was also administered as above and insulin-induced drinking was observed according to the standard protocol. After 1 month of recovery, these rats received SC injections of 200 μg of estradiol valerianate (PROGYNON-DEPOT, Schering España, S.A.) for 4 days. On the fourth day, insulin was again administered and insulin-induced drinking recorded.

Experiment 3: Prepubertally Castrated Male and Female Rats Insulin-Induced Drinking: Effects of Gonadal Hormones

Male (n = 12) and female (n = 12) rats were castrated at the age of 25–30 days, as described in Experiment 1B. Males with testicular descent and females with opening of the vagina were discarded. The castrated animals were placed in collective cages separated by sex, until they reached 3 months of age. Then the protocols of Experiments 1 (insulin-induced drinking test) and 2B (insulin-induced drinking test after sexual hormones administration) were performed on these rats.

Experiment 4: Perinatally Castrated Male and Female Rats Insulin-Induced Drinking: Effects of Gonadal Hormones

Male (n = 11) and female (n = 10) rats were castrated at the age of 2–3 days, under hypothermic anesthesia. For this the pups were maintained in the freezer at −15°C for 5 min approximately. They were then placed on a covered Petri dish containing ground ice. Testicles were removed through a single ventrolateral incision performed in an inguinal fossa. Ovaries were removed through two dorsolateral incisions. In all cases the borders of the surgical incisions were immediately glued with collodion. The animals were returned to the dams until the age of 21 days, when they were weaned, separated by sex, and placed in collective cages until they reached the adult age. All the steps (sex hormone administration and insulin-induced drinking tests) refered to in Experiment 3 were then followed in this group of rats.

Statistics

ANOVA, Fisher's exact probability, Student's paired or unpaired two-tail t-tests, and Mann–Whitney or Wilcoxon Signed Rank Test were used when appropriate. All values of $p < 0.05$ were deemed statistically significant. Values were always expressed as mean ± standard error of the mean.

RESULTS

Experiment 1: Intact Adult Male and Female Rats

There was a significant insulin-induced drinking in male rats at 60 min (insulin vs. saline; U = 50; n = 15, 15; $p < 0.01$) and at 120 min (insulin vs. saline; U = 52; n = 15, 15; $p < 0.001$). The dipsogenic effect of insulin appeared to last at least 2 h, as the total intake of males after 2 h was significantly higher than after 1 hour (S = 0; n = 15; < 0.05) (Fig. 1).

Female rats behaved in a similar way (Fig. 2). In fact, insulin-induced drinking was significant after 1 and 2 h in every one of the phases of the estrous cycle ($p < 0.001$). Nevertheless, insulin-induced drinking among different phases of the estrous cycle did not show significant differences, F (3, 50) = 1.01, NS, at 60 min; F (3, 50) = 0.83, NS, at 120 min.

Insulin-induced drinking in males was significantly lower than in female rats in estrous, t (27) = 2.4, $p < 0.05$ at 60′;

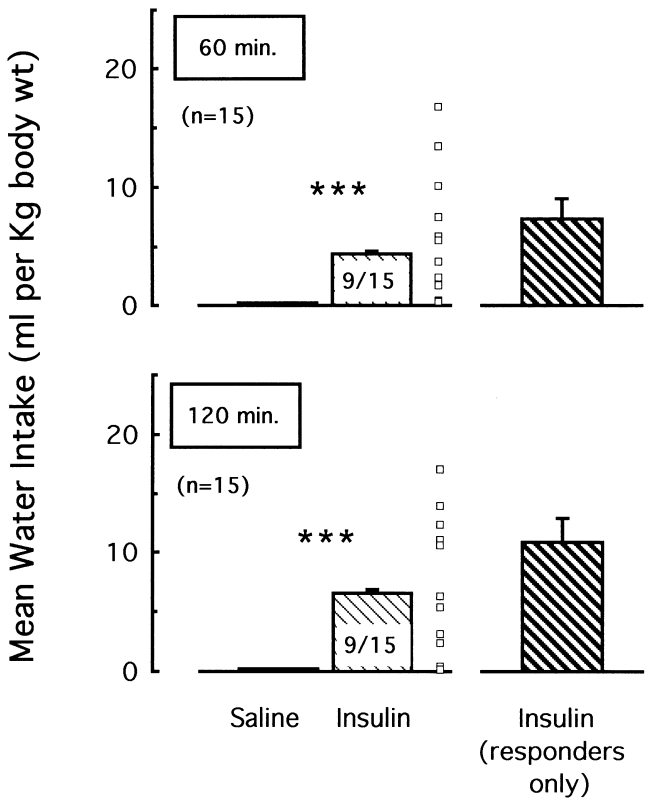

FIG. 1. Water drunk by adult male rats during 1 and 2 h after IP administration of either 5 U/kg b.wt. of Insulin or an identical volume of 0.9% saline (1.0 ml/kg b.wt.). Empty boxes represent individual data of the water drunk after administration of insulin. The fraction included in the bar represents the number of rats that drank more than 1 ml/kg after insulin administration. ***$p < 0.001$ insulin vs. saline.

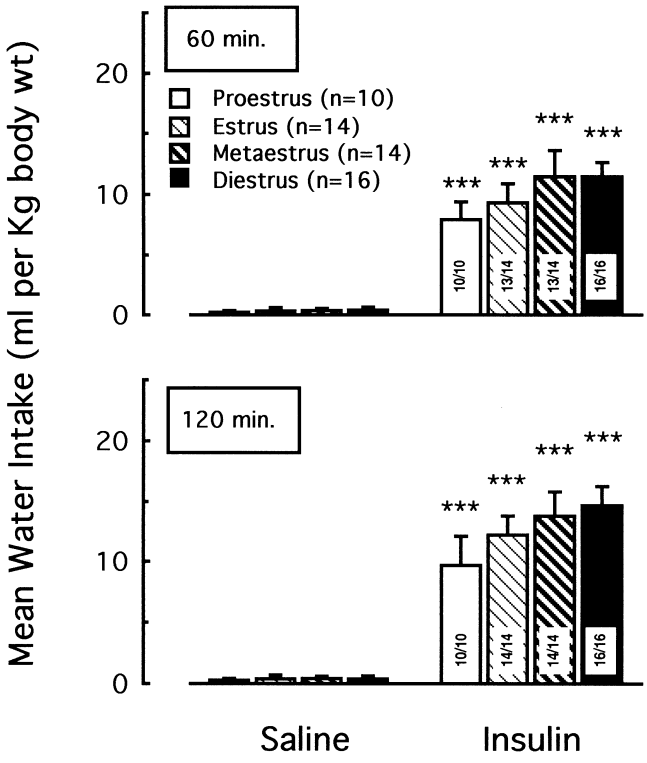

FIG. 2. Water drunk by adult female rats in different phases of their estrous cycle during 1 and 2 h after IP administration of either 5 U/kg b.wt. of Insulin or an identical volume of 0.9% saline (1.0 ml/kg b.wt.). The fraction included in the bar represents the number of rats that drank more than 1 ml/kg after Insulin administration. ***$p <$ 0.001 insulin vs. saline.

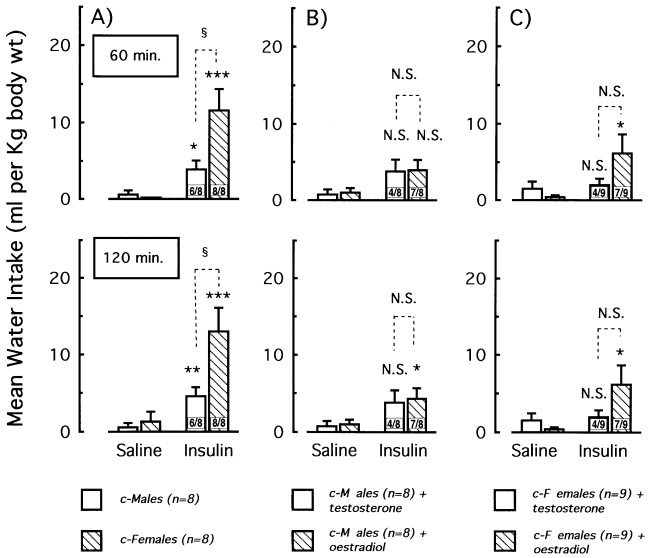

FIG. 3. Water drunk by adult castrated male (c-male) and female (c-female) rats treated and nontreated with testosterone or estradiol, during 1 and 2 h after IP administration of either 5 U/kg b.wt. of Insulin or an identical volume of 0.9% saline (1.0 ml/kg b.wt.). The fraction included in the bar represents the number of rats that drank more than 1 ml/kg after insulin administration. *$p < 0.05$ insulin vs. saline. **$p < 0.01$ insulin vs. saline. ***$p < 0.001$ insulin vs. saline. $^{\S}p < 0.05$ male vs. female. N.S. not significant.

t (27) = 2.29, $p < 0.05$, at 120′, diestrous, t (29) = 3.92, $p <$ 0.001 at 60′; t (29) = 3.33, $p < 0.01$, at 120′, and metaestrous, t (27) = 2.8, $p < 0.01$, at 60′; t (27) = 2.6, $p < 0.05$, at 120′. However, the differences were not significant amongst males vs. females in proestrous, t (23) = 1.71, NS at 60′; t (23) = 1.05, NS at 120′.

There are evident sensitivity differences to insulin among individuals in both sexes, as only 9 out of 15 males responded to the insulin with an intake equal to or higher than 1 ml/kg, whereas cycling females responded in a bigger proportion (10 out of 10 during proestrous, 13 out of 14 during estrous, 13 out of 14 during metaestrous and 16 out of 16 during diestrous) after 1 h, and in all these cases after 2 h. The difference in the proportion of male and female responders reached statistical significance (Fisher's exact probability test: p = 0.028 to 0.007) in all phases of the estrous cycle, 1 and 2 h after insulin injection.

Experiment 2A: Castrated Adult Male and Female Rats

There was significant insulin-induced drinking in castrated adult male rats at 60′, t (14) = 2.55, $p < 0.05$, and at 120′, t (14) = 3.07, $p < 0.01$. Castrated adult female rats receiving insulin also drank significantly more than controls at 60′, t *(14)* = 4.11, $p < 0.001$, and 120′, t (14) = 4.15, $p < 0.001$ (Fig. 3A).

Insulin-induced drinking was significantly different among both sexes. The average volume of water drunk in 1 h by castrated adult female rats was 11.6 ± 2.8 ml vs. 3.9 ± 1.2 ml in castrated adult male rats, t (14) = 2.53, $p < 0.05$. After 120′ the results were 13.0 ± 3.09 ml vs. 4.60 ± 1.17 ml, t (14) = 2.54, $p < 0.05$. Although some differences in individual sensitivity to insulin among the sexes persisted (eight out of eight females responded against only six out of eight males), the difference in that proportions was not statistically significant.

Experiment 2B: Testosterone or Estradiol Effects on Insulin-Induced Drinking in Castrated Adult Male and Female rats

Castrated adult male rats treated with testosterone, failed to drink more after insulin than after saline at 60′, t (14) = 1.76, NS, and 120′, t (14) = 1.80, NS. Castrated male rats treated with estradiol did not drink significantly more than those receiving saline after 60′, t (14) = 1.98, NS, although insulin-induced drinking became significant after 120′, t (14) = 2.18, $p < 0.05$ (Fig. 3B).

The average volume of water drank in response to insulin by castrated adult male rats treated with testosterone or oestradiol, was similar at 60″, t (14) = 0.08, NS, and at 120′, t (14) = 0.23, NS (Fig. 3B).

Testosterone administered to castrated adult female rats also abolished insulin-induced drinking at 60′, t (8) = 0.34, NS, and 120′, t (18) = 1.63, NS, only four out of nine rats responding to insulin at 120′. These castrated adult female rats when treated with estradiol showed a significant insulin-induced drinking at 60′, t (8) = 2.29, $p < 0.05$, and at 120′, t (8) = 2.32, $p < 0.05$, seven out of nine rats responding at both times (Fig. 3C).

Castrated adult female rats treated with testosterone drank significantly less water after insulin than noncastrated rats in any of the phases or their sexual cycle: Diestrous, t (23) = 5.63, $p < 0.001$ at 60′, and t (23) = 4.69, $p < 0.001$ at 120′, me-

taestrous, t (21) = 3.41, $p < 0.01$ at 60′, and t (21) = 3.65, $p < 0.01$ at 120′, estrous, t (21) 3.61, $p < 0.01$ at 60′, and t (21) = 3.8, $p < 0.01$ at 120′, and proestrous, t (17) = 3.42, $p < 0.01$ at 60′, and t (17) = 2.21, $p < 0.05$ at 120′.

Castrated adult female rats treated with estradiol showed a similar level of insulin-induced drinking than intact female in estrous, t (20) = 1.34, NS, in proestrous, t (11) = 0.58, NS, and in metaestrous, t (21) = 1.78, NS. On the contrary in these rats insulin-induced drinking was significantly lower than in intact rats during diestrous, t (23) = 2.32, $p < 0.05$. The above results refer to intakes 2 h after insulin injection. Results concerning 1 h response were the same.

Experiment 3: Prepubertally Castrated Male and Female Rats Insulin-Induced Drinking: Effect of Gonadal Hormones

Insulin-induced drinking in prepubertally castrated male rats, tested when adults, showed a statistical significance at 60′, t (21) = 2.05, $p < 0.05$, and at 120′, t (21) = 2.26, $p < 0.05$. Prepubertally castrated female rats, also showed a significant insulin-induced drinking at 60′, t (22) = 2.17, $p < 0.05$, and at 120′, t (22) = 2.33, $p < 0.05$. Sexual differences on insulin-induced drinking were not found after 60′, t (22) = 0.85, NS, nor after 120′, t (22) = 1.49, NS (Fig. 4A).

Insulin-induced drinking in prepubertally castrated male rats, treated with testosterone and tested when adults was statistically significant at 60′, t (20) = 2.39, $p < 0.05$, and at 120′, t (20) = 2.47, $p < 0.05$. These rats treated with estradiol also showed a significant insulin-induced drinking, t (18) 4.44, $p < 0.001$, at 60′; t (18) 4.10, $p < 0.001$, at 120′ (Fig. 7). Response to insulin with testosterone or estradiol did not differ 1 h after insulin injection, t (19) = 1.83, NS, nor after 2 h, t (19) = 1.49, NS (Fig. 4B).

A significant insulin-induced drinking persisted in prepubertally castrated female rats when treated with testosterone, t (18) = 2.21, $p < 0.05$ after 60′; t (18) = 2.70, $p < 0.05$ after 120′. There was also a significant insulin-induced drinking when they were treated with estradiol, t (8) = 2.01, $p < 0.05$ after 60′; t (8) = 2.334, $p < 0.05$ at 120′ (Fig. 4, B and C). Response to insulin with testosterone or estradiol did not differ 1 h after insulin injection, t (14) = 0.66, NS, but was significantly higher in estradiol-treated rats 2 h after insulin injection, t (14) = 2.47, $p < 0.05$ (Fig. 4C).

Experiment 4: Perinatally Castrated Male and Female Rats Insulin-Induced Drinking: Effects of Gonadal Hormones

Male rats castrated at newborn age and then tested in the adulthood showed a significant insulin-induced drinking, t (20) = 2.69, $p < 0.05$, at 60′; t (20) = 2.32, $p < 0.05$, at 120′, whereas females did not, t (18) = 1.37, NS, at 60′, t (18) = 1.91, NS, at 120′. In these rats sexual differences on insulin-induced drinking were not found after 60′ minutes, t (19) = 1.05, NS, nor at 120′, t (19) = 0.72, NS (Fig. 5A).

Insulin-induced drinking disappeared in males castrated when new born, treated with testosterone and tested in the adulthood, t (15) = 0.94, NS, at 60′; t (15) = 1.64, NS, at 120′. On the contrary, when these rats were treated with estradiol, a significant insulin-induced drinking was found again, t (7) = 2.83, $p < 0.05$, at 60′; t (7) = 2.60, $p < 0.05$, at 120′. The response to insulin in this group was significantly higher in estradiol-treated rats, 1 h, t (15) = 2.79, $p < 0.05$ and 2 h after insulin injection, t (15) = 2.17, $p < 0.05$ (Fig. 5B).

In female rats castrated when newborn, after treatment with testosterone, there is not a significant insulin-induced drinking after 60′, t (9) = 1.72, NS, although it became signifi-

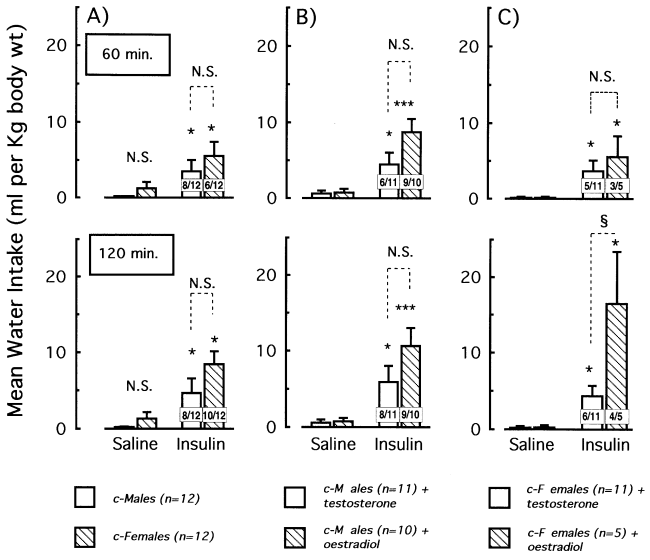

FIG. 4. Water drunk by prepubertally castrated male (c-male) and female (c-female) rats treated and nontreated with testosterone or estradiol, during 1 and 2 h after IP administration of either 5 U/kg b.wt. of Insulin or an identical volume of 0.9% saline (1.0 ml.kg b.wt.). The fraction included in the bar represents the number of rats that drank more than 1 ml/kg after Insulin administration. *$p < 0.05$ insulin vs. saline. ***$p < 0.001$ insulin vs. saline. §$p < 0.05$ female treated with testosterone vs. female treated with estradiol. N.S. not significant.

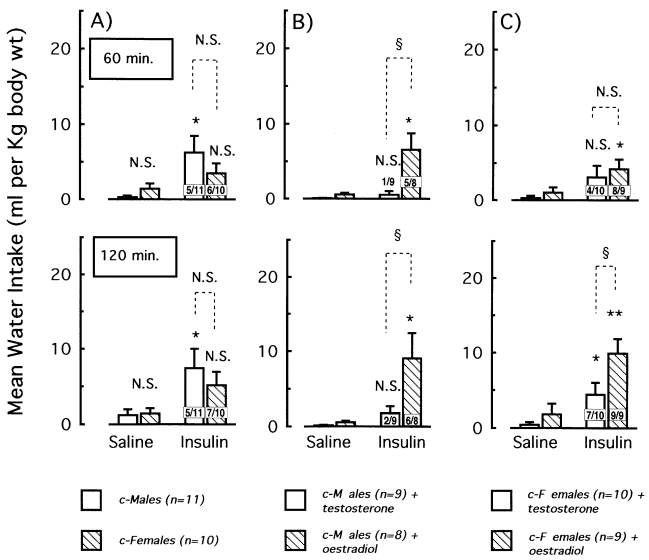

FIG. 5. Water drunk by perinatally castrated male (c-male) and female (c-female) rats treated and nontreated with testosterone or estradiol, during 1 and 2 h after IP administration of either 5 U/kg b.wt. of Insulin or an identical volume of 0.9% saline (1.0 ml/kg b.wt.). The fraction included in the bar represents the number of rats that drank more than 1 ml/kg after Insulin administration. *$p < 0.05$ insulin vs. saline. **$p < 0.01$ insulin vs. saline. §$p < 0.05$ male or female treated with testosterone vs. male or female treated with estradiol. N.S. not significant.

cant after 2 h, t (9) = 2.32, $p < 0.05$. When these rats were treated with estradiol, insulin-induced drinking was significant (1 h: t (16) = 2.14, $p < 0.05$; 2 h: t (16) = 3.36, $p < 0.01$). Response to insulin with testosterone or estradiol did not differ 1 h after insulin injection, t (17) = 0.53, NS, but was significantly higher in estradiol treated rats after 2 h, t (17) = 2.18, $p < 0.05$ (Fig. 5C).

DISCUSSION

Our results indicate that insulin-induced drinking is a phenomenon sensible to gonadal hormones. From the experiments whose results are described above, it can be considered that a difference in thirst response to insulin could be dependent on early endocrine environment, which induced a more vigorous response in females than in males. At the same time, a direct effect of exogenous gonadal hormones seems to affect in a more immediate way the insulin-induced drinking response. Testosterone reduces intake, whereas estradiol tends to increase it. A summary of the results is shown in Fig. 6 and can be followed to illustrate and direct this discussion. Water intakes in all experiments are expressed as ml/kg b.wt. Considering that female rats weigh less than males, some significant differences in thirst could diminish or even disappear had the intake been expressed as actual absolute volumes.

Our results are in agreement with reports that indicate that insulin administration induces water intake in rats (2, 20,26,27) and humans (31,32). Different thirst stimuli are more effective in female than in male rats, as has been reported for the responses to Angiotensin II (AII) (30), or polyethyleneglycol (29). In our study insulin-induced drinking in females was significantly greater than in males, independently of their sexual phase. These sexual differences in water intake after insulin appear to be related to some extent to differences in individual sensitivity or threshold because a significantly higher proportion of females showed response to insulin than males.

The relation between exogenous AII and thirst is well known (4,6,10–12,33,34). A great number of experimental situations in which the animals show a dipsic response has been related to increased endogenous AII levels (5,9,15,29). Previous studies in our laboratory (20,31,32) showed that injections of 5 U/kg of insulin induced an increase in plasmatic renin activity (PRA), in agreement with results of others who also reported an increase in PRA after insulin administration in humans (22) and in rats (3). Because sarile-AII (an AII brain receptor blocker) diminishes insulin-induced drinking (20), central AII receptors seem to be involved. In addition, sexual differences in drinking response to AII or in the renin-angiotensin system (RAS) (8,13,18,29,30) have also been reported. The insulin-induced drinking differences described here are compatible with the hypothesis of an activation of the endogenous RAS as the cause of insulin drinking. Moreover, the sexual differences observed in insulin-induced drinking match, to a great extent, those already observed in response to exogenous AII. In fact, sexual differences in insulin-induced drinking were not affected by castration in adult rats. This is in agreement with results reported elsewhere in which using Angiotensin as a thirst stimulator (30), the manifestation of sexual differences in drinking behavior was affected by differentiation that takes place just before or during puberty. On the other hand, when castration is performed before adulthood, sexual differences in insulin-induced drinking tend to disappear (Fig. 6). The pubertal period (puberty), according to our results, is the critical moment in which the influence of sexual factors, presumably gonadal hormones, determine the future pattern of dipsogenic responses to insulin. According to this, female rats that pass the pubertal crisis having an intact sexual status will become more sensitive to insulin when adult than those who have undergone castration before that moment (prepubertally or when new born). The role of the gonadal hormones in sexual differentiation has been amply documented in different fields (1,21,23,25,28), including insulin hepatocyte dynamics in rats (19), and this seems also to be the case of insulin-induced drinking.

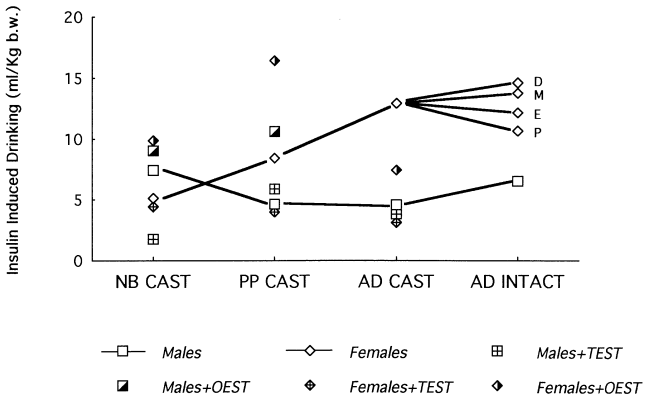

FIG. 6. Summary diagram showing results of insulin induced drinking during 120′ in several groups of male and female rats, castrated and noncastrated, treated and nontreated with sex hormones. D = Diestrous; P = proestrous; E = estrous; M = metaestrous.

The direct role of gonadal hormones in the insulin-induced drinking response was also investigated. The most common pattern of influence of gonadal hormones seen in our experiments is that exogenous estradiol induced a bigger responsiveness to insulin in rats (male and female) in comparison to testosterone treatment.

Accepting the above hypothesis of a role for gonadal hormones in the determination of insulin-induced drinking response, noncastrated adult male and female rats responded as was expected. Male rats (endogenous testosterone present) always drank less than females rats did in any of their phases of the sexual cycle. On the contrary, the lack of differences in insulin-induced drinking responses of female rats in different sexual phases could indicate that the titre of estradiol in these rats is in a different range than that induced by external source in the other experiments, or that these rats have reached already a high sensitive status as a consequence of their maturation as intact females.

We can conclude that insulin-induced drinking has a sex-dependent component and its differentiation seems to take place during puberty. The sexual differences in response to insulin could be related to several factors including differences in central thirst mechanisms, which could be determined during sexual maturation. Further research is necessary to clarify the immediate cause of the phenomenon described here, and in particular why almost half of the intact male rats did not responded to insulin.

ACKNOWLEDGEMENTS

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