Original Article

Assessment of the interaction of progesterone and estrogen administered intraperitoneally on food intake, water intake and body weight in ovariectomized albino rats

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Abstract

Background and Aim: The gonadal hormones like estrogen and progesterone play important role in the regulation of ingestive behaviors and body weight. Recent report from our laboratory indicates the inhibitory role of intraperitoneally injected estrogen on food intake in rats. However, the reports of progesterone, the other important female gonadal hormone on ingestive behaviors are not clearly elucidated. Moreover, the interaction of progesterone with estrogen in mediating ingestive behaviors is also not clear. Therefore in the present study, we have assessed the effects of progesterone injected alone and in combination with estrogen on ingestive behaviors and body weight in ovariectomized albino rats.

Methods: Bilateral ovariectomy was performed in 24 rats, and postovariectomy basal food and water intake and body weight were recorded. The animals were divided into two groups: progesterone group (n = 12) and estrogen-progesterone group (n = 12). Three different dosages (5, 10 and 15 μg/ml) of progesterone were injected intraperitoneal (i.p.) separately and in combination with estrogen on 5 days, following which 24 h food and water intake and body weight were recorded for each injection.

Results: It was observed that ovariectomy significantly increases food intake, water intake and body weight. I.p. injection of progesterone resulted in a dose-dependent increase in water intake without significant alteration in food intake and body weight. Anorectic effect of estrogen and dipsogenic effect of progesterone were prominent when estrogen and progesterone were injected combinely.

Conclusion: It was concluded that estrogen inhibits food and water intake and progesterone facilitates water intake without affecting body weight. The effects of estrogen and progesterone are independent of each other.

Key words: Body weight, estrogen, food intake, ovariectomy, progesterone, water intake

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INTRODUCTION

Gonadal hormones, especially estrogen has been described to affect food intake and body weight in animal models.[1,2] Recently conducted study from our laboratory demonstrates that the ovariectomy results in a significant increase in appetite and sustained increase in body weight indicating that ovarian hormones in general inhibit food intake and body weight gain.[3] Report of the same study also suggests that intraperitoneal (i.p.) injection of estrogen for a shorter duration inhibits food intake without significantly altering body weight in ovariectomized rats. Studies in various animal models following ovariectomy show that gonadal hormones significantly influence the control of food intake, body weight and metabolism of adipose tissue.[4-6] However, reports of earlier studies on the effects of progesterone on food intake and body weight gain are limited and conflicting.[7-10] Moreover, to the best of our knowledge, there is no report of progesterone and combined effects of estrogen and progesterone on the process of dipsogenesis.

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Expression of appetite and the motivational drive toward an energy source are highly regulated phenomenon invertebrates. These are considered as cornerstones for maintenance of energy homeostasis and for strictly guarding the body weight around a set point. The control of body weight is multifactorial, in which food intake plays an important role in the regulation of this important physiological parameter. Many hormones like the cholecystokinin, ghrelin, neuropeptide Y, leptin etc., are known to influence body weight mainly by influencing food intake.[11] Obesity is more prevalent in females than in males.[12] Estrogen, the major female gonadal hormone, is known to influence food intake by altering the plasma level of CCK, neuropeptide Y, angiotensin II and leptin.[13-17] However, the role of progesterone on food intake and body weight, and especially the interaction of progesterone with estrogen on mediating these effects have not been elucidated. It has been observed that changes occur in ingestive behaviors and body weight gain during different phases of the menstrual cycle in women.[18-24] These changes are suggested to be in response to the fluctuations in the hormonal status of women during the cycle. A study in human beings reveals that food intake is less during the peri-ovulatory period during which the estrogen level is high, and water intake is more in the postovulatory period during which the progesterone level is high.[19] However, no study has been done to correlate the hormonal changes with the changes in the pattern of these ingestive behaviors. Reports also indicate that progesterone *per se* does not significantly influence food intake and body weight, rather influences these parameters in the presence of estrogen.[7,8] However, no systematic work has been done to demonstrate the involvement of gonadal hormones and their interactions in these behavioral changes. Therefore, in the present study, we have attempted to assess the individual and combined effects of these ovarian hormones on ingestive behaviors and body weight.

Alteration in food intake is known to be associated with change in water intake. This type of alteration is called as prandial drinking.[25-27] Therefore, in the present study, we have included water intake with food intake and body weight measurements to assess the changes in water intake if present as prandial drinking or induced primarily by gonadal hormones.

MATERIALS AND METHODS

Institute-bred 24 female albino rats of Wistar strain with body weights of 200-240 g were used for this study after obtaining approval of JIPMER Animal Ethics Committee (CPCSEA). Each animal was kept in a separate cage. The temperature of the room in which the animals were kept was between 28°C and 32°C. Animals were exposed to 24 h natural light-dark cycle. Food and water were provided *ad lib*.

Basal food intake, water intake and body weight measurements

The food and water were provided at 10.00 h, and every day following which 24 h food and water intakes were measured for each animal. For water intake, the tap water at room temperature (28-32°C) was provided in a calibrated glass cylinder with a sprout. The cylinder had the provision of measuring the minimum of 0.5 ml. The food was given in the form of standard rodent chow (food pellets). The food ingested in 24 h was measured by weighing the food before placing food in the cage and again after 24 h by collecting the remaining food from the cage. The difference between these two weights was taken as a 24 h intake. Weight of the food was measured by using electronic weighing machine. Daily food and water intakes were recorded for 7 consecutive days to determine the 24 h mean basal food intake of each animal. Body weight of each rat was measured for 7 days to obtain the mean body weight.

Ovariectomy

Bilateral ovariectomy was performed in all animals using aseptic precautions under ketamine anesthesia (100 mg/kg body weight, i.p.). For performing ovariectomy, a paramedian midline incision was made in the lower abdomen of the animal and the peritoneum was cut. The didelphus uterus of the rat was identified, and the fallopian tubes were traced upwards and laterally to locate the ovarian end of the tube. The ovary was identified as a yellowish flowery 1-2 mm structure and was carefully excised from the surrounding fibro-fatty tissue. Gentle pressure was applied for 15-30 s to avoid bleeding from the raw area. Similarly, the other side ovary was also removed. The abdominal wall was closed in layers. Prophylactic antibiotic cover was provided by administering penicillin locally at the site of the abdominal incision and as a single i.p. injection in the postoperative state. The removed ovaries were later confirmed by histological examination under light microscope by the presence of the characteristic follicular arrangement of cells.

Postovariectomy basal measurements

Animals were allowed 2 weeks for recovery, after which the postovariectomy basal mean food and water intake and body weight were recorded in a similar way to that of their preovariectomy measurements.

Preparation of different dosages of chemicals

Progesterone powder (Sigma, USA) was dissolved in a given volume of sesame oil to obtain solutions with progesterone concentrations of 5 μg/ml, 10 μg/ml and

15 μg/ml. Estradiol benzoate (Sigma, USA) solution was also diluted in sesame oil to make the solution of 15 μg/ml dosage. The solutions were stored in the refrigerator. At the time of injection, the necessary volume of solution to be injected was taken out of the refrigerator and was allowed to reach the room temperature before injecting into the animals.

Experimental protocol

The animals were divided into two groups; progesterone group (n = 12) and estrogen-progesterone group (n = 12).

Progesterone group

Animals were divided equally into experimental (n = 6) and control (n = 6) groups. In experimental group, three different dosages (5, 10 and 15 μg; each dissolved in 1 ml of sesame oil) of progesterone were injected i.p., every day at 10 h following which 24 h food and water intake, and body weight were recorded. The dosages were injected randomly to the animals in such a way that all animals received all the 3 doses of progesterone 5 times on different days. The interval between administrations of the two different doses of the chemical was 24 h. The control animal received equal volume (1 ml) of sesame oil without any chemical following which their 24 h food and water intake and body weight were recorded.

Estrogen-progesterone group

24 h food and water intake and body weight were measured following the i.p. injection of maximum effective dose of estrogen (15 μg/ml) and progesterone (15 μg/ml) alone and then in combination. The maximum effective dose of estrogen was detected in our previous study[3] and the maximum effective dose of progesterone was detected in the present study (as observed in the above experiment). In the control group of 6 animals, an equal volume (2 ml) of sesame oil without any chemical was injected intraperitoneally.

Statistical analysis of data

Statistical Package for Social Sciences (SPSS) version 19 (SPSS Software Inc, Chicago, IL, USA) was used for statistical analysis. All the data were presented as mean ± standard error. The P values less than 0.05 was considered statistically significant. The level of significance between the groups was assessed by using one-way ANOVA.

RESULTS

The basal food and water intake and body weight (mean ± standard error) of all rats (n = 18) was 18.12 ± 0.45 g, 26.10 ± 0.64 ml and 210.60 ± 3.80 g, respectively.

Effects of ovariectomy

Following ovariectomy, food intake and body weight were increased very significantly (P < 0.001), whereas increase in water intake was just significant (P < 0.05) [Table 1].

Progesterone group

There was a dose-dependent increase in water intake without change in food intake following injection of doses of progesterone [Table 2]. No significant change was observed in the body weight. No significant change in these parameters was detected in the control group.

Estrogen-progesterone group

This experiment compares the maximum effective doses of estrogen and progesterone injected separately and in

Table 1: Change in 24 h food intake (g), water intake (ml) and body weight (g) following ovariectomy in albino Wistar rats (n=18)

	Mean±SE		
	Food intake	Water intake	Body weight
Preovariectomy	18.12±0.45	26.10±0.64	210.60±3.80
Postovariectomy	23.86±0.50**	28.74±0.70*	248.10±4.10**

*P<0.05, **P<0.0001. SE: Standard error

Table 2: Change in 24 h food intake (g), water intakes (ml) and body weight (g) following injection of different dosages of progesterone intraperitoneally in ovariectomised experimental rats (n=6)

Progesterone doses (μg/ml)	Mean±SE		
	Food intake	Water intake	Body weight
Ovariectomy basal (zero dose)	23.52±0.58	28.46±0.68	244.70±3.70
5	23.98±0.60	29.70±0.66	245.80±3.20
10	24.16±0.72	31.80±0.60	249.60±3.60
15	24.24±0.56	32.84±0.80	251.3±3.80
dF/F	3.20/0.2717	3.20/8.294	3.20/0.7
P	>0.05	0.0009	>0.05
Inter-group comparison			
Ovariectomy basal versus 5 μg dose	NS	NS	NS
Ovariectomy basal versus 10 μg dose	NS	<0.05	NS
Ovariectomy basal versus 15 μg dose	NS	<0.01	NS
5 μg dose versus 10 μg dose	NS	NS	NS
5 μg dose versus 15 μg dose	NS	<0.05	NS
10 μg dose versus 15 μg dose	NS	NS	NS

The values are mean±SEM, ovariectomy basal is the mean of 10 days basal recording following ovariectomy. Test for comparison was done by one-way ANOVA (Tukey–Kramer multiple comparison test). NS change was observed in control rats that received equal volume of vehicle. SEM: Standard error of the mean, SE: Standard error, NS: No statistically significant

Table 3: Change in 24 h food intake (g), water intakes (ml) and body weight (g) following injection of maximum dosages of estrogen (15 μg) and progesterone (15 μg) intraperitoneally (individually and combinely) in ovariectomized albino rats

	Mean±SE		
	Food intake	Water intake	Body weight
Ovariectomy basal	23.80±0.56	28.60±0.62	247.90±3.68
Estrogen (E)	17.60±0.70	25.58±0.40	238.64±3.20
Progesterone (P)	24.96±0.64	33.14±0.90	252.10±4.26
E+P	20.72±0.52	32.56±0.84	248.60±3.90
df/F	320/27.632	320/24.535	320/1.212
P	<0.0001	<0.0001	>0.05
Inter-group comparison			
Ovariectomy basal versus estrogen	<0.001	<0.05	NS
Ovariectomy basal versus progesterone	NS	<0.01	NS
Ovariectomy basal versus E+P	<0.01	<0.01	NS
Estrogen versus progesterone	<0.001	<0.001	NS
Estrogen versus E+P	NS	<0.001	NS
Progesterone versus E+P	<0.001	NS	NS

The values are mean±SEM; ovariectomy basal is the mean of 10 days basal recording following ovariectomy. Test for comparison was done by one-way ANOVA (Tukey–Kramer multiple comparison test). NS change was observed in control rats that received equal volume of vehicle. SEM: Standard error of the mean, SE: Standard error, NS: No statistically significant

combination on ingestive behaviors and body weight [Table 3].

The maximum effective dose of estrogen was detected in our previous study[3] and the maximum effective dose of progesterone was detected in the present study [Table 2]. When injected alone, estrogen had significant inhibition on food intake, mild inhibition on water intake and no statistically significant on body weight, and progesterone had moderate inhibitory effect on water intake without significantly altering food intake and body weight. When injected combined, the degree of inhibition of food intake was reduced than the inhibition produced by estrogen alone. However, the facilitation of water intake on combined injection was similar to the facilitation produced by progesterone alone. No statistically significant change was observed in the body weight on combined injection.

DISCUSSION

Ovariectomy resulted in an increase in food and water intake and body weight. The increase in food intake and body weight ($P < 0.0001$) was more significant than the increase in water intake ($P < 0.05$). This suggests that normally the ovarian hormones strongly inhibit food intake and body weight and mildly inhibit water intake. The important hormones secreted from the ovaries are estrogen and progesterone. Estrogen is known to inhibit food intake and body weight gain.[5,6,28,29] Result of our previous study also indicates the same.[3] However, it is not known whether estrogen interacts with progesterone in intact animals in eliciting these effects. It has been observed in earlier studies that withdrawal of ovarian hormones in adult female rats by ovariectomy induces the hyperphagia and rapid weight gain, which lasts for about a month. The hyperphagia subsides thereafter but, increase in body weight is maintained above 20% of normal for a longer period.[6,30] The findings of our previous study and the present study are almost similar to the reports of these earlier studies.

In the present study, i.p. injections of progesterone resulted in an increase in water intake in a dose dependent manner without significant change in food intake and body weight. This indicates the profound influence of progesterone on water intake. As water intake was not associated with an increase in food intake, it can be concluded that it was not a prandial drinking. However, progesterone injection did not result in a significant change in food intake and body weight. It may be due to the fact that progesterone injected alone (in the absence of estrogen) in ovariectomized rats does not affect food intake and body weight as reported earlier.[7,8] Therefore, progesterone should be injected with estrogen in different combinations to see its interaction with estrogen on these parameters, for which the third experiment was performed in the present study.

It is interesting to note that in pregnant women, weight gain occurs slowly in the first trimester, which becomes faster in the later part of pregnancy. Pregnancy is an ideal example for a physiological process causing changes in the plasma gonadal hormone concentration. Progesterone concentration increases in the early pregnancy and then falls, and the estrogen concentration rises toward the later part of pregnancy.[31] Weight gain in the later part of pregnancy is due to the rapid growth of the fetus and enlarging uterus and placenta. However, the mechanism of weight gain in the early part of the pregnancy is not known. Usually, hormones that increase water intake like angiotensin II and antidiuretic hormone also increase water reabsorption from the kidney. Therefore, it should be investigated whether progesterone also promotes water retention in addition to its dipsogenic property and contributes to weight gain in pregnancy.

In the present study, treatment of estrogen alone in ovariectomized rats not only abolished hyperphagia, but also produced significant hypophagia, though this change was not associated with proportionate loss of body weight [Table 3]. This indicates that the estrogen (not the other ovarian hormones) profoundly inhibits food intake. Normally, estrogen replacement following ovariectomy decreases the appetite and body weight,[5,6] and therefore estrogen therapy had been advocated treatment of obesity in rats.[32,33] However, estrogen at anorectic and body weight inhibiting dose produces widespread metabolic effects.[34,35] Therefore, the use of estrogen in the management of obesity has not been extrapolated to human beings. In the present study, the inhibitory effect of estrogen was not significantly evident on body weight, which may probably be due to the assessment of estrogen effects for a shorter duration in this experiment. As change in body weight takes longer time to manifest, we plan to assess the long-term effects of estrogen in our future studies.

When estrogen and progesterone were injected combined, the degree of inhibition of food intake was less ($P < 0.01$) than the degree of inhibition produced by injection of estrogen alone ($P < 0.001$). This indicates that progesterone opposes the hypophagic effect of estrogen, but estrogen effect predominates over progesterone effect. However, the facilitator effect of progesterone on water intake was almost similar to the facilitation produced by combined injection. This indicates that the hypodipsic effect of estrogen is not strong enough to oppose the hyperdipsic effect of progesterone. Therefore, experiments should be performed in the future to assess the effects of progesterone injected into the known dipsogenic areas in the brain on water intake. The absence of significant change in body weight by combined injection of estrogen and progesterone could be due to the short duration of the study.

Though, literature is abundant to implicate gonadal hormones in the regulation of body weight, the role of progesterone in this regard is not clearly defined. In the present study, except following ovariectomy, alteration in body weight was not observed on i.p. injections of estrogen and progesterone separately and in combination. Body weight was not altered even in the presence of significant alteration in food intake. One possibility for absence of significant body weight gain could be the short-term nature of the study; but other possibilities like ponderostatic hypothesis should also be excluded. It has been suggested that separate structures in the brain exclusively control of body weight. These structures are collectively known its ponderostat. The nuclei in the hypothalamus especially in the medial hypothalamus and the nucleus tractus solitarious are suggested to be the structures mediating the control of body weight without affecting food intake.[36] We presume that estrogen, but not the progesterone might inhibit the ponderostatic activity when injected chronically. Hence, in our future study we plan to inject ovarian hormones into these areas of the brain on a long-term basis to assess their effect on food intake and body weight.

In the present study, progesterone injected alone did not suppress food intake though estrogen strongly inhibited the same. This correlates well with a report of the previous study, which indicates that progesterone inhibits food intake only in the presence of estrogen, but not when injected alone.[37] However, the effect of progesterone and estrogen on water intake was of the opposite nature, estrogen mildly inhibiting it and progesterone moderately facilitating it. This indicates that the estrogen mainly controls food intake and progesterone controls water intake, and these influences are independent of each other. This means estrogen controlling food intake is not dependent on water intake and progesterone controlling water intake is independent of food intake. Therefore, we suggest that the stimulatory effect of progesterone on water intake does not require the presence of estrogen. This also suggests that progesterone could be among the potent dipsogenic neurotransmitters in the brain. However, there is scarcity of reports on the effect of ovarian hormones on ingestive behaviors following injection into different areas of the brain. Though, one study suggests that ovarian hormones can act on brain centers to alter food intake,[38] it does not provide clear location of brain sites for their action. Therefore, future studies should assess the intracerebral injections of gonadal hormones into various mesolimbic areas on ingestive behaviors and body weights.

The control of food intake and body weight is profoundly influenced by alteration in substrate metabolisms.[39,40] Presently due to lack of facilities for measuring metabolism in rats in our laboratory, we could not assess metabolic activities in the experimental animals. Earlier we had reported the effects of administration estrogen and progesterone separately on ingestive behaviors in ovariectomized rats.[3,41] In the present study, we have reported the concurrent administration of estrogen and progesterone, and we found that anorectic effect of estrogen and dipsogenic effect of progesterone are predominant when injected separately.

Limitations of the study

The effects of estrogen and progesterone have been studied in the ovariectomized rat model that does not closely mimic post-menopausal condition in human being.

CONCLUSION

It may be concluded that ovariectomy significantly increases food intake, water intake and body weight. Estrogen on short-term i.p. injection inhibits food intake and water intake without affecting body weight, whereas progesterone increases water intake without affecting food intake and body weight. Anorectic effect of estrogen and dipsogenic effect of progesterone predominate when estrogen and progesterone are injected combinely. The control of food and water intake by ovarian hormones may be independent of each other. Future studies should address if anorectic and body weight inhibiting effects of estrogen are more effective in long-term administration and ovariectomized rats, and if these findings can be utilized in the management of obesity in post-menopausal women.

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