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Research article

## EFFECT OF GONADAL HORMONES ON HYPOPHAGIC PROPERTY OF OPIOID ANTAGONIST NALOXONE

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### ABSTRACT

**Background:** Studies have shown that hormonal fluctuations that occur over the estrous cycle in rats affect food intake. It is possible that estrogen affects food intake via Opioid system and other brain areas which are involved in regulation of food intake. Therefore it may affect the sensitivity of female rats to hypophagic effect of Opioid antagonist Naloxone. Testosterone in male rats also changes food intake. However, little is known about how these Gonadal hormones interact with Opioid receptors to modulate food intake. **Objective:** The aim of the study was to find out how Gonadal hormones affect hypophagic property of Naloxone. **Methods:** Basal food intake of 40 healthy adult females and 20 healthy adult male rats was recorded. Then they were injected intraperitoneally with Naloxone after fasting for 24 hrs. In female rats food intake was measured during different phases of the estrous cycle. All the rats were then subjected to gonadectomy. The food intake was measured after gonadectomy. The effect of Naloxone was also measured in deprivation paradigm after gonadectomy. **Results:** Female rats showed decreased food intake during proestrous and estrous phases. In female rats there was no hypophagia after Naloxone injection during these phases. Male rats showed hypophagia on Naloxone injection. Male rats showed increased food intake after gonadectomy. In female rats the increase in food intake was not significant when gonadectomy was done during metestrous and diestrous. However, Naloxone could induce hypophagia in all female rats after gonadectomy. **Conclusion:** Estrogen decreases food intake, it decreases sensitivity of female rats to hypophagic effects of Naloxone. Testosterone decreases food intake. Testosterone does not interfere with hypophagic effect of Naloxone.

**Keywords:** Food intake, Gonadal hormones, Naloxone, Hypophagia.

### INTRODUCTION

Appetite, energy balance and body weight gain are modulated by diverse neurochemical and neuroendocrine signals from different organs in the body and diverse regions in the brain. Alterations in the regulation of food intake and energy expenditure underlie the development, progression and recurrence of obesity.<sup>1,2</sup> This has been the cause of obesity related complications like diabetes and hypertension etc.

This energy balance and fuel utilization are significantly affected by gonadal hormones, estrogen in females and testosterone in males. Estrous cycle related effects on food intake have been linked to the effects of estrogen on central nervous system and peripheral tissues.<sup>3,4,5</sup> Rodents typically cycle over 4-5 days and phases of estrous cycle are commonly classified by histological changes in vaginal cytology which is roughly divided into estrous, metestrous,

diestrous and proestrous.<sup>6</sup> Food intake is generally increased during diestrous and decreased during estrous.<sup>7</sup> So we confirmed these findings in our laboratory by conducting the present study.

Previous studies suggest that estradiol acts on mu-opioid receptors to modulate the antinociception in rats.<sup>8</sup> So estradiol may also modulate food intake by its action on mu-opioid receptors. Therefore it may affect the anorectic property of Opioid antagonist naloxone during different phases of estrous cycle in female rats.

There are studies which show that in male rats testosterone reduces food intake. Other studies also show that opioid antagonist naloxone facilitates sexual behaviour<sup>9</sup> and there is no satisfactory explanation to this. However, possible explanation could be the interaction between testosterone and Naloxone which can affect food intake as well.

So the present study was undertaken to evaluate if Gonadal hormones alter food intake by acting on opioid receptors in the central nervous system in addition to their action on other parts of the CNS.

## MATERIALS AND METHODS

The present study was approved Institutional Animal Ethics Committee of KIMS, Karad. 40 healthy adult female and 40 healthy adult male Wistar rats were used for the study. The average age was 3-4 months old. Animals were weighed, marked and housed in separate polyvinyl cages in animal room having controlled room temp ( $25 \pm 2^{\circ}\text{C}$ ). They were maintained on 12 hrs dark and 12 hrs light cycle with standard laboratory diet and water ad lib.

First 8 days baseline food intake in all animals were recorded. In female rats food intake was recorded during different phases of estrous cycle.

In deprivation paradigm, the animals were kept fasting for 24 hrs. Then saline 2ml was injected intraperitoneally at 9 am on the day of test. 30 mins after injection the food was weighed and introduced into the cage. Then the food intake was measured at an interval of ½ hr, 1hr, 2hrs and 24hrs. These values were considered as control. Same animals were used as control on 1<sup>st</sup> 24 hrs deprivation with saline injection and on 2<sup>nd</sup> 24 hrs deprivation they were injected with naloxone

The animals were kept fasting for 24 hrs again on the next day. Then 2.5 mg/kg naloxone was injected intraperitoneally at 9 am on the day of test. 30 mins after injection the food was weighed and introduced

into the cage. Then the food intake was measured at an interval of ½ hr, 1hr, 2hrs and 24hrs.

Every time the intake was measured by weighing food prior to and after each condition and adjusting for spillage that was collected in paper towel under wire mesh.

Food intake in deprivation paradigm after saline and after naloxone injection in male and female rats was recorded. In addition to this the female rats' food intake was recorded during different phases of estrous cycle after naloxone injection in deprivation paradigm.

**Vaginal cytology for stage of estrous cycle:** Daily vaginal smears were obtained at 8.30am to assess the stage of estrous cycle. Smears were examined under light microscope. Stage of the cycle was assigned using the following criteria as previously described<sup>6</sup>

1) Proestrous when predominantly nucleated epithelial cells in the absence of leukocytes were present.

2) Estrous when sheets of nonnucleated squamous cornified cells in absence of leucocytes were present.

3) Metestrous (D1) when there was an equal distribution of leucocytes and cornified and nucleated epithelial cells.

4) Diestrous (D2) when a mixture of epithelial cells and leucocytes with predominance of leucocytes was present.

Then both male and female rats were gonadectomised.

**Procedure for ovariectomy:** The female rats were weighed and then injected with atropine sulphate in a dose of 0.25mg subcutaneously to minimise the respiratory discomfort. Intraperitoneal Sodium pentobarbitone in the dose of 35mg/kg body weight was injected for anaesthesia, whereas Ether inhalation was used to maintain a steady level of anaesthesia while doing gonadectomy.

Anaesthetised rat was placed on a rat operating table with ventral surface facing towards operator. Animal was secured properly to the operation table.

Midline incision was taken on lower abdomen extending for 2cms lengthwise. A snip was made through the fascia of abdominal rectus muscle. The points of forceps were forced through the snip and hole was extended opening the forceps. The ovary was found embedded in the fat lying just below the dorsal muscle mass. It was identified by fimbrial end. The ovary was drawn through the incision, uterus clamped in a haemostat and a ligature placed around the uterus just below fallopian tube and was tied tightly. The ovary was removed. Similarly other ovary was also removed. The muscle incision was closed with catgut

and skin incision with thread. Powder Nebasulf was sprinkled over the sutures and Benzathin Penicillin, 3 lakh units was injected intramuscularly to prevent infection. Animal was allowed to recover from anaesthesia and then was transferred to respective cage.

A period of 10 days was allowed for recovery from operative injury following which vaginal smears were examined for 1 week. Continuous diestrous was taken as an indication for successful gonadectomy.

**Procedure for Orchidectomy:** The male rats were weighed and then injected with atropine sulphate in dose of 0.25mg subcutaneously to minimise the respiratory discomfort. Then they were anaesthetised as in female rats. Anaesthetised rat was kept on the rat operating table. Part to be operated was shaved properly. Under aseptic precautions ventral midline incision was made through the skin of the scrotum. The slight pressure was given on abdomen as rats are able to retract testes in abdominal cavity. They were freely movable within the scrotum. One testis was drawn through a skin incision. A slit was made

through tunica and the testis was freed. The spermatic cord which was attached to the testis was doubly ligated and cord was cut between the ties. Other testis was removed similarly. Skin incision was closed with thread sutures. Powder Nebasulf was sprinkled on sutured skin and rat was injected with 3 lakh units of Benzathin Penicillin intramuscularly to prevent infection. 10 days were allowed for recovery from operative injury.

After measuring 8 days basal food intake, all gonadectomised rats were injected with 2.5mg/kg intraperitoneally naloxone after keeping them fasting for 24 hrs. The food intake was measured as was done before gonadectomy. Food intake was measured in grams.

**Statistical analysis:** For data analysis all the values were expressed in terms of mean  $\pm$  standard error of mean. Differences between means were compared by applying paired 't' test. The effect was considered statistically significant if the probability of chance was less than 0.05 ( $p < 0.005$ ).

## RESULT

**Table 1: Food intake in female rats during different phases of estrous cycle**

Phase of estrous cycle	Food intake (in gms.) at different time of the day			
	1hr	1.5hr	2.5hr	24hr
Proestrous	0.6 $\pm$ 0.44*	1.1 $\pm$ 0.30*	2.0 $\pm$ 0.71*	7.1 $\pm$ 0.24*
Estrous	1.35 $\pm$ 0.43	1.86 $\pm$ 0.53	3.3 $\pm$ 0.71	9.5 $\pm$ 0.98
Metestrous	2.87 $\pm$ 0.55*	4.16 $\pm$ 0.98*	6.29 $\pm$ 0.92*	13.16 $\pm$ 0.71*
Diestrous	2.5 $\pm$ 0.15*	3.9 $\pm$ 0.24*	5.3 $\pm$ 0.23*	13.82 $\pm$ 0.39

\*P < 0.05, data presented as Mean  $\pm$  SEM

**Table 2: Effect of different phases of estrous cycle on Naloxone induced hypophagia in deprivation paradigm in female rats.**

Phase of estrous cycle	Food intake (in gms.)			
	1hr	1.5hr	2.5hr	24hr.
<b>Proestrous</b>				
After saline injection	1.7 $\pm$ 0.11	2.75 $\pm$ 0	4.2 $\pm$ 0.37	10.25 $\pm$ 0.33
After Naloxone injection	1.2 $\pm$ 0.32	2.5 $\pm$ 0.16	3.9 $\pm$ 0.24	19.0 $\pm$ 0.39
<b>Estrous</b>				
After saline injection	1.33 $\pm$ 0.40	1.98 $\pm$ 0.46	3.45 $\pm$ 0.42	8.23 $\pm$ 0.77
After Naloxone injection	0.93 $\pm$ 0.09	1.75 $\pm$ 0.27	3.00 $\pm$ 0.42	10.11 $\pm$ 0.29
<b>Metestrous</b>				
After saline injection	3.9 $\pm$ 0.55	5.0 $\pm$ 0.93	7.1 $\pm$ 0.92	15.2 $\pm$ 0.71
After Naloxone injection	1.2 $\pm$ 0.31*	1.98 $\pm$ 0.23*	3.56 $\pm$ 0.53*	14.7 $\pm$ 0.29
<b>Diestrous</b>				
After saline injection	3.1 $\pm$ 0.22	4.5 $\pm$ 0.62	6.8 $\pm$ 1.1	14.3 $\pm$ 0.35
After Naloxone injection	1.5 $\pm$ 0.44*	2.25 $\pm$ 0.33*	4.25 $\pm$ 0.53*	15.2 $\pm$ 0.75

\*P < 0.05, data presented as Mean  $\pm$  SEM

Table 2 shows the effect of food deprivation on food intake in different phases of estrous cycle. After 24 hrs

fasting in female rats during estrous phases there was no significant increase in food intake.

However, in metestrous, diestrous and proestrous phases the food intake was significantly increased after 24 hrs fasting.

This table also shows effect of naloxone on food intake in different phases of estrous cycle. It was seen

that Naloxone induced significant hypophagia in rats after 24hrs fasting in metestrous and diestrous. In proestrous and estrous phases naloxone could not induce hypophagia in deprivation paradigm.

**Table 3: Effect of gonadectomy on food intake and naloxone induced hypophagia in female rats in deprivation paradigm.**

Phase of estrous cycle	Food intake (in gms)			
	1hr	1.5hr	2.5hr	24hr.
<b>Proestrous</b>				
saline injection before gonadectomy	1.7 ± 0.11	2.75 ± 0	4.2 ± 0.37	10.25 ± 0.33
saline injection after gonadectomy	4.8 ± 1.1*	5.89 ± 0.76*	6.7 ± 0.83*	14.6 ± 0.45*
Naloxone injection after gonadectomy	2.9 ± 0.32*	4.2 ± 0.16*	5.3 ± 0.24*	13.9 ± 0.39
<b>Estrous</b>				
saline injection before gonadectomy	1.33 ± 0.40	1.98 ± 0.46	3.45 ± 0.42	8.23 ± 0.77
saline injection after gonadectomy	3.16 ± 0.42*	3.6 ± 0.53*	5.06 ± 0.71*	13.66 ± 0.98*
Naloxone injection after gonadectomy	1.58 ± 0.29*	2.5 ± 0.15*	3.75 ± 0.53*	12.95 ± 1.46
<b>Metestrous</b>				
saline injection before gonadectomy	3.9 ± 0.55	5.0 ± 0.93	6.1 ± 0.92	15.2 ± 0.71
saline injection after gonadectomy	4.4 ± 0.55	5.2 ± 0.44	6.7 ± 0.13	15.6 ± 0.89
Naloxone injection after gonadectomy	2.12 ± 0.31*	3.75 ± 0.23*	4.25 ± 0.53*	15.7 ± 0.29
<b>Diestrous</b>				
saline injection before gonadectomy	3.1 ± 0.22	4.5 ± 0.62	6.8 ± 1.1	14.3 ± 0.35
saline injection after gonadectomy	4.9 ± 0.33*	5.5 ± 0.9*	7.7 ± 0.24*	15.4 ± 0.56*
Naloxone injection after gonadectomy	2.15 ± 0.65*	3.96 ± 1.07*	5.43 ± 0.47*	15.9 ± 0.98

\*P < 0.05, data presented as Mean ± SEM

**Table 4: Food intake after orchidectomy and naloxone injection**

Before Gonadectomy	Food intake (in gms.)			
	1hr	1.5hrs	2.5hrs	24hrs
Basal food intake	2.2 ± 0.27	3.2 ± 0.46	4.6 ± 0.80	15.1 ± 0.82
<b>After 24 hrs fasting</b>				
After Saline injection	3.0 ± 0.36*	4.3 ± 0.55*	5.7 ± 0.82*	15.6 ± 0.82
After Naloxone injection	0.6 ± 0.24*	1.7 ± 0.28*	3.0 ± 0.59*	11.8 ± 1.1
<b>After Gonadectomy</b>				
Basal food intake	4.2 ± 0.48*	5.5 ± 0.45*	6.5 ± 0.60*	17.0 ± 0.71*
<b>After 24hrs fasting</b>	<b>1hr</b>	<b>1.5hrs</b>	<b>2.5hrs</b>	<b>24hrs</b>
After Saline injection	4.9 ± 0.57*	6.2 ± 0.42*	7.2 ± 0.76*	17.2 ± 1.88
After Naloxone injection	1.0 ± 0.19*	1.6 ± 0.42*	3.0 ± 0.59*	11.2 ± 1.37

\*P < 0.05, data presented as Mean ± SEM

Table 3 shows the effect of ovariectomy on food intake and hypophagia induced by Naloxone in female rats. It is seen that the food intake was significantly increased after ovariectomy in all female rats. However, the increase was not significant when ovariectomy was done during metestrous phase.

This increase was more pronounced in the female rats in which ovariectomy was done in estrous and proestrous phases. Naloxone induced significant hypophagia in all rats after ovariectomy in initial period after 24hrs food deprivation.

Table 4 shows food intake in male rats. Food intake is significantly increased after orchidectomy. In

deprivation paradigm naloxone induced hypophagia in initial period of the day before and after orchidectomy.

## DISCUSSION

The present study was designed to examine whether the gonadal hormones affect the hypophagic properties of naloxone upon food deprivation induced hyperphagia. It appears that naloxone induces hypophagia in food deprived male and female rats as compared to controls (saline). These concur with the earlier studies.<sup>10</sup>

It is known that after puberty male rats weigh and eat more than do female rats of same age. This sex difference is more pronounced with age. We also studied the role of sex hormones in regulation of food intake. We found that during the estrous phase the food intake of female rats was less and least in proestrous phase. The food intake was increased during diestrous but it was highest during metestrous. These findings are consistent with other workers.<sup>11-13</sup>

This could be because of wide variations in estrogen levels during the phases of estrous cycle. The sequence of phases in the cycle is proestrous, estrous, metestrous and diestrous. The estrogen levels start rising in diestrous reaching its peak in proestrous and start declining during estrous decreasing to lowest level during metestrous.<sup>14,15</sup> Estrogen is known to affect food intake thorough central and peripheral mechanisms. Several lines of evidence indicate that the effects of estradiol on food intake are mediated by its actions on estrogen receptors within the brain. In the early 1970's, Wade and Zucker were the first to report that direct stimulation of the ventromedial hypothalamus (VMH) by estradiol influenced feeding behavior in female rats. They found that central implants of undiluted estradiol benzoate (EB) in the VMH decreased food intake in ovariectomized rats.<sup>16</sup> In this study we found that after gonadectomy in female rats there was a significant increase in food intake. This increase was more pronounced in female rats where gonadectomy was done during proestrous and estrous. Perhaps this explains the effect of withdrawal of high estrogen after gonadectomy.

In our study we found that Naloxone which is an opioid receptor antagonist blocking mu- receptors, induces hypophagia in food deprived male and female rats ( $p < 0.05$ ) as compared to controls (saline). It appears that naloxone induces hypophagia in food deprived male and female rats as compared to controls

(saline injection). These concur with the earlier studies.<sup>17,18</sup> One of the main functions proposed for opioid peptides in the CNS is involvement in mediation of hunger component in the control of food intake. Changes in the beta endorphin content of pituitary or hypothalamus have been demonstrated under condition designed to reflect changes in the state of hunger or satiety in rats. In normal rats fasted for 2-3 days beta endorphin content of the whole hypothalamus is decreased.<sup>19</sup> Several investigators have also reported that administration of beta endorphins in CNS increased food intake.<sup>20</sup> Intake of palatable food containing sugar or high fat is selectively increased by mu-opioid agonist when injected into ventromedial striatum including nucleus accumbens.<sup>10</sup> Other studies also show that agonists of mu, delta, kappa and ORL Opioid receptors increase food intake while Opioid receptor blockade decreases food intake.<sup>21</sup>

In female rats we studied the effect of estrous cycle phases on the hypophagic effect of Naloxone. It was observed that during proestrous and estrous phases the Naloxone failed to induce hypophagia in these rats. Our findings are consistent with earlier studies.<sup>22</sup> It is seen from the previous studies that gonadal steroids modulate opioid peptides and receptors in the central nervous system.<sup>23-25</sup> Ovariectomy in rats results in an increased sensitivity to suppressive effects of Naloxone on food intake compared with estradiol-treated ovariectomized rats.<sup>26-29</sup> The probable explanation for this may be that estrogen acts on mu-opioid receptors in the brain to modulate the functions of Opioid peptides.<sup>8</sup> When Naloxone is injected it fails to block the Opioid receptors which are already blocked competitively by estrogen. So Naloxone fails to induce hypophagia in the presence of high estrogen. In this study we found that after gonadectomy in all the female rats naloxone induced significant hypophagia when estrogen was no more there for competitive blockade of the receptors.

In male rats also Naloxone induced hypophagia in deprivation paradigm. After gonadectomy the basal food intake of male rats was increased. After gonadectomy in these male rats food intake was significantly increased. In all these rats Naloxone induced significant hypophagia before and after gonadectomy. These findings suggest that testosterone in males interferes with the mechanisms on energy intake however unlike estrogen it does not interact

with opioid receptors to alter the hypophagic effect of Naloxone. Our findings concur with the other studies. Gonadectomised male rats treated with testosterone propionate showed decrease in food intake.<sup>21</sup>

## CONCLUSION

Amongst the Gonadal hormones estrogen in females and testosterone in males modulates food intake. However, estrogen interferes with the hypophagic effects of naloxone perhaps by competitive blockade while there is no such alteration caused by testosterone. How do these Gonadal hormones and Opioid receptors interact to modulate food intake needs to be further investigated.

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**Conflict of interest**-None declared.

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