# EFFECT OF THIRD VENTRICLE INJECTION OF GABA TO FEEDING RESPONSES AND BLOOD SUGAR IN INTACT AND OVARIECTOMIZED FEMALE RATS

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**Summary** : Injections into third ventricle of brain of either 4 m Molar or 8 m Molar of GABA were given to intact and ovariectomized female rats to evaluate the effects of these dosages on body weight, food intake, water intake and blood glucose levels. Statistically significant reduction in food and water intake with 8 m Molar GABA was observed in intact female rats. Very precise alterations seen in ovariectomized female rats suggested that GABA could demonstrate more profound effects on body weight, food intake and water intake. Blood glucose level was elevated in both the groups with 4 m Molar GABA, while inhibition was seen with 8 m Molar GABA. These observations have been correlated with trophic hormonal profiles, which are modulated by third ventricle GABA injection or it might have had some intervention on hypothalamic mechanisms regulating these functions.

**Key words** : third ventricle, GABA, body weight, food intake, water intake, blood glucose

## INTRODUCTION

Hypothalamus is made up of a great number of neuronal network containing a variety of neurotransmitters in high concentration. Gamma-aminobutyric acid (GABA) has been an important inhibitory neurotransmitter in the mammalian central nervous system (19) and highest amount is found in hypothalamus (12), particularly in median eminence (15). GABA has been reported to involve hypothalamus in depicting behavioural changes (7), temperature regulation (2) and anterior pituitary functions (8, 9, 10) Dietary GABA reduces food intake and growth of young rats (16), cats (18, 19) or of lean and obese mice (17). Present report is an attempt to enunciate the mechanisms that regulate body weight, food intake, water intake and blood sugar levels in intact and ovariectomized female rats treated with third ventricle injection of GABA. Intact and ovariectomized rats were chosen due to the reason that

GABAergic influences on brain vary with variation of gonadal hormonal feedback to brain (8).

## MATERIAL AND METHODS

Healthy female rats (Wistar strain) weighing between 150±25 *g* obtained from Disease Free Animal House, Haryana Agricultural University, India were used throughout the study. They were maintained at constant temperature 22±1°C and 14L : 10D cycle. All rats were fed with feed pellets, a mixture of natural products (Hindustan Lever Ltd., Bombay; India) and water *ad libitum*. All rats were acclimatized with animal house conditions for one week.

After one week of acclimatization, rats were divided into two major groups : one with feeding (body weight, food intake and water intake) and another with biochemical (blood glucose) studies. Each of this major group was further divided into two groups : one with intact ovaries (OV) and other with ovaries removed (ovariectomized, OVX) (Table I). OVX was performed, while rats, were anaesthetized with ether. After OVX, the rats were kept for 3-4 weeks.

TABLE I : Details of experimental plan in feeding and biochemical groups showing the mode of injection.

| *Number of days* | *Major groups* | | | |
|---|---|---|---|---|
| | *Feeding group (Body weight/Food intake/Water intake) Group* | | *Biochemical group (Blood glucose) Group* | |
| | *Intact ovaries* | *Ovariectomized* | *Intact ovaries* | *Ovariectomized* |
| 1 | + | + | (i) 4 μl 4 mM GABA<br>(ii) 4 μl 8 mM GABA | (i) Injection E. B.* |
| 2 | + | + | | |
| 3 | + | + | | (i) 4 μl 4 mM GABA<br>(ii) 4 μl 8 mM GABA |
| 4 | + | + | | |
| 5 | + | (i) Injection E. B.* | | |
| 6 | + | + | | |
| 7 9 AM | + | + | | |
| 10 AM | (i) 4 μl saline<br>(ii) 4 μl 4 mM GABA<br>(iii) 4 μl 8 mM GABA | (i) 4 μl saline<br>(ii) 4 μl 4 mM GABA<br>(iii) 4 μl 8 mM GABA | | |
| 8-14 | + | + | | |

* Injection of estradiol benzoate was given subcutaneously.

+= This sign denotes that the feeding observation continued before and after injection from 1 to 14 days. All GABA/saline injections were made in third ventricle of brain.

Then after 3-4 weeks, a 23-gauge stainless steel cannula, 17 mm in length was implanted stereotaxically in the third ventricle (AP-2, L-0, D-2 mm above base) according to the atlas of Fifkova and Marsala (4), while rats were anaesthetized with Nembutal* (35 *mg/kg* b.wt., i.p.). Continuous oozing out of cerebrospinal fluid from the tip of cannula, when mandril was removed, certified its placement in third ventricle and only those rats were used. For biochemical estimation of blood glucose, under ether anaesthesia, indwelling catheters were placed in external jugular vein 24 h prior to experimentation (6), which allowed a serial blood sampling before and after third ventricle injection.

Records of body weight, food intake and water intake were taken daily throughout the period of study both before and 24 h after administration of GABA upto a total period of 14 days. Each of this group (OV or OVX) was further divided into three : (i) control group was injected with 0.9 per cent physiological saline, (ii) 4 $\mu l$ of 4 m Molar GABA treated group, and (iii) 4 $\mu l$ of 8 m Molar GABA treated group (see Table I).

Three to four weeks after ovariectomy, 10 $\mu g$ of estradiol benzoate in 0.1 *ml* castor oil was injected subcutaneously 48 h before the GABA injection into third ventricle in all the groups (feeding and biochemical). Blood glucose levels were estimated (21) after third ventricle injection of 4 m Molar and 8 m Molar GABA in OV and OVX female rats. Blood samples were withdrawn after 15 and 30 min of third ventricle injection and read at 680 nm after colour development. Details of the experimental plan are given in Table I.

All the data were computed for significance following the method of Students 't' test and paired 't' test (14) as indicated in appropriate figures.

## RESULTS

Third ventricle administration of 4 m Molar GABA caused non-significant decrease in body weight, food intake and water intake in OV intact female rats, while 8 m Molar dosage caused a significant reduction in food intake ($P<0.001$, on 8th, 9th and 10th day) and water intake ($P<0.001$, on 9th and 10th day) with almost no change in body weight in OV intact female rats. These results are shown in Fig. 1. In case of OVX female rats, 4 m Molar GABA again caused a non-significant reduction in body weight and water intake, and significant ($P<0.001$, on 8th and 9th day) decrease in food intake, while with 8 m Molar dosage significantly decreases body weight, food intake and water intake (Fig. 1).

*Registered Trade Mark

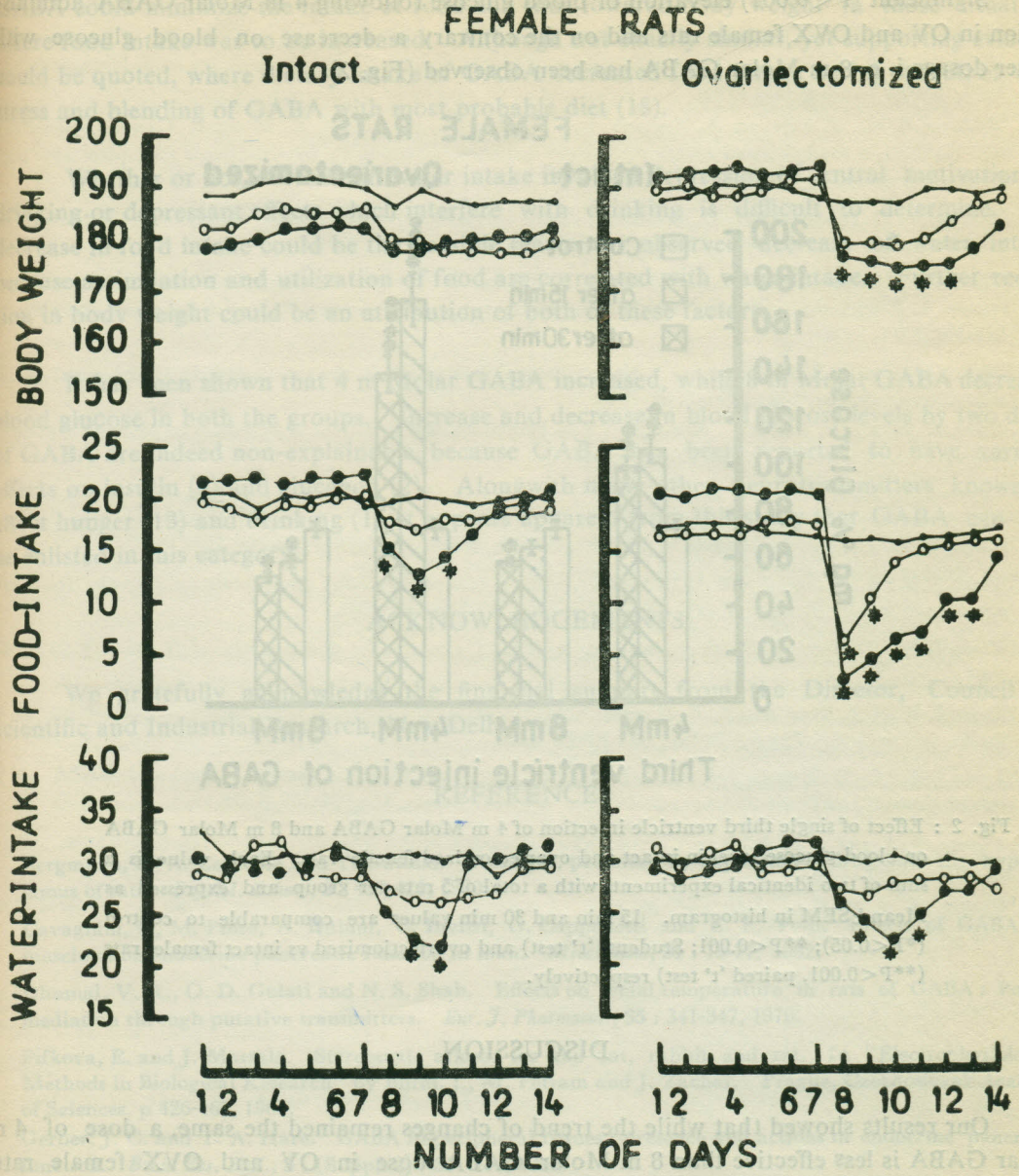


Fig. 1 : Effect of single third ventricle injection (on 7th day) of saline (●———●), 4 m Molar GABA (o———o) and 8 m Molar GABA (●———●) on body weight, food intake and water intake in intact and ovariectomized female rats. Each value is a sum of two identical experiments with a total of 5 rats per group and expressed as Mean. GABA group is compared with saline treatment (*$P<0.001$, Students 't' test).

Significant ($P<0.001$) elevation of blood glucose following 4 m Molar GABA administration in OV and OVX female rats and on the contrary a decrease on blood glucose with higher dosage i. e. 8 m Molar GABA has been observed (Fig. 2).

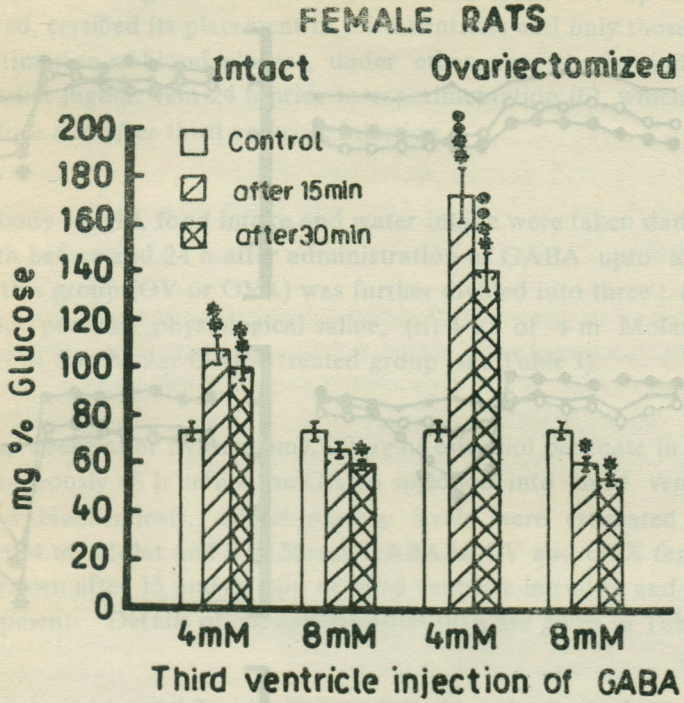


Fig. 2 : Effect of single third ventricle injection of 4 m Molar GABA and 8 m Molar GABA on blood glucose level in intact and ovariectomized female rats. Each value is a sum of two identical experiments with a total of 5 rats per group and expressed as Mean±SEM in histogram. 15 min and 30 min values are comparable to control (*$P<0.05$); **$P<0.001$; Students 't' test) and ovariectiomized vs intact female rats (**$P<0.001$, paired 't' test) respectively.

## DISCUSSION

Our results showed that while the trend of changes remained the same, a dose of 4 m Molar GABA is less effective than 8 m Molar GABA dose in OV and OVX female rats. Although priming of rats with estrogen results in many-fold increased titres of prolactin (8-10), yet decrease in body weight, food intake and water intake was observed by us. Whereas in weanling rats, when prolactin levels are increased, self selection of proteins and energy has been demonstrated (11). Thus our studies of OVX rats simply suggested that

GABA could minimize the intake of food and thus lowered body weight in those situations where food intake was to be increased. Although not exactly similar, yet supporting evidence could be quoted, where dietary intake of GABA decreased food intake during stimuli of cold stress and blending of GABA with most probable diet (18).

Whether or not, decrease in water intake involved depression of central motivation to drinking or depressant effects which interfere with drinking is difficult to determine. The decrease in food intake could be the possible reason for observed decrease of water intake, because assimilation and utilization of food are correlated with water intake. Further reduction in body weight could be an attribution of both of these factors.

It has been shown that 4 m Molar GABA increased, while 8 m Molar GABA decreased blood glucose in both the groups. Increase and decrease in blood glucose levels by two doses of GABA are indeed non-explainable, because GABA has been reported to have variable effects on insulin (5) and glucagon (2). Alongwith many other neurotransmitters known to affect hunger (13) and drinking (1), it appears apparent from this study that GABA can also be enlisted in this category.

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