

Inhibition of deprivation-induced food intake by GABA_A antagonists: roles of the hypothalamic, endocrine and alimentary mechanisms

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The role of gamma amino butyric acid A receptors/neurons of the hypothalamic, endocrine and alimentary systems in the food intake seen in hunger was studied in 20 h food-deprived rats. Food deprivation decreased blood glucose, serum insulin and produced hyperphagia. The hyperphagia was inhibited by subcutaneous or ventromedial hypothalamic administration of gamma amino butyric acid A antagonists picrotoxin or bicuculline. Although results of blood glucose was variable, insulin level was increased by picrotoxin or bicuculline. In contrast, lateral hypothalamic administration of these agents failed to reproduce the above changes. Subcutaneous administration of picrotoxin or bicuculline increased gastric content, decreased gastric motility and small bowel transit. In contrast, ventromedial or lateral hypothalamic administration of picrotoxin or bicuculline failed to alter the gastric content but decreased the small bowel transit. The results of alimentary studies suggest that gamma amino butyric acid neurons of both ventromedial and lateral hypothalamus selectively regulate small bowel transit but not the gastric content. It may be concluded that ventromedial hypothalamus plays a dominant role in the regulation of food intake and that picrotoxin or bicuculline inhibited food intake by inhibiting gamma amino butyric acid receptors of the ventromedial hypothalamus, increasing insulin level and decreasing the gut motility.

Key Words: GABA_A receptors, food deprivation, food intake, hypothalamus, satiety

The search for food and the act of feeding are the most important part of the lives of animals. Hence it is maintained with great precision by the involvement of the nervous, endocrine and alimentary systems and metabolic activities. Further, this system is fine-tuned by the nutrients in the blood, especially glucose, neurotransmitters, peptides and hormones.⁽¹⁻³⁾ The coordination of all these systems and factors towards the regulation of food intake appears to lie in the hypothalamus and its surrounding regions of the brain.^(4,5) Among the hypothalamic nuclei, ventromedial hypothalamus (VMH) and lateral hypothalamus (LH), considered the satiety and appetite center respectively, play critical role in the energy homeostasis, possess receptors for nutritional signals and receive firsthand information on the nutritional status of the organism.^(2,6-8)

Several studies suggested that the organisms' nutritional information reaches the brain through blood glucose and the sensor for glucose is present in the hypothalamus.⁽⁹⁻¹¹⁾ Flooding of the VMH area of the brain with glucose led to inhibition of food intake while its deprivation produced hyperphagia.⁽¹²⁾ Glucoprivation was accompanied by the increased levels of gamma aminobutyric acid (GABA), an inhibitory neurotransmitter, in the VMH; this

finding suggests that GABA may be a link between glucose and the VMH.⁽¹³⁻¹⁶⁾ The functional relationship between glucose and GABA is supported by one of the recent publications also.⁽¹¹⁾ The link between glucose and GABA was substantiated as the injection of GABA or muscimol, a GABA_A agonist or L-glutamic acid, a precursor for GABA, into the VMH facilitated feeding; in contrast, injection of these agents into the LH inhibited the food intake.⁽¹⁷⁾ On the other hand, subcutaneous (SC) or VMH injection of GABA_A antagonists inhibited glucoprivation-induced food intake while LH administration of these drugs failed to modulate the food intake.^(18,19) Further studies supported the above findings by showing that insulin or 2-deoxy-D-glucose (2-DG) led to an increase in VMH glutamate decarboxylase (GAD), the synthetic enzyme for GABA which was accompanied by increased food intake.^(20,21)

The evidence shown above studied the relation between GABA, hypothalamus and food intake following glucoprivation induced by pharmacological means. However, the contribution of GABA and hypothalamus is not known in the glucoprivation caused by fasting, a physiological means. In other words, the present study examines the molecular mechanisms underlying hunger. Hence, in this investigation, we studied the roles of GABA and hypothalamus in food deprivation employing rats and GABA_A antagonists. Further, we also examined the contribution of hormonal and alimentary systems in this mechanism.

Materials and Methods

Animals. Adult male Sprague-Dawley rats weighing 200–300 g were used throughout the study. All the animals were housed individually in plastic cages with bedding. They were transferred to clean plastic cages for the duration of the food intake measurement. The temperature of the vivarium was maintained at 24–27°C with 10 h artificial light (08:00–18:00 h). Animals were trained to take the food pellets for 4 h (10:00 to 14:00 h), while they were deprived of food for the rest of the day. Water was provided *ad libitum*. The animal protocol for this research was approved by the Animal Care and Use Committee (ACUC) of University of Virginia, Charlottesville, VA.

Cannula implantation. Animals were implanted with a stainless steel cannula (22 ga; Plastics One Inc., Roanoke, VA) either in VMH or LH under pentobarbital sodium (40 mg/kg i.p.) anesthesia as described before.^(18,19) Briefly, these unilaterally placed cannulae were fastened to the skull with dental cement such that the tip was 3.0 mm above VMH (with skull flat: 2.7 mm

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behind bregma, 0.5 mm lateral to sagittal suture and 8.5 mm vertical) or LH (with skull flat: 2.8 mm behind bregma, 1.6 mm lateral to sagittal suture and 8.5 mm vertical) according to the stereotaxic coordinates of König and Klippel.⁽²²⁾ A separate group of rats was implanted with the cannula in the lateral ventricle (ICV) (with skull flat: 2.0 mm posterior to bregma; 2.0 mm lateral to sagittal suture and 4.0 mm vertical) for testing the extra-hypothalamic sites of action of the administered drugs. The animals were allowed a week for recovery following surgery. The food and water intake and gross behaviors were monitored during this period.

General Procedure

Food intake measurement schedule. Pre-drug baseline was obtained in the week prior to the commencement of drug treatment with vehicle injections and employing the 4 h feeding and 20 h fasting schedule. The drug treatment was performed after the food intake during the 4 h period had been stabilized. Each animal served as its own control. The food was presented to the animals 15 min after the administration of the drug. Cumulative food intake was measured at 1, 2 and 4 h (i.e. 11:00, 12:00 and 14:00 h) after subtracting the spillage. In peripheral as well as VMH studies, different groups of animals were used for picrotoxin (Fluka AG, Switzerland) and bicuculline methiodide (Sigma, St. Louis, MO). However, in LH study, one group of LH cannulated rat was used for both picrotoxin and bicuculline. If the animals underwent more than one treatment, an interval of 3 days was allowed between each testing. Moreover, vehicle was injected a day prior to each treatment to ascertain the return of food intake scores to pre-drug baseline. Picrotoxin or bicuculline methiodide were dissolved in saline; peripheral studies were conducted after the subcutaneous (SC) injection of these drugs in a volume of 0.1 ml. VMH or LH injection was made with a 10 μ l microsyringe which extended 3.0 mm beyond the tip of the cannula, whose total length was 5.5 mm. Further, these injections were made over a period of 15 s in a volume of 0.5 μ l. The volume of the ICV injection was 2 μ l. The doses of picrotoxin and bicuculline used were determined in a separate study. They were well below their ED 50 (3.1 and 3.4 mg/kg SC respectively) for convulsive effect. The concentration of picrotoxin or bicuculline found to have produced the maximum effect in peripheral or central study (of food intake determination) were employed in the endocrine and alimentary studies.

Endocrine study. Blood glucose and serum insulin concentration were determined in this study. These animals were not fed on the day of the experiment and only water was made available to them. Blood samples were collected from tail vein of these animals at 1/2, 1, 2 and 4 h respectively (i.e. 10:30, 11:00, 12:00 and 14:00 h). In contrast to the food intake experiments, 1/2 h value was also taken, as significant changes in both blood glucose and serum insulin were expected within an hour. The blood glucose level was determined using a Glucometer (Glucometer Elite, Bayer Corporation Diagnostics GMBH, Leverkusen, Germany). The serum was separated and stored at -20°C for the estimation of insulin. The insulin concentration was examined using immunoassay [Insulin (Rat) Ultra-Sensitive ELISA, Alpco Diagnostics, Salem, NH] following the supplier's instructions.

Alimentary study. The animals were not fed while only water was available on the day of the experiment. Two parameters viz., gastric content (an index of gastric emptying and distention) and SBT (which indicates intestinal motility) were determined. Charcoal meal (gastric load; 10 ml/kg) consisting of 20% activated vegetable charcoal and 5% gum acacia in water was used as a marker. The vehicle or the drug injections were made 15 min (at 9:45 h) before the gastric load which was administered intragastrically. Followed by these procedures, the animals were sacrificed by cervical dislocation at 1/2, 1, 2 and 4 h (10:30, 11:00,

12:00 and 14:00 h). The 1/2 h value was also included as the gastric emptying and SBT are very fast in small animals. The abdominal cavity was dissected open and the pyloric and esophageal ends were ligated. The stomach was dissected out and the net weight was determined in a sensitive balance to the nearest 50 mg. It was opened along its greater curvature, contents removed, rinsed with water, blotted and the empty stomach was weighed again. Gastric content was determined by subtracting the weight of the empty stomach from the net weight. The percent change was calculated, by taking the load given as 100%.

In SBT study, the movement of the charcoal meal from the duodenum to the ileocaecal junction was measured at the time intervals mentioned above. From this value the percent SBT was calculated by taking the total length of the small intestine as 100% and was plotted against time.

Histology. At the conclusion of the study, the cannulated animals were killed by decapitation and the brain was processed for histological verification. Serial sections of 10 μ m were cut, stained with toluidine blue and examined under light microscope. The greatest diameter of non-specific damage around the cannulation site was 1.0 mm (including the cannula). Analysis of neuropathological changes showed a small glial infiltration with an occasional cavitation along the cannula pathway.

Statistical analysis. The results were analyzed by one-way ANOVA and expressed as mean \pm SEM.

Results

SC or VMH administration of GABA_A antagonists inhibit deprivation-induced food intake. Following their habituation, animals which underwent 4 h feeding and 20 h fasting schedule showed uniform and stable food intake. In these animals, the food intake was in the range of 5 to 12 g in the 4 h test period. SC administration of either picrotoxin or bicuculline inhibited the food intake in these animals in a dose dependent fashion throughout the 4 h feeding period (Fig. 1). Similar results were obtained when either of these agents was administered in the VMH (Fig. 2). Contrarily, even the maximal effective dose (in the VMH) of picrotoxin or bicuculline failed to significantly alter the food intake when injected into the LH (Fig. 3). In addition, injection of these agents into the ICV also did not affect the food intake in the food-deprived rats (data not shown).

SC, VMH or LH administration of GABA_A antagonists produced varying results on blood glucose and serum insulin levels. Blood glucose and serum insulin levels were estimated in the free feeding and fasted animals in order to determine the effect of food deprivation on these two parameters. Food deprivation produced a significant ($p < 0.05$) decrease in both blood glucose and serum insulin levels when compared to free feeding rats (free feeding: glucose = 84.6 ± 3.6 mg/dl and insulin = 11.04 ± 0.86 μ U/ml; food deprivation: glucose = 61.2 ± 3.6 mg/dl and insulin = 6.21 ± 0.51 μ U/ml). SC administration of either picrotoxin or bicuculline showed contrasting results in both the blood glucose and serum insulin levels (Fig. 4). Picrotoxin decreased the serum insulin levels throughout the 4 h study period though it was significant at the 2nd and 4th h only. Although the decreased insulin level was accompanied with increased glucose it was significant at the 4th h only. Conversely, bicuculline led to a sharp increase in insulin which was accompanied with decreased glucose level at 1/2 h (Fig. 4).

In contrast to peripheral studies, VMH injection of picrotoxin or bicuculline increased serum insulin concentration throughout the 4 h study period, though it was significant in the 1/2 and 1 h only (Fig. 5). The blood glucose concentrations were not consistent with the increased serum insulin levels seen with the VMH administration of these agents. Picrotoxin-induced increase in insulin level was accompanied with a generalized decrease in blood glucose concentration which was significant only at the 1/2 h.

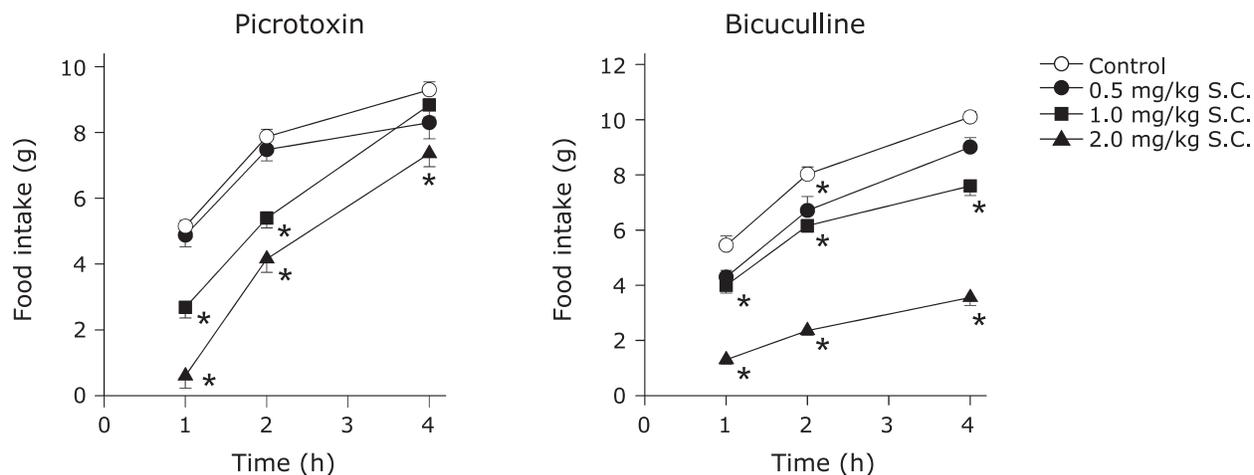


Fig. 1. Cumulative food intake by 20 h food deprived rats treated with SC administration of picrotoxin or bicuculline. The rats underwent 20 h fasting and 4 h feeding as mentioned in the methods. Each point represents mean \pm SEM; for picrotoxin, $n = 5$ to 6 ; for bicuculline, $n = 6$; * $p < 0.05$ compared to control.

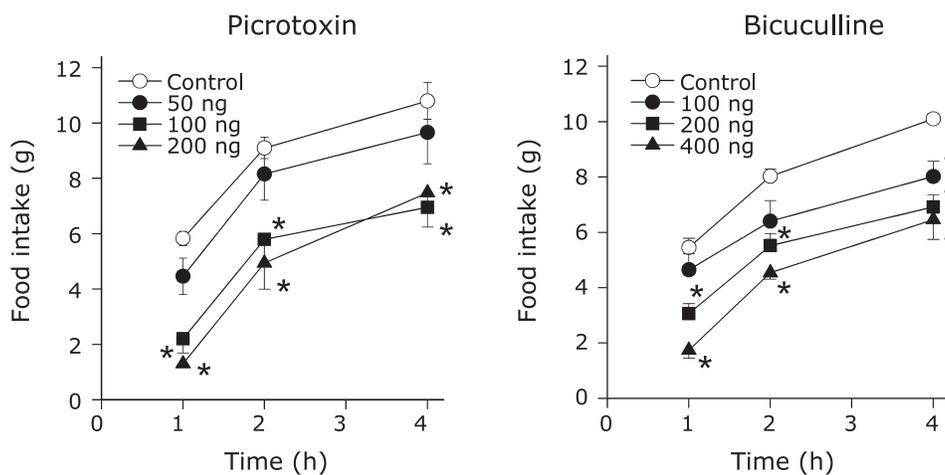


Fig. 2. Cumulative food intake by 20 h food deprived rats treated with VMH administration of picrotoxin or bicuculline. The unilateral cannula was implanted in VMH following the stereotaxic coordinates as mentioned under cannula implantation. The drug testing was started following the recovery from surgery and the stabilization of food intake during the 4 h period. Separate groups of rats were used for picrotoxin and bicuculline. Each point represents mean \pm SEM; $n = 5$ to 6 ; * $p < 0.05$ compared to control.

Contrary to picrotoxin, bicuculline produced a sharp increase in the blood glucose concentration at 1 h (Fig. 5). LH administration of these agents did not alter either serum insulin or blood glucose levels at the times tested (Fig. 6).

Gastric content and SBT are differentially affected after SC, VMH or LH administration of GABA_A antagonists.

Figs. 7–9 display the effect of the GABA_A antagonists on gastric content and SBT. The amount of orally administered charcoal meal together with the possible gastric secretion retained in the stomach (at various time intervals) was taken as the gastric content. As expected, the gastric content was high at the 1/2 h and at its lowest at the 4th h. Although the control gastric content varied between the SC, VMH and LH injection groups in the 1/2 and 1 h periods, it declined and consistently reached its lowest level at the 4th h in all these groups. In contrast, the control SBT was stable and similar in the peripheral, VMH and LH studies (Figs. 7–9). Further, the control SBT was below 100% in the 1/2 h

period and reached 100% in the 1st h.

SC administration of picrotoxin or bicuculline increased the gastric content and decreased the SBT (Fig. 7). The changes in the gastric content and SBT were significantly different from the control for a period of 2 h only (Fig. 7). In the VMH study, the gastric content was more than 100% in the control, picrotoxin and bicuculline groups. However, it sharply decreased in the picrotoxin group at the 2nd h (Fig. 8). Contrary to the gastric content, SBT was decreased in both the picrotoxin and bicuculline groups in the 1/2 h period (Fig. 8). In the LH study also, the gastric content was more than 100% in the control, picrotoxin and bicuculline groups. In contrast, SBT was decreased significantly in both the picrotoxin and bicuculline groups in the 1/2 h period compared to the control (Fig. 9).

Behavioral changes after SC or VMH administration of GABA_A antagonists.

Administration of 2 mg/kg of picrotoxin showed hypermotility and hyperexcitability in five rats. VMH injection of 400 ng of bicuculline produced frothy salivary

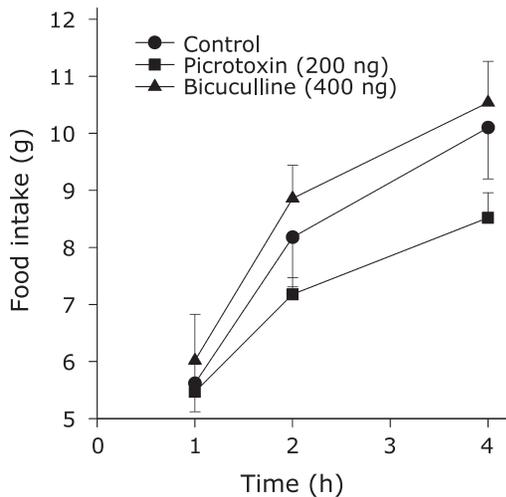


Fig. 3. Cumulative food intake by 20 h food deprived rats treated with LH administration of picrotoxin or bicuculline. Unilateral cannula in LH was implanted as mentioned in the methods. The drug testing was started following the recovery from surgery and the stabilization of food intake during the 4 h period. Both picrotoxin and bicuculline were tested in the same group of cannulated rats. However, an interval of 3 days was allowed between the testing with these agents. Each point represents mean \pm SEM; $n = 5$ to 6.

secretion in two rats. These changes occurred 5 min after the administration of drugs and lasted for 20 min. Even though no significant change in the feeding behavior of these rats was observed, the data obtained from these rats were not included in the results.

Discussion

The presence of GABAergic neurons in various hypothalamic areas and their contribution in the regulation of food intake is well known.⁽²³⁻²⁵⁾ An increase and a decrease of GABAergic activity

was observed in the VMH and LH respectively during insulin-induced hypoglycemia and hyperphagia.⁽²⁶⁾ The above finding was supported by our earlier observation that insulin-induced hyperphagia was inhibited by SC or VMH, but not in LH administration of GABA_A antagonists.⁽¹⁸⁾ Similarly, hyperphagia induced by other types of hypoglycemic agents *viz.*, 2-DG or 5-thio glucose was also inhibited by the SC or VMH, but not LH administration of GABA_A antagonists.⁽¹⁹⁾ Other investigators showed that 2-DG induced food intake was associated with a simultaneous increase of GABA and GAD in VMH.^(14,21) Further, VMH injection of l-glutamic acid, a precursor of GABA produced an increase of food intake in satiated sheep.⁽¹⁷⁾ Such elevated GABA activity in VMH would have inhibited the VMH satiety center from sending inhibitory output to LH feeding center, thereby facilitating a desire for food intake.

In the present study, both hypoglycemia and hyperphagia were induced by physiological means *i.e.*, food deprivation. Hence, as seen with insulin or 2-DG, food deprivation may have also increased the GABAergic activity in the VMH. The increased GABAergic activity in the VMH may have inhibited VMH and the subsequent activation of LH and the food intake (or hyperphagia) in hunger. The GABA_A receptor-induced inhibition of VMH was blocked by the SC or VMH GABA_A antagonists and thus the food intake caused by hunger or deprivation was decreased (Figs. 1 and 2). This view is in agreement with the report that bicuculline mainly influences those postsynaptic neurons in which the GABAergic inputs prevail.⁽²⁷⁾ The failure of the GABA_A antagonists to influence food intake, when administered through LH, may be due to the lack of GABA activity in that area (Fig. 3). The specificity of VMH may be further stressed as, ICV administration of picrotoxin or bicuculline failed to alter the food intake under a similar condition (data not shown).

Insulin is important in the regulation of food intake as it acts as a central satiety factor in normal animals, a process that is perturbed in obesity.⁽²⁸⁾ Although the satiety caused by the GABA_A antagonists may be related to insulin, the differential levels of insulin after the SC administration of these agents (Fig. 4) is not in agreement and suggest that the inhibition of food intake and endocrine effects may be parallel. This may be agreeable since VMH administration of picrotoxin or bicuculline

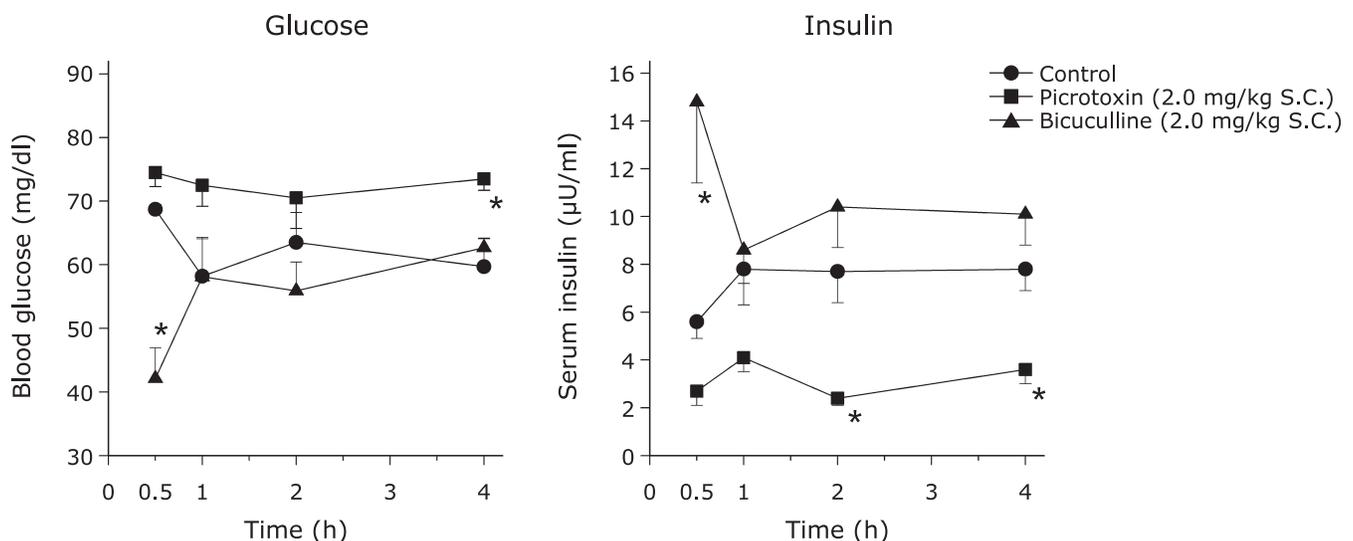


Fig. 4. The effect of SC administration of picrotoxin or bicuculline on the blood glucose and serum insulin levels in rats deprived of food for 20 h as described in methods. Same group of rats was used for bleeding at the 4 different time intervals. However, different groups of rats were used for picrotoxin and bicuculline. The animals were not fed on the day of the experiment whereas water was available *ad libitum*. Each point represents mean \pm SEM; $n = 5$ or 6; * $p < 0.05$ compared to control.

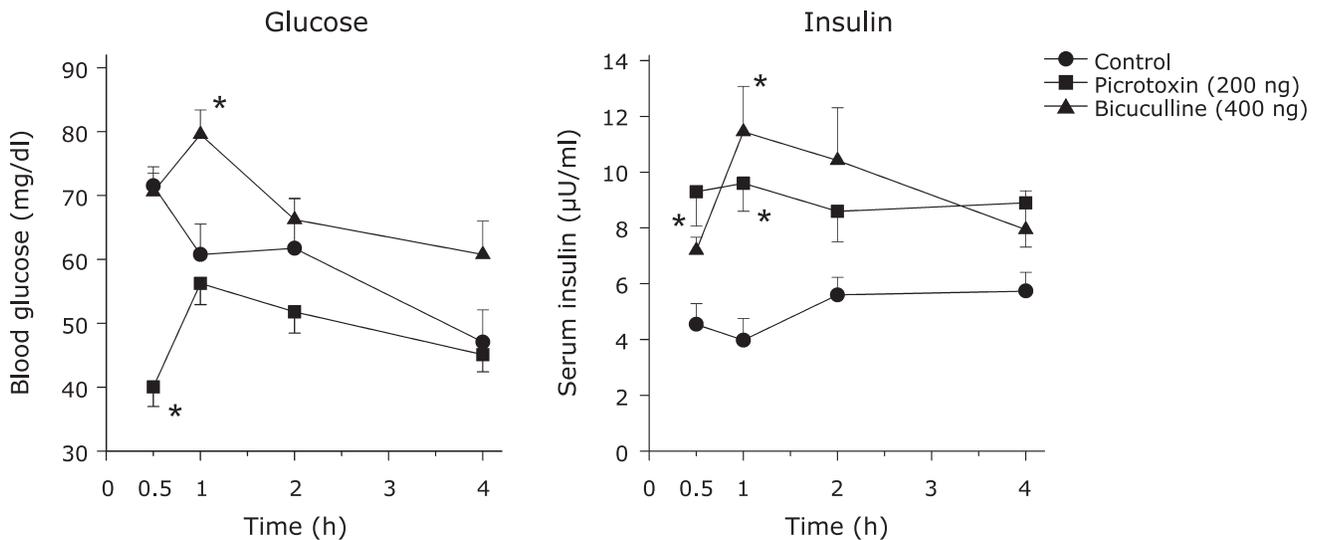


Fig. 5. The effect of VMH administration of picrotoxin or bicuculline on the blood glucose and serum insulin levels in food deprived rats. Unilateral cannula was implanted in VMH as mentioned in methods. After a week period of recovery the animals underwent 20 h fasting and 4 h feeding schedule. The animals were not fed on the day of the experiment whereas water was available *ad libitum*. Each point represents mean \pm SEM; $n = 5$; * $p < 0.05$ compared to control.

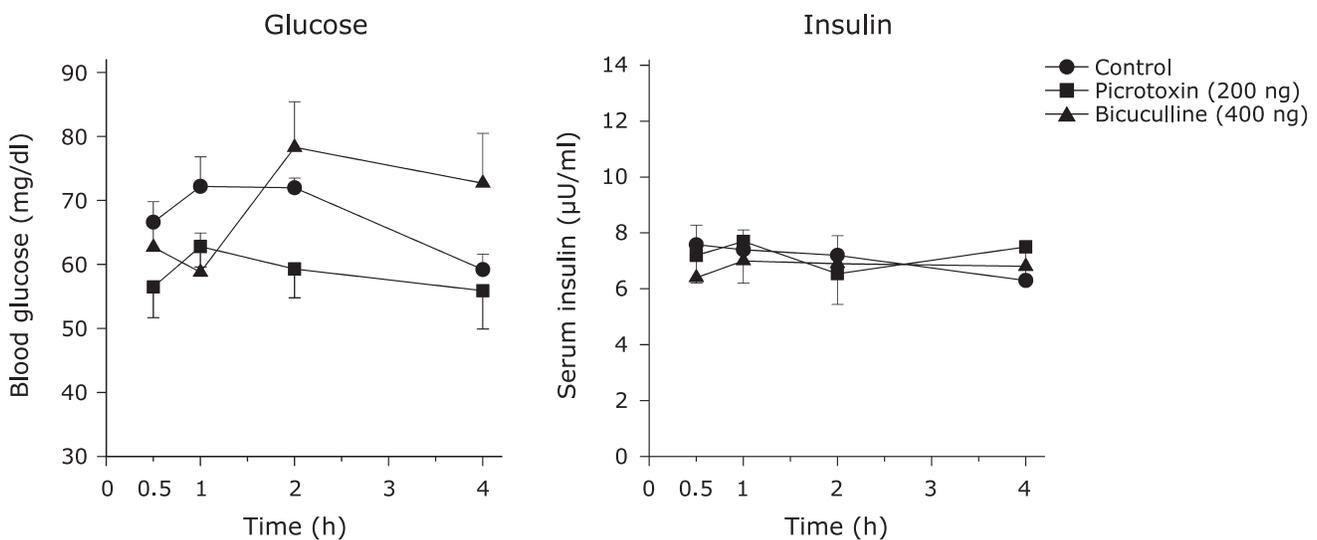


Fig. 6. The effect of LH administration of picrotoxin or bicuculline on the blood glucose and serum insulin levels in food deprived rats. The animals had unilateral cannula and underwent 20 h fasting and 4 h feeding schedule after one week period of recovery. The animals were not fed on the day of the experiment whereas water was available *ad libitum*. Each point represents mean \pm SEM; $n = 5$.

increased insulin for a short duration (0.5 to 1.0 h) (Fig. 5) whereas the inhibition of food intake was observed for 4 h (Fig. 2). The contribution of extra-hypothalamic factors such as the pancreas, liver and skeletal muscle and the different mechanisms of action of these agents⁽²⁹⁾ have to be considered in this context. In spite of the possible parallel action, the contribution of insulin to satiety cannot be neglected. The link between insulin and VMH may be explained from the observation that bilateral destruction of VMH produced a syndrome characterized by hyperinsulinemia, hyperphagia and obesity and these changes were reversed by vagotomy.^(30,31) The role of VMH in the food intake as well as in the insulin secretion is further stressed since the injection of the

GABA_A antagonists in the LH neither affected the food intake nor insulin levels (Figs. 3 and 6).

It has been reported that in normal condition, GABA might inhibit the insulin release through the vagal pathway.^(32,33) Thus in normal rats, the efferent vagus that reaches the pancreas is under the powerful tonic GABA inhibitory action, probably to prevent hypoglycemia.⁽³⁴⁾ The increased insulin level produced by the VMH administration of either bicuculline or picrotoxin (Fig. 5) may be the result of the blockade of the tonic inhibitory action of GABA. It may have happened at the level of the VMH since GABAergic inhibitory tone within the VMH is hypothesized to modulate glucose counter regulatory responses.⁽³⁵⁾ Direct action

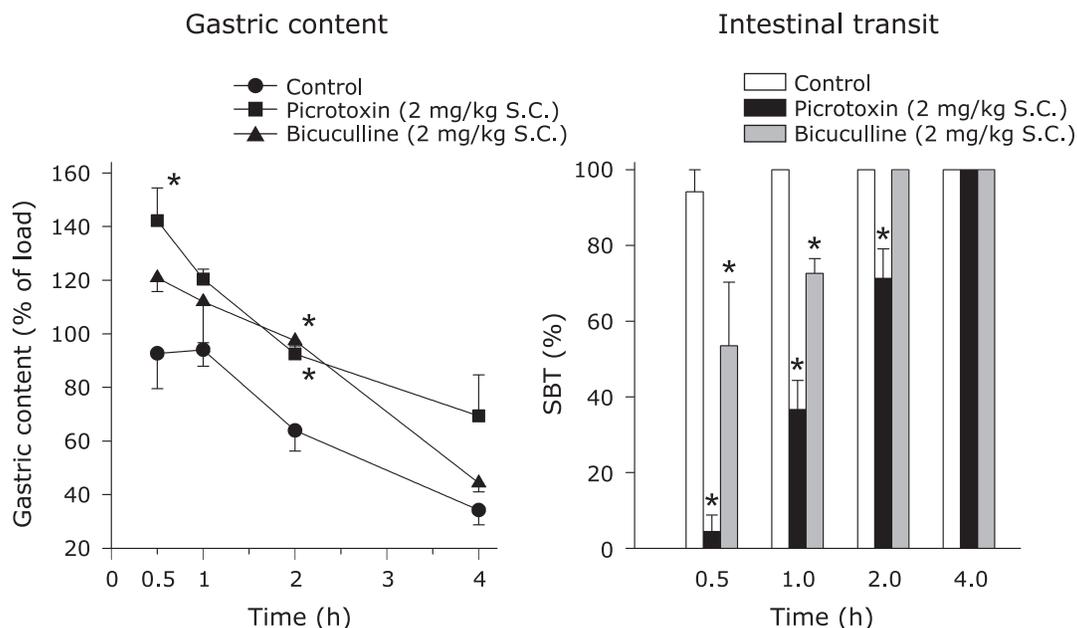


Fig. 7. The effect of SC administration of picrotoxin or bicuculline on gastric content and SBT in rats. All these animals were trained to take the food pellets in the 4 h period as described in methods. The experiments were followed after the stabilization of food intake during this period. The animals were not fed on the day of the experiment whereas water was available *ad libitum*. Each point represents mean \pm SEM; $n = 5$; * $p < 0.05$ compared to control.

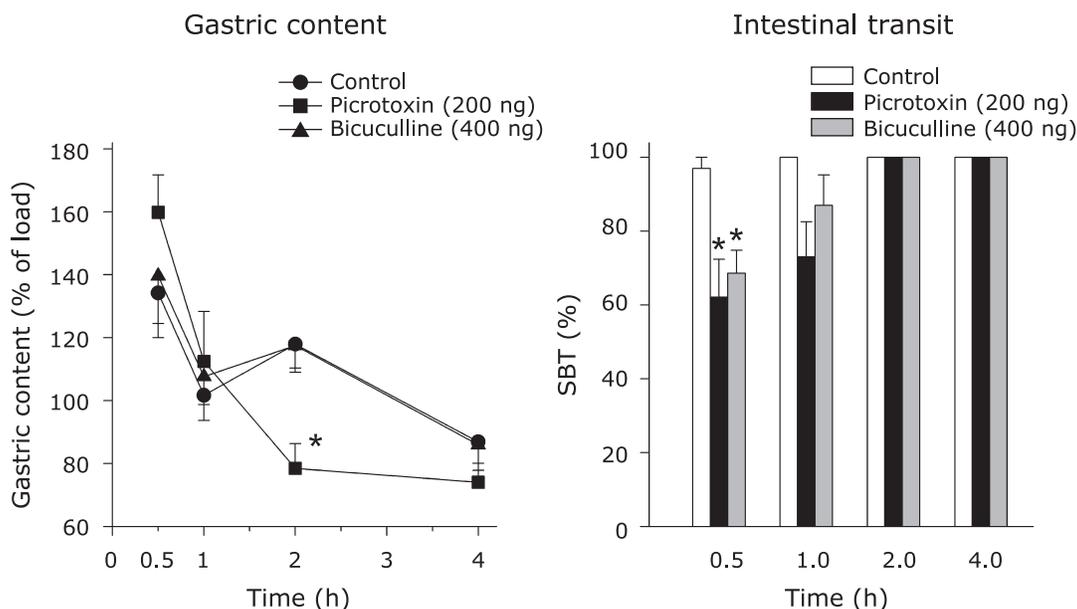


Fig. 8. The effect of VMH administration of picrotoxin or bicuculline on gastric content and SBT in rats deprived of food. The animals had unilateral cannula and underwent training to take food in the 4 h period that was preceded by 20 h food deprivation. The experiments were followed after the stabilization of food intake during this period. The animals were not fed on the day of the experiment whereas water was available *ad libitum*. Each point represents mean \pm SEM; $n = 5$; * $p < 0.05$ compared to control.

of GABA_A antagonists at the pancreas is also possible since mRNA for GABA_A receptors and secretion of GABA has been demonstrated in the pancreas.^(36,37) Further, perfusion of the isolated pancreas with GABA inhibited insulin release.⁽³⁸⁾

Although picrotoxin at VMH decreased glucose level based on the modulation of insulin, it was not so with bicuculline. The

increased insulin level was accompanied with enhanced glucose concentration after VMH administration of bicuculline. It has been reported that ICV administration of bicuculline methiodide (10 nmol) increased glucose level due to an enhanced hepatic gluconeogenesis and glycogenolysis.^(39,40) Direct evidence for the role of VMH in the increased glucose level is available as, VMH

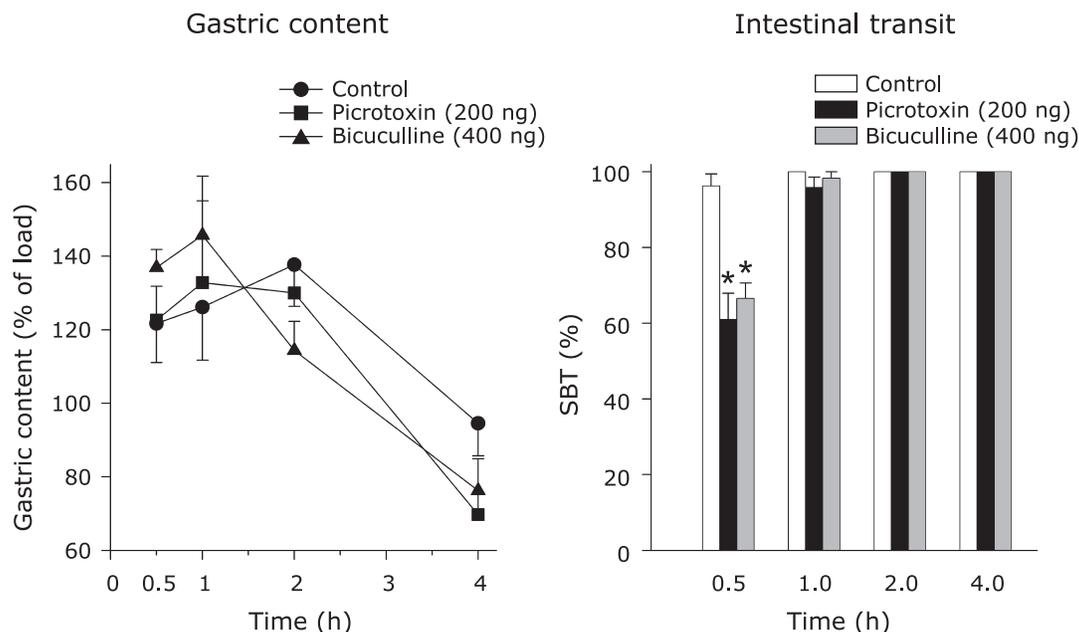


Fig. 9. The effect of LH administration of picrotoxin or bicuculline on gastric content and SBT. The rats had unilateral cannula and deprived of food for 20 h and fed for 4 h as described in methods. The experiments were followed after the stabilization of food intake during this period. The animals were not fed on the day of the experiment whereas water was available *ad libitum*. Each point represents mean \pm SEM; $n = 5$; $*p < 0.05$ compared to control.

administration of 500 μ M bicuculline led to an increase in the glucose concentration.⁽⁴¹⁾

Although GABA_A antagonists produced varying effects on the gastric content, they consistently decreased gastric emptying as can be seen from the decreased intestinal transit after the SC, VMH or LH administration of these agents (Figs. 7–9). However SC GABA_A antagonists were most potent in decreasing the intestinal transit compared to the VMH or LH administrations. These changes may have increased the gastric distension and led to the stimulation of the distension receptors located in the esophagus, stomach, duodenum, jejunum and small intestine.⁽⁴²⁾ It is known that the stimulation of distension receptors is one of the reasons for satiety.⁽⁴³⁾ Gastric distension induced by GABA_A antagonists is substantiated as stimulation of GABA receptors of intestine led to the contraction of ileum.^(44,45) Considerable amount of GABA is present in the gut as it is synthesized by the bacteria in the gut and metabolized by the hepatocytes in the liver.^(46,47) In addition, GABA, GAD and high affinity GABA uptake sites are all present in the myenteric plexus (Auerbach's plexus) of rat, guinea-pig and cat.^(48–50)

GABAergic neurons of the VMH may not contribute to the gastric content or emptying since there was no significant increase in the gastric content after VMH administration of GABA_A antagonists. Moreover, a decrease in the gastric content was found in the second h after VMH administration of picrotoxin (Fig. 8). As bicuculline did not show a similar change it is hard to explain this on the basis of GABA_A receptor blockade. Furthermore, the gastric content was more than the load given in the control as well as in the VMH and LH groups. Since the gastric content was uniformly increased in the control as well as in the treatment groups, it may be a nonspecific effect due to the microinjection in these brain regions. Contrary to the gastric content, SBT was delayed in the 1/2 h after the VMH and LH administration of GABA_A antagonists (Figs. 8 and 9). It is possible that the GABAergic neurons of both these areas are involved in the intestinal motility. It also suggests that gastric motility and SBT are regulated by mechanisms that are independent of each other.

Taken together food intake and insulin secretion seem to be controlled almost similarly by the peripheral mechanisms and VMH; in contrast, gastrointestinal activity may be modulated differently. While the GABAergic neurons of VMH and LH seem to contribute equally to the intestinal transit, it appears that VMH plays a dominant role in the regulation of food intake and insulin secretion compared to LH. Evidence of VMH's dominance over LH comes from the observation that axons from VMH project laterally and caudally and produce inhibition of the LH feeding center, either by inhibition of the facilitatory area (feeding center), or by projecting caudally into the brainstem and inhibiting feeding reflexes directly, or both.⁽⁵¹⁾ Another possibility is that VMH GABA_A antagonists interfered with the transmission of impulses at the level of the brain stem, since unilateral injection of these agents modified food intake and insulin secretion in this study.

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Abbreviations

ANOVA	analysis of variance
2-DG	2-deoxy-D-glucose
GABA _A	gamma amino butyric acid A
GAD	glutamate decarboxylase
ICV	intra-cerebro ventricle or lateral ventricle
LH	lateral hypothalamus
SBT	small bowel transit
SC	subcutaneous
VMH	ventromedial hypothalamus

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