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Antagonism of Acute Feeding Response to 2-Deoxyglucose and 5-Thioglucose by GABA Antagonists: The Relative Role of Ventromedial and Lateral Hypothalamus

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LENIN KAMATCHI, G., K. VEERARAGAVAN, DINESH CHANDRA AND J. S. BAPNA. *Antagonism of acute feeding response to 2-deoxyglucose and 5-thioglucose by GABA antagonists: The relative role of ventromedial and lateral hypothalamus.* PHARMACOL BIOCHEM BEHAV **25**(1) 59–62, 1986.—The effect of GABA antagonists picrotoxin and bicuculline was studied on hyperphagia caused by 2-DG and 5-TG. The GABA antagonists were administered either SC or into the VMH or LH through stereotaxically implanted chronic cannulae. The peripheral as well as VMH injection antagonised the hyperphagia significantly. In contrast, injection of these agents into the LH failed to produce any effect. These findings show that in a glucoprivic state there might be an increased GABAergic activity in the VMH.

2-DG 5-TG Hyperglycemia Hyperphagia Hypothalamus GABA Picrotoxin Bicuculline

GAMMA aminobutyric acid (GABA), an inhibitory neurotransmitter [1], has been shown to play an important role in the hypothalamic control of feeding behavior [13]. Injection of GABA or its agonist muscimol into the ventromedial hypothalamic (VMH) satiety center facilitated feeding while inhibition was observed when injected into the lateral hypothalamic (LH) feeding center [4,7].

The role of blood glucose concentration in the regulation of feeding behavior is well established [6,11]. It has been shown that hypoglycemia induced by insulin which is known to produce hyperphagia, increased the GABA content in VMH and decreased the same in LH [8]. Further, we have shown that the hyperphagia produced by insulin was antagonised by GABA antagonists, picrotoxin and bicuculline, when injected SC or in VMH but not in LH [10].

Another method for studying glucoregulatory feeding is the use of 2-deoxy-D-glucose (2-DG) and 5-thio-D-glucose (5-TG). The administration of 2-DG and 5-TG cause hyperglycemia, but produce hyperphagia due to cellular glucoprivation [3, 16, 17]. Since GABA has been suggested to be a mediator in hyperphagia induced by insulin, it was proposed to study its role on hyperphagia caused by 2-DG and 5-TG by using GABA antagonists picrotoxin and bicuculline. They were administered systemically by SC route and intracranially in VMH and LH.

METHOD

Animals

A total number of 60 adult male Sprague Dawley rats (175–200 g) were used. They were individually housed in round metabolic cages with wire mesh sides and bottom. They had free access to food pellets (Gold Mohr; Hindustan Lever, India) and water ad lib. The temperature of the vivarium was maintained at 24–27°C with 10 hr artificial light (8 hr to 18 hr).

Drugs and Chemicals

2-DG and 5-TG (Sigma, USA) were dissolved in normal saline. Bicuculline (Sigma, USA) was dissolved in diluted HCl pH 4. Picrotoxin (Fluka AG, Switzerland) was dissolved in warm normal saline.

The doses of both 2-DG and 5-TG (200 and 100 mg/kg IP, respectively) were determined during the preliminary studies. The doses of picrotoxin and bicuculline used in this study were well below their ED 50 (3.1 and 3.4 mg/kg SC respectively) for convulsive effect.

Cannula Implantation

Two groups of twelve rats each were implanted with a

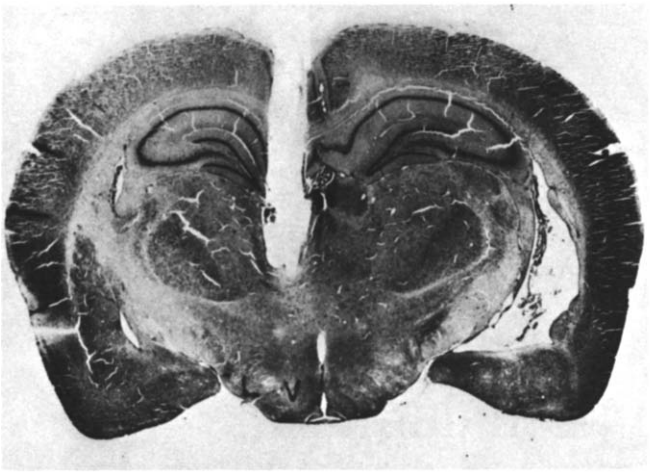

FIG. 1. Coronal section (10 μ thickness) of the rat brain showing the ventromedial and lateral hypothalamus. The pathway of the guide cannula is pointed towards the ventromedial hypothalamus; V—ventromedial hypothalamus, L—lateral hypothalamus (Toluidine blue ×8).

stainless steel cannula (23 ga) in the VMH and LH respectively under pentobarbital sodium (40 mg/kg IP) anaesthesia. 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 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 [9]. Similarly, two groups of six rats each were implanted with the cannula in the lateral ventricle (ICV) for testing the extra-hypothalamic site of action of picrotoxin and bicuculline. This cannula was inserted 2.0 mm posterior to bregma and 2.0 mm lateral to sagittal suture, so that it reached the ICV 4.0 mm below the surface of the skull. This was secured to the skull surface with dental cement. Following surgery, the animals were allowed a week period for recovery. During this period the food and water intake and gross behaviors were monitored. At the conclusion of the study the placement of cannula was verified histologically (Fig. 1).

General Procedure

Three studies (peripheral, VMH and LH) were conducted. A group of 12 rats were used in each study. All these rats were acclimated to the experimental conditions for a period of ten days by repeated handling and vehicle (normal saline) injections. All the experiments were conducted between 10 and 14 hr. The cumulative food intake was measured at 1, 2 and 4 hr. The spillage was collected at the end of each hour and deducted from the amount eaten. The control food intake was determined after vehicle injections either peripherally or centrally. The peripheral administration of drug was made either SC or IP in a volume of 0.1 ml. The intracranial injection (in VMH or LH) was made with a 10 μl microsyringe which extended 3.0 mm beyond the tip of the cannula which was 5.5 mm. The maximum volume injected intracranially was 0.5 μl over a period of 15 sec. Similarly, in the ICV study a volume of 2 μl was injected into the ventricle.

TABLE 1

EFFECT OF 2-DG AND 5-TG ON BLOOD GLUCOSE CONCENTRATION

Treatment (Dose)	Glucose Concentration (mg/100 ml)
Saline (SC)	74.2 ± 1.72
2-DG (200 mg/kg IP)	139.2 ± 7.30*
5-TG (100 mg/kg IP)	225.5 ± 13.46*

Values are mean ± SEM of 6 animals.
*$p<0.001$.

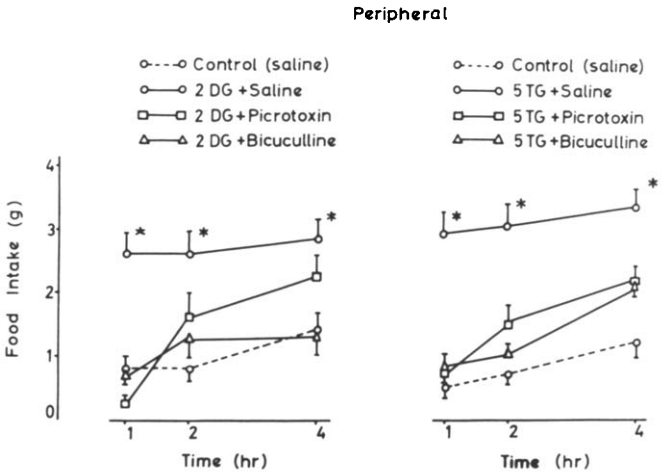

FIG. 2. The effect of SC administration of picrotoxin and bicuculline on the 4 hr food intake in 2-DG or 5-TG treated rats. Each point represents mean±SEM; n=6; *$p<0.05$.

Drug Treatment

In all these studies, the glucoprivic substances (2-DG and 5-TG) were administered IP 45 min before keeping the food pellets in the cage. The GABA antagonists (picrotoxin and bicuculline) were administered SC in the peripheral study and in VMH or LH in the central study 30 min after the administration of the glucoprivic substances. A latent period of 15 min was given to these GABA antagonists and the food intake measurements were started.

In the peripheral study, following the control food intake measurements the first group of six rats received 2-DG (200 mg/kg IP), 2-DG plus picrotoxin (2.0 mg/kg SC) and 2-DG plus bicuculline (2.0 mg/kg SC) consecutively maintaining 3 days interval between each treatment. The second group of rats received the same treatment as above except that 2-DG was replaced by 5-TG (100 mg/kg IP). In both the groups vehicle injection was made a day before each treatment to ascertain the return of food intake to the pretreatment value.

The central study was carried out in two groups of six rats each with cannula in the VMH and LH, respectively. These rats were administered with 2-DG, 2-DG plus picrotoxin (100 ng), and 2-DG plus bicuculline (200 ng) consecutively after an interval of 3 days for each treatment. The other two groups of rats with cannula in the VMH and LH respectively were treated with 5-TG in the same sequence in place of

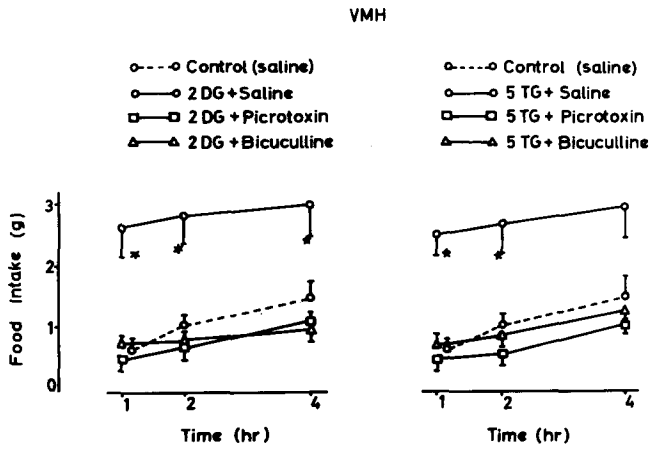

FIG. 3. The effect of VMH administration of picrotoxin and bicuculline on the 4 hr food intake in 2-DG or 5-TG treated rats. Each point represents mean±SEM; n=6; *$p<0.05$.

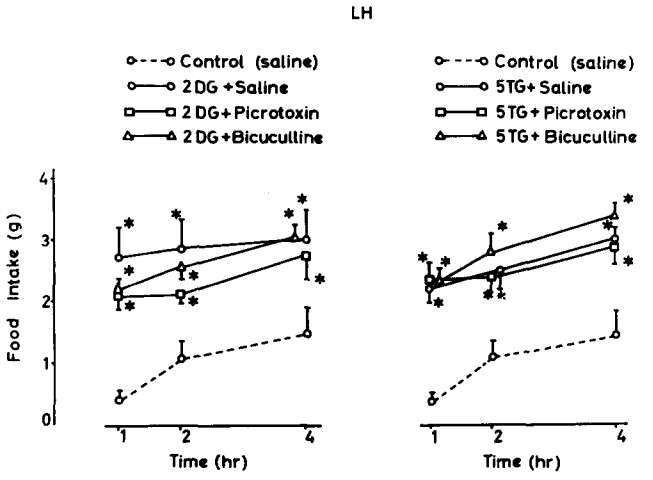

FIG. 4. The effect of LH administration of picrotoxin and bicuculline on the 4 hr food intake in the 2-DG or 5-TG treated rats. Each point represents mean±SEM; n=6; *$p<0.05$.

2-DG and the cumulative food intake was recorded for each treatment.

Two groups of six rats each with ICV cannulae were acclimated to take the food pellets only during a specified 4 hr period (10 to 14 hr) in a day. The first and second group of rats were administered picrotoxin (100 ng) and bicuculline (200 ng), respectively. The food intake was recorded as mentioned above.

Glucose Estimation

This was done to determine the extent of hyperglycemia in 2-DG and 5-TG treated rats. Both the drug treated and the freely fed control rats kept under the identical experimental conditions were used. Blood samples were taken from the retro-orbital venous plexus 30 min after the administration of 2-DG or 5-TG, using a capillary tube. The blood glucose was estimated by glucose oxidase-peroxidase method [5].

All the results of food intake studies were analysed by Dunnett's *t*-test and the results of glucose estimation was analysed by Student's *t*-test.

RESULTS

Both 2-DG and 5-TG caused a significant hyperglycemia (Table 1). This was followed by hyperphagia.

After the administration of both picrotoxin and bicuculline by SC route to the 2-DG or 5-TG treated rats, no significant hyperphagia was found. In other words, the hyperphagia induced by 2-DG or 5-TG was antagonised by these agents (Fig. 2). Similar results were obtained when these GABA antagonists were administered in the VMH of 2-DG or 5-TG treated reats (Fig. 3). In contrast to this, the injection of these agents in LH failed to antagonise the hyperphagia produced by 2-DG or 5-TG (Fig. 4).

Peripheral and central administration of diluted HCl (vehicle for bicuculline) did not produce any significant change in food intake (not shown). Both picrotoxin and bicuculline when injected alone in the ICV did not affect the food intake of fasted rats (not shown).

Behavioral Changes

Administration of 2 mg/kg SC of picrotoxin showed hypermotility in three rats.

DISCUSSION

The glucoprivic state and its role in the stimulation of food intake is well known [11,17]. Substances like insulin, 2-DG and 5-TG produce hyperphagia by means of their glucoprivic action [3, 6, 16]. Kimura and Kuriyama [8] had shown a correlation between the hyperphagic action of insulin with that of an increased GABA level in the VMH after insulin administration. However, the mechanism has not been demonstrated. In our earlier study with GABA antagonists we have suggested that GABA activity in VMH may be responsible for the hyperphagia induced by insulin [10]. Further, Tappaz *et al.* [18] reported that both GABA and its synthetic enzyme, glutamicacid decarboxylase (GAD), are rich in the hypothalamic regions which are considered to be involved in the feeding behavior. These GAD-containing cells have their origin inside the hypothalamus. In this region GABAergic neurons are likely to be short interneurons providing intrahypothalamic connections [19]. These data suggest that GABA in the hypothalamic neurons act as a separate entity in the vital function of feeding behavior.

It has been shown that glucose stimulates VMH by its action on glucoreceptors which are found in abundance in the VMH neurons leading to satiety [15]. A link between GABA and the glucoreceptors of the hypothalamus has been suggested. These hypothalamic neurons are sensitive to glucose metabolism and may encode metabolic information through the GABA shunt which parallels energy flow through the TCA cycle [12].

In the present study, the hyperphagia produced by 2-DG and 5-TG was antagonised by both picrotoxin and bicuculline after peripheral administration. Similar results were obtained when these agents were administered in the VMH. This antagonism at the level of VMH may be due to an increased GABA activity with 2-DG and 5-TG. Both pic-

rotoxin and bicuculline which are known to cross the bloodbrain barrier after peripheral administration might have exerted their action at the level of VMH. This is in agreement with the view of Olgiati *et al.* [14] who have suggested that bicuculline mainly influences those postsynaptic neurons in which the GABAergic inputs prevail. The failure of any action of these GABA antagonists on hyperphagia when administered through LH may be due to low GABA activity in that area. Moreover, the ICV administration of picrotoxin and bicuculline failed to alter the food intake of fasted rats. It can be considered that these antagonists in the concentration employed do not act at extrahypothalamic sites to change the feeding behavior.

These results suggest that there might be an increased GABAergic activity in VMH in a glucoprivic state. Such elevated GABA activity in VMH would have inhibited the VMH satiety center leading to a decrease in the inhibitory output to LH feeding center, thereby facilitating a desire for food intake.

Thus, the present findings suggest that VMH plays a dominant role in regulating food intake, probably by means of an increased GABAergic activity. Further evidence regarding VMH's dominance over LH comes from the observation of De Groot [2] 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. Apart from GABA certain other putative substances such as TRH, substance P, enkephalins, calcitonin, cholecystokinin, catecholamines etc. seem to act in VMH in the control of food intake [13]. Further studies are necessary to elucidate the possible interactions between GABA and the other substances.

REFERENCES

1. Curtis, D. R. and J. C. Watkins. The pharmacology of amino acids related to gamma aminobutyric acid. *Pharmacol Rev* **17:** 347–391, 1965.
2. De Groot, J. Organisation of hypothalamic feeding mechanisms. In: *Handbook of Physiology, Sec 6, Alimentary Canal, Vol I, Control of Food and Water Intake*, edited by C. F. Code. Washington DC: American Physiological Society, 1967, pp. 239–247.
3. Desiraju, T., M. G. Banerjee and B. K. Anand. Activity of single neurons in the hypothalamic feeding centers: Effect of 2-deoxy-D-glucose. *Physiol Behav* **3:** 757–760, 1968.
4. Grandison, L. and A. Guidotti. Stimulation of food intake by muscimol and beta endorphin. *Neuropharmacology* **16:** 533–536, 1977.
5. Huggett, A. St. G. and D. A. Nixon. Use of glucose oxidase, peroxidase and O-dianisidine in determination of blood glucose. *Lancet* **2:** 368–370, 1957.
6. Janowitz, H. D. and A. C. Ivy. Role of blood sugar levels in spontaneous and insulin induced hunger in man. *J Appl Physiol* **1:** 643–645, 1948.
7. Kelly, J., G. F. Alheid, A. Newberg and S. P. Grossman. GABA stimulation and blockade in the hypothalamus and mid brain: Effects of feeding and locomotor activity. *Pharmacol Biochem Behav* **7:** 537–541, 1977.
8. Kimura, H. and K. Kuriyama. Distribution of gamma aminobutyric acid (GABA) in the rat hypothalamus: Functional correlates of GABA with activities of appetite controlling mechanism. *J Neurochem* **24:** 903–907, 1975.
9. König, J. F. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas*. Baltimore: Williams and Wilkins, 1963.
10. Lenin Kamatchi, G., P. Bhakthavatsalam, Dinesh Chandra and J. S. Bapna. Inhibition of insulin hyperphagia by gamma aminobutyric acid antagonists in rats. *Life Sci* **34:** 2297–2301, 1984.
11. Mayer, J. Regulation of energy intake and the body weight: The glucostatic theory and the lipostatic hypothesis. *Ann NY Acad Sci* **63:** 15–43, 1955.
12. Meeker, R. and R. D. Myers. In Vivo (^{14}C) amino acid profiles in discrete hypothalamic regions during push-pull perfusion in the unrestrained rat. *Neuroscience* **4:** 495–506, 1979.
13. Morley, J. E. The neuroendocrine control of appetite: The role of the endogenous opiates, cholecystokinin, TRH, gamma aminobutyric acid and the diazepam receptor. *Life Sci* **27:** 355–368, 1980.
14. Olgiati, V. R., C. Netti, F. Guidobono and A. Pecile. The central GABAergic system and control of food intake under different experimental conditions. *Psychopharmacology (Berlin)* **68:** 163–167, 1980.
15. Oomura, Y., M. Ohta, S. Ishibashi, H. Kita, T. Okajima and T. Ono. Activities of chemosensitive neurons related to neurophysiological mechanism of feeding. In: *Recent Advances in Obesity Research. II. Proceedings of the 2nd International Congress of Obesity*, edited by G. A. Bray. Washington: Newman Publishers, 1978, pp. 17–26.
16. Ritter, R. C. and P. Slusser. 5-TG causes increased feeding and hyperglycemia in the rat. *Am J Physiol* **238:** E141–E144, 1980.
17. Smith, G. P. and A. N. Epstein. Increased feeding in response to decreased glucose utilization in the rat and monkey. *Am J Physiol* **217:** 1083–1087, 1969.
18. Tappaz, M. L., M. J. Brownstein and M. Palkovits. Distribution of glutamate decarboxylase in discrete brain nuclei. *Brain Res* **108:** 371–379, 1976.
19. Tappaz, M. L. and M. J. Brownstein. Origin of glutamate decarboxylase (GAD)-containing cells in discrete hypothalamic nuclei. *Brain Res* **132:** 95–106, 1977.