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GABA Stimulation and Blockade in the Hypothalamus and Midbrain: Effects on Feeding and Locomotor Activity[1]

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KELLY, J., G. F. ALHEID, A. NEWBERG & S. P. GROSSMAN. *GABA stimulation and blockade in the hypothalamus and midbrain: effects on feeding and locomotor activity.* PHARMAC. BIOCHEM. BEHAV. 7(6) 537–541, 1977. – Microinjections of the gamma-aminobutyric acid (GABA) antagonist, bicuculline methiodide (BM) (100 ng), into the anterolateral hypothalamus (LH) increased ingestion of sweet milk. A subsequent injection of BM 48 hrs. later produced a type of kindling effect consisting of feeding related automatisms, such as gnawing and biting. The behavioral effects of injections of 100 ng of GABA into the LH were variable. GABA injections into the ventromedial hypothalamus (VMH) reliably increased food intake. GABA injections into the origin of the nigrostriatal dopamine (DA) neurons in the substantia nigra (SN) suppressed it. Similar injections into the origin of the mesolimbic DA cells in the ventral tegmental area (VTA) had no effect on feeding behavior. Following BM injections into the SN, a moderate increase in tilt box activity was observed. A second injection of the GABA blocker 6 days later exaggerated this effect. Short latency extreme hyperactivation was accompanied by unidirectional barrel rolling which persisted until blocked by local injections of GABA.

Gamma-aminobutyric acid Bicuculline methiodide Lateral hypothalamus Ventromedial hypothalamus
Substantia nigra Ventral tegmental area Feeding Locomotor activity

THE PRESENT experiment was undertaken to investigate the possible involvement of GABAergic neurons in the hypothalamus and mesencephalon in the regulation of feeding behavior. There is strong evidence that GABA serves as a neurotransmitter at inhibitory synapses in the mammalian brain [13] and at certain loci in the invertebrate nervous system [14]. GABA and its synthetic enzyme, glutamic acid decarboxylase (GAD), have quantitatively corresponding regional distributions which vary greatly from one location to another in mammalian brain [4]. High concentrations of GABA and GAD have been demonstrated in regions considered to be related to feeding behavior, particularly the hypothalamus [12] and the substantia nigra [5]. Nigral GABA appears to be concentrated in the terminals of axons which originate in the caudate nucleus, globus pallidus or both [1,8]. It may thus provide a feedback modulation of the ascending DA projection [3,11]. Recent evidence suggests that hypothalamic GABA neurons originate within the hypothalamus itself [20].

The ventromedial and lateral hypothalamus have been implicated in the regulation of ingestive behavior by the results of a large number of studies of the effects of electrolytic or chemical lesions, electrical or chemical stimulation and single cell recordings [7]. The results of recent experiments have also implicated a number of extrahypothalamic structures, particularly those related to ascending catecholamine systems [19]. Because of the intimate anatomical relationship between GABA and catecholamine systems in the midbrain [8] and because hypothalamic GABA concentrations vary systematically with blood glucose [12], we have investigated the effects of direct injections of GABA and its putative antagonist, bicuculline methiodide (BM), into these areas. The following is a preliminary report of our findings.

METHOD

Animals

Adult male or female rats (250–325 g) of the Sprague-Dawley strain (Holtzman, Madison, Wisc.) were used. The animals were maintained on ad lib food (Teklad, 6% fat diet) and tap water while housed individually in stainless steel cages. The temperature of the vivarium was main-

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tained at 21–24°C. A 12 hr light-dark cycle (0700–1900 hr light) was employed.

Surgery

Twenty-three ga. stainless steel cannulas (with 30 ga. inner obdurators) were implanted stereotaxically into several brain regions. The cannulas were fastened to the skull such that the tips of the cannulas were 1 mm above the intended site of injection. Chemicals were administered with a 30 ga. injector which extended 1 mm beyond the tip of the outer cannula. Bilateral cannula placements were made in the LH (AP = 6.2, H = −0.5, L = ± 2.5 mm), in the VMH (AP = 6.2, H = −1.5, L = ± 1.0 mm) and in the SN (AP = 2.2, H = −1.5, L = ± 2.0 mm), using coordinates from the de Groot [2] atlas of the rat brain. A single cannula was placed above the midline ventral tegmental area (VTA) at an angle of 15° to the sagittal plane (AP = 1.8, H = −2.5, L = 0.0 mm). Surgery was conducted under Ketamine plus Acepromazine anesthesia.

Postsurgical Procedure

Following surgery food and water intake and general motor ability were monitored. Particular note was taken of ambulatory ability (the presence of akinesia) and of the visual placing response (rats normally extend their forelimbs as they are brought near a table edge when held in a head-down position). All drug tests were deferred until ingestive behavior was normal and no gross motor abnormalities were present.

Chemical Injections and Ingestive Behavior

Intracranial injections of chemicals and subsequent observations of ingestive behavior were conducted in 12 translucent Plexiglas boxes (22 × 26 × 35 cm high) with floors made of 0.5 cm stainless steel rods placed 2 cm apart. Ingestion of sweetened milk was determined by reading calibrated drinking tubes (Wahmann, Baltimore, Md.) to the nearest ml. This procedure allowed frequent recordings without interrupting the animal. The sweetened milk consisted of half Carnation evaporated milk and half sucrose solution (50% aqueous sucrose wt./vol.). All animals were given a pretest experience with the sweet milk by presentation of the milk in drinking tubes while the animals were in their home cages. Intracranial injections were administered with a 50 μl syringe. The animals were placed into the Plexiglas observation chambers 15 min prior to the injections. They were then taken from the chambers, the obdurators were removed and the injector inserted into the outer cannula. A 1 μl volume was injected and 15 sec were allowed for diffusion of the compound from the injector before it was removed from the brain. The animals were then returned to the observation chambers. This procedure was completed in 1–2 min. Each drug injection was preceded by a saline injection of equal volume the day before. In each test, the cumulative volume of milk ingested was determined every 15 min for the first hour after the injection. A final reading 3 hr postinjection was also recorded. Rats with VMH and SN implants were each tested twice for the effects of GABA on feeding. Rats with VTA implants were tested 4 times for the effects of BM on feeding. The effects of all other drugs on feeding were observed once. The effects of GABA and BM on activity were tested once in rats with SN and VTA implants only.

The following chemicals were injected in 1.0 μl volumes of 0.9% saline at each brain location: bicuculline methiodide (BM; 100 ng/μl; pH approx. 5.0) prepared from bicuculline (Sigma Chem. Co., St. Louis, MO.) according to Pong & Graham [16]; and GABA (100 ng/μl; pH approx. 6.0; Sigma Chem. Co., St. Louis, MO.).

Activity

The effects of intracranial injection on activity were measured in rats with SN and VTA cannula placements. Activity was monitored in eight tilt cages located in a sound attenuating room (see [10], for a detailed description). Briefly, a microswitch recorded each crossing from one side of the cage to the other. Readings were taken every 15 min for the first hour and once more 3 hr postinjection.

Histology

Animals were sacrificed with an overdose of Nembutal and perfused trans-cardially with isotonic saline followed by a 10% formol-saline solution. After fixation in Formalin, brains were sectioned on a freezing microtome. Fifty micron sections were made and every other section in the area of the cannula tract was saved. These sections were mounted on glass slides and stained with cresyl violet.

Statistical Analysis

T-tests for correlated samples were used to compare behavior after chemical injections with that after placebo (vehicle) injections. When multiple drug or saline injections were administered median scores were calculated and used for the statistical analysis. For comparative purposes, a single control median was derived from the saline injections prior to both GABA and BM injections for a given group.

RESULTS

Histology

LH. Five female rats with LH implants had cannula placements which were dorsal to the optic tract in the anterior aspect of the dorsolateral LH and medial forebrain bundle (AP = 6.8 –6.2, L = 2.0 –2.5, see Fig. 1). One animal had asymmetrical placements in the lateral dimension. The left cannula was properly placed, but the right cannula was displaced laterally almost to the amygdala. Another animal had one cannula positioned slightly more posteriorly (AP = 5.8). Most placements were in the vicinity of the highest hypothalamic GABA concentrations [12].

VMH. Four female rats had bilaterally symmetrical cannula placements. A fifth had the left cannula slightly more medial (L = 0.7). All other cannula tips were just dorsal to the region between the rostral tip of the VMH and LH at approximately AP = 6.8 –6.2, L = 1.0 –1.4 (see Fig. 1). Placements were in the region with intermediate hypothalamic GABA concentrations [12].

SN. Five female rats with SN cannulas had similar bilaterally symmetrical placements which varied slightly in the AP along the dorsal aspect of the SN (AP = 3.0 –1.8). The cannula tips were located over the middle of the nucleus (L = 2.0, see Fig. 1). One exception was an animal with the right cannula at L = 1.2.

VTA. Six female rats with cannulas intended for the

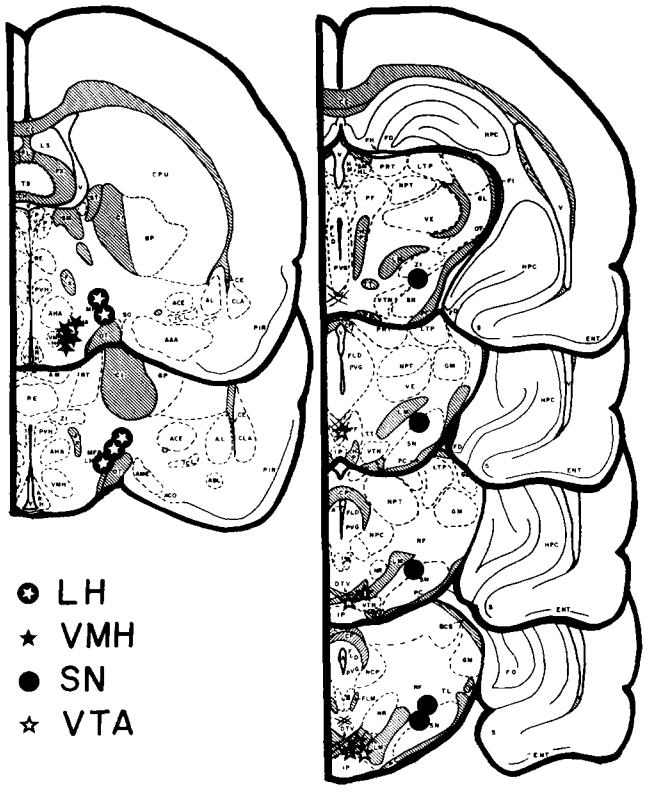

FIG. 1. Diagram of cannula placements in the lateral hypothalamus (LH), ventromedial hypothalamus (VMH), substantia nigra (SN) and in the ventral tegmental area (VTA).

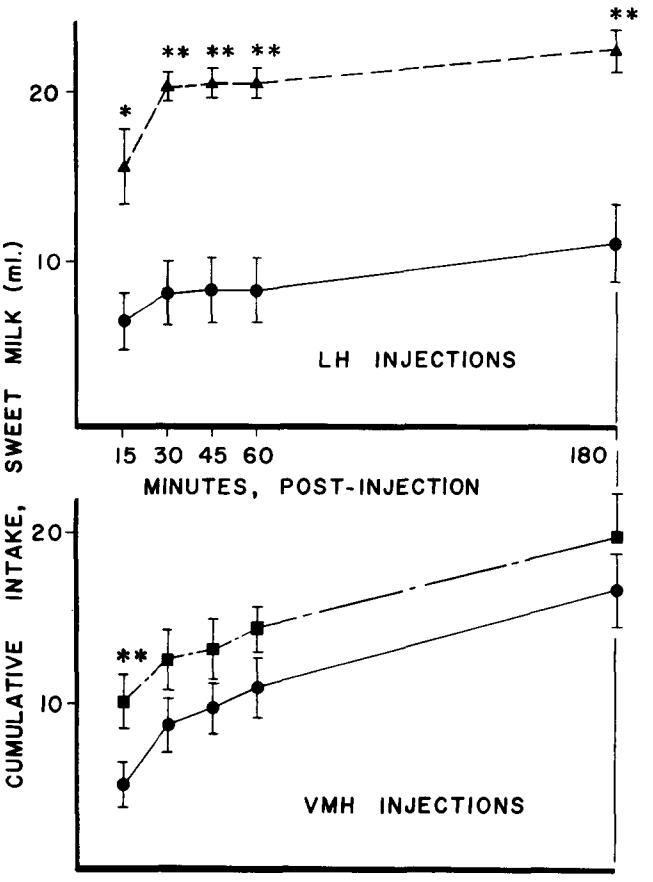

FIG. 2. Cumulative intake of sweetened milk after hypothalamic injections of isotonic saline (•), bicuculline methiodide (▲), or GABA (■). *Significantly different from response to isotonic saline ($p<0.05$). **Significantly different from response to isotonic saline ($p<0.01$).

VTA were found to have similar placements dorsal to the interpeduncular nucleus (L = 0.0 −0.2, see Fig. 1) which varied slightly in the AP (2.8 −1.4).

Postoperative Condition

No persisting disabilities occurred as a result of the surgical procedure. Of the 6 animals in each group, 2 LH rats, 2 VMH rats, 4 VTA rats and 1 SN rat were aphagic on the first operative day. All animals recovered voluntary feeding 48 hr after surgery. Akinesia was noted in 6 rats, but this was not characteristic of any of the four groups, and none of the hypoactive animals remained so for longer than a 48 hr period. In the immediate postoperative period, 17 of the 24 operated animals experienced difficulty with the visual placing response. In some animals this persisted for several days, but all animals responded normally by the fifth postoperative day. LH rats had difficulty in visual placing for an average of 1.33 ± 0.49 days (SEM), VMH rats for 2.16 ± 0.75 days, VTA rats for 1.0 ± 0.37 days and SN rats for 3.0 ± 0.82 days. In all respects all animals had recovered from the effects of surgery at least 1–5 weeks prior to testing.

Chemical Injections

LH injections. Injections of 100 ng of bicuculline methiodide (BM) into the LH reliably ($p<0.01$) increased the intake of sweet milk (Fig. 2). Most of the increase occurred during the first 15 min ($\bar{X}$ = 6.3 ml for saline; $\bar{X}$ = 15.3 ml for BM) with a smaller increase from 15–30 min ($\bar{X}$ = 1.7 ml for saline; $\bar{X}$ = 4.7 ml for BM). After 30 min most animals groomed and retired to the rear of the cage. The food intake of the experimental animals was normal ($\bar{X}$ = 5.4 cc for the first 15 min), when saline injections were given the next day. A second injection of bicuculline 48 hr after the first injection resulted in a type of kindling effect. All animals with LH cannulas engaged in vigorous and persistent (30–45 min), short-latency (1–2 min) feeding automatisms. This consisted of gnawing (the animal's tail, the grid bars of the floor, or the milk spout) accompanied by licking, sniffing and other behaviors apparently related to but incompatible with eating.

The effects of GABA (100 ng) injections into the LH were observed in only three animals. Of these three rats one demonstrated a 50% decrease from baseline food intake; another showed a small initial decrease (15 min) followed by an increase (60 min). The last animal increased its intake above baseline across the entire first 60 min. Because the GABA test followed the bicuculline test, it is possible that persisting effects of bicuculline (i.e., kindling) might have influenced the results of GABA treatment.

VMH injections. Injections of GABA into the VMH resulted in a significant ($p<0.01$) increase in food intake during the first 15 min. The increases were smaller and more variable during the first hour than those produced by

bicuculline injections into the LH, but all animals showed reliable increases (approx. 4.0 ml above baseline in the first 15 min). Before the effect of bicuculline could be observed, two VMH animals had to be discarded due to loose or obstructed cannulas. In the remaining three, bicuculline injections into the VMH resulted in decreases in food intake in two (4.0 and 9.0 cc in the first 15 min); the remaining rat showed no effect in this test.

In another group of animals (males, n = 5) bicuculline injections into the VMH significantly ($p<0.02$) suppressed the intake of Noyes pellets (decreases of 1.4–3.0 g in the first 15 min) and increased the latencies to feed (9–30 min; $p<0.02$). Because further testing with these animals is in progress histological data are not presently available.

SN injections. GABA injections into the SN resulted in a significant ($p<0.05$) decrease in the cumulative intake of sweet milk 30 and 60 min postinjection (see Fig. 3). Intake was also suppressed 45 min after the injection, but the effect failed to meet customary levels of statistical significance ($0.10>p>0.05$). Injections of GABA into the SN failed to affect tilt box activity levels ($\bar{X}$ = 1.70 ± 2.37 crossings for saline; $\bar{X}$ = 1.60 ± 3.51 for GABA). Injections of bicuculline into the SN produced no change in activity in 2 rats; the remaining 2 rats increased their activity (250% and 850%, respectively) in the 3 hr test period. A second injection of bicuculline, given 6 days later, produced extreme and persistent hyperactivation in all animals (characterized by gnawing and violent rolling over the longitudinal body axis). Feeding could not be observed under these conditions. The animals persisted in these barrel rolls continuously for 15 min after the bicuculline injection. At this time they were given a 2 μl injection of GABA (100 ng/μl) into the SN. Within 1–2 min barrel rolling and hyperactivity diminished, and within 10 min. the effect had disappeared completely. The animals then rested in the rear of the observation cages.

VTA injections. Bicuculline injections into the VTA produced a reliable ($p<0.01$) increase in the intake of sweet milk after 3 hr (see Fig. 3). Immediately following bicuculline injections the animals sniffed, groomed and explored. The intense activation observed after repeated bicuculline injections into the SN did not occur. There was no significant effect of GABA injections in the VTA on milk ingestion or tilt box activity. Injections of bicuculline into the VTA increased tilt-box activity in all three rats tested ($\bar{X}$ = 72.2 ± 22.8 crossings for saline; $\bar{X}$ = 185.7 ± 89.8 for bicuculline after 3 hr).

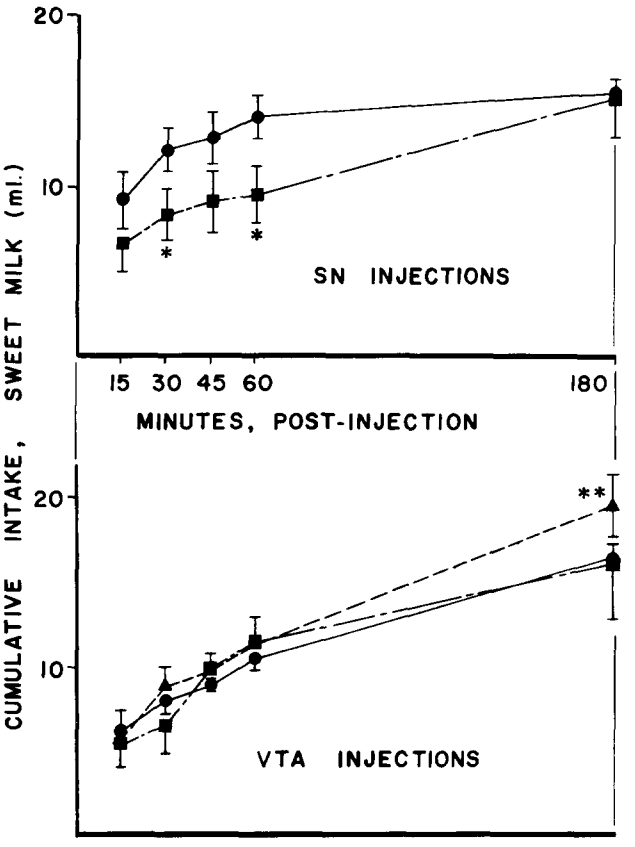

FIG. 3. Cumulative intake of sweetened milk after SN or VTA injections of isotonic saline (●), bicuculline methiodide (▲), or GABA (■). *Significantly different from response to isotonic saline ($p<0.05$). **Significantly different from response to isotonic saline ($p<0.01$).

DISCUSSION

The results of the present preliminary investigation provide evidence for the possible involvement of GABAergic mechanisms in the central control of ingestive behavior. We would like to suggest that GABAergic neurons in the LH and VMH may serve as modulators of afferent inputs to feeding control neurons (i.e., primary efferents).

Bicuculline methiodide, a putative GABA antagonist, increased ingestion of sweet milk, when injected into the LH and suppressed feeding, when administered into the VMH. GABA injections into the VMH produced a complementary increase in intake. The effects of GABA in the LH were variable, possibly because of the interaction with persisting effects of preceding bicuculline methiodide injections into the area, or because of leakage to adjacent components of the VMH. On balance, these results are congruent with the traditional view that the LH and VMH interact reciprocally in the control of ingestive behavior. Our data are also consistent with the report that GABA concentrations in the LH and VMH vary inversely with changes in blood glucose levels [12].

The functions of the GABAergic neurons in the hypothalamus may be similar to that proposed for other brain regions, particularly those utilizing catecholamines as neurotransmitters (e.g., substantia nigra, cerebellum). Roberts [17] suggests a primary role for GABA in the mediation of afferent inputs to control or command neurons within a given neuronal substrate. GABAergic mechanisms in the extrapyramidal system may serve as a model for GABAergic systems in other brain regions. Gale, *et al.* [6] have provided neurochemical evidence that a system similar to that proposed by Roberts exists within the nigrostriatal-strionigral loop. They presented evidence that changes in DA-sensitive adenylate cyclase in the SN depend on GABAergic or Substance P neurons and suggest that this interaction reflects the activation of DA receptors on GABA or Substance P neurons which modulate the activity of the ascending DA projections. This model is analagous to the probable relationship between the noradrenergic projection from the locus coeruleus to the cerebellum. The cerebellum receives terminals from the locus coeruleus [15] which, it is assumed, terminate on

inhibitory neurons which most likely utilize GABA as the neurotransmitter [18]. These interneurons control the output of cerebellar Purkinje cells.

We suggest that a similar relationship may exist in the noradrenergic or adrenergic control of hypothalamic feeding command efferents. The hypothalamus receives several aminergic afferents from the midbrain and caudal diencephalon [15]. These may terminate on GABAergic neurons in the LH and VMH. A recent report suggests that such hypothalamic GABAergic neurons are restricted to an interneuronal system [20]. Although the precise locations of GABA and GAD in specific interneurons has not been determined, we suggest that the relationship between the theoretical GABA mechanisms and aminergic afferents is similar to that described above for the extrapyramidal system and the cerebellum.

The nature of the contribution of GABAergic mechanisms in the midbrain to the regulation of ingestive behavior is uncertain. We have previously reported that GABA blockade in the VTA produced more reliable feeding than more lateral placements in the SN [9]. In the present study we have observed similar effects, but only after long delays. However, in both the VTA and the SN, the effects of bicuculline on feeding were complicated by increased activity and a peculiar kindling effect at the SN involving barrel rolling.

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