The purpose of this study was to clarify further the site of action in the amygdala as well as functional characteristics of feeding in response to two GABA receptor agonists. Guide cannulae for microinjection were implanted stereotaxically in the rat just above the central nucleus of the amygdala (CNA). Microinjections of 0.05, 0.25, 0.5 or 1.0 nmol muscimol, a GABA_A-selective receptor agonist, produced a dose- and time-dependent decrease of food intake in both the satiated and fasted rat. The bilateral injection of muscimol into the amygdala was more effective than a unilateral injection during the first 2 h, although the overall effects were similar. Microinjection of 0.1 nmol bicuculline methiodide, a GABA_A receptor antagonist, into the CNA significantly blocked this inhibitory effect of 0.05 and 0.5 nmol muscimol again in both the satiated and fasted rat. Doses of 0.05, 0.5, 0.0 and 1.0 nmol of the selective GABA_A agonist, baclofen, injected into homologous sites in the CNA did not alter food intake. These findings support the viewpoint that the amygdala and its central nucleus comprise a pivotal region involved in the mechanisms underlying the control of feeding behavior. Further, it is envisaged that hypophagic or anorexic responses are induced through the activation of GABA_A receptors by the presynaptic release of GABA from neurons which form a component of the anatomical system for hunger and satiety.

INTRODUCTION

Historically, neurochemical mechanisms within different regions of the hypothalamus have been implicated in the control of ingestive behavior. Structures including the paraventricular (PVN), dorsomedial (DMN) and ventromedial (VMN) nuclei as well as the lateral hypothalamic area appear to play a key role in this function in terms of both the monitoring of circulating nutrients and the control of feeding and alimentary physiology. Generally, it is believed that the neuronal mechanisms for feeding and satiety are contingent upon the synaptic activity of norepinephrine, dopamine and serotonin, since the profile of their endogenous release and metabolism within the hypothalamus depends on specific physiological stimuli.

The amino acid neurotransmitter, GABA, also is thought to play a role in the central regulation of feeding behavior. From studies on the differential effects of receptor agonists and antagonists of GABA on the intake of food, two reciprocal GABA-sensitive feeding systems presently appear to exist in the hypothalamus. When muscimol is injected into the medial hypothalamus of the rat, eating is stimulated, but when this GABA_A receptor agonist is applied to the lateral area, feeding is suppressed. Pretreatment of the injection site with the GABA_A receptor antagonist, bicuculline, attenuates these effects, which suggests that GABA_A receptors in the hypothalamus are involved in the differential mechanism of appetite regulation. Since binding sites and immunoreactive GABA exist in other regions of the brain involved in the control of feeding, GABA may function in structures other than the hypothalamus to modulate food intake. For example, the amygdala possesses moderate to high densities of GABA-containing cell bodies, nerve fibers, GABA_A receptors and a large amount of the...
enzymatic intermediary of GABA, L-glutamic acid decarboxylase. In addition, electrical stimulation or lesioning of circumscribed nuclei of the amygdala influences both feeding and drinking behaviors, ostensibly by way of its synaptic connections to distinct parts of the hypothalamus. Further, electrical stimulation of efferent pathways arising from the amygdala predominantly inhibits the activity of neurons in the VMN, which implies that a satiety signal originating within the amygdala can act by way of the VMN through the stria terminalis.

The purpose of the present experiments was to investigate the role of GABA in the amygdala in the control of feeding. In this study, the GABA agonists, muscimol, and the GABA agonist, baclofen, were injected directly into the central nucleus of the amygdala (CNA) in both food-satiated and food-deprived rats. In addition, the pharmacological effects of bicuculline and muscimol injected in the same region of the amygdala were characterized under both experimental conditions.

MATERIALS AND METHODS

Female Wistar rats weighing 270-320 g at the time of surgery were housed individually. The animals were maintained at 21 ± 1°C with a 12-h light cycle with lights on at 07.00 h. Purina rat pellets and tap water were provided ad lib throughout the experiments unless specified otherwise. A period of at least 1 week elapsed for acclimation to the laboratory condition prior to surgery.

Surgery

Each of 28 rats was anesthetized with 40 mg/kg sodium pentobarbital given intraperitoneally. Following aseptic surgical procedures described previously, a craniotomy hole was drilled in the calvarium and a chronic 23-gauge stainless-steel guide cannula was stereotaxically implanted unilaterally or bilaterally 1.0 mm dorsal to the CNA. With the nose bar at 3.3 mm below horizontal and 0.0 mm from the midline and dura, the coordinates were AP = -2.3 mm from bregma; LAT = ± 3.8 mm from midline and HOR 7.0 mm from dura. After the cannulae were fixed to the skull with dental acrylic, a 30-gauge stylet was inserted into the guide cannula and a protective cap was screwed onto the pedestal. During the recovery period of at least 7 days each animal was habituated to the experimental procedures.

Experimental design

Rats were maintained and tested on a freshly prepared milk-mash diet consisting of 50 ml sweetened condensed milk, 200 ml tap water and 100 g of ground Purina chow. Prior to an experimental session, free access to water and the highly palatable diet, placed in food cups in the home cages, was given for 2.0 h to ensure maximum satiation. Intakes of food and water were monitored at 0.5, 1.0, 2.0, 4.0 and 24 h after a microinjection of a given drug.

Each compound was microinjected into the CNA by means of a stainless-steel, heat-sterilized 30-ga injector cannula which extended 1.0 mm beyond the tip of the guide cannula. The injector was connected to prefilled polyethylene tubing which was affixed to a 10 μl Hamilton microsyringe. The syringe was then mounted on a Harvard Model 22 infusion pump calibrated to deliver a volume of 0.5 μl at a rate of 1.0 μl/min. Following an injection, the needle was kept in place for an additional 2.0 min, to maximize infiltration of the solution into the tissue, and then replaced by the stylet.

The solution of each drug was prepared as the base immediately before the experiment with pyrogen-free 0.9% sodium chloride and passed through a 0.22 μm Millipore filter. Muscimol (Sigma) was prepared and injected in a concentration of 0.05, 0.25, 0.5 or 1.0 nmol/0.5 μl (1 nmol = 114.10 ng). Baclofen (Ciba-Geigy) was prepared in a concentration of 0.05, 0.5, 5.0 or 10.0 nmol/0.5 μl (1 nmol = 213.67 ng). Each rat served as its own control and received a given dose of a drug or control saline vehicle in a randomized sequence. Groups of five rats were tested once or twice weekly between 09.00 h and 11.00 h under either a condition of 18-h food deprivation or in the presence of the ad lib milk-mash diet.

Histological analysis

At the conclusion of the experiments, each rat was given an overdose of sodium pentobarbital injected intraperitoneally. The heart was perfused transcardially with isotonic saline followed by 10% formalin. After the brain was removed and stored in formalin for at least 48 h, coronal sections then were cut at 40 μm on a freezing microtome. Each section was mounted and stained with Cresyl violet according to standard procedures. The sites of injection were identified under light microscopy and mapped on histological reconstructions.

Statistical analysis

Data from all experiments were analyzed using the StatSoft CSS Statistica software program. A two-way (time and dose) analysis of variance with repeated measures was performed on the grouped data, followed by Newman–Keuls post hoc tests for either means or interaction effects. The 95% level of confidence was accepted as statistically significant in all analyses.

RESULTS

A composite histological analysis of the sites of micro-injection of each of the drugs is presented in Fig. 1. In 20 of the 28 rats studied, the tip of the injector needle was positioned accurately within the CNA between AP -2.1 and -2.8 mm from bregma. Those rats in which the sites were not localized in this region of the amygdala were not considered further.

Muscimol effects: satiated state

As shown in Fig. 2, 0.05, 0.25, 0.5 or 1.0 nmol muscimol microinjected unilaterally into the CNA produced a dose-dependent decrease in intake of the palatable food in the satiated rat. An analysis of variance revealed significant effects of dose ($F_{20,40} = 4.06$, $P < 0.01$), time ($F_{4,80} = 635.70$, $P < 0.001$), and a dose-by-time interaction ($F_{4,80} = 9.39$, $P < 0.025$). The ingestion of food was reduced significantly at the 0.5, 1.0, 2.0 and 4.0 h interval after injection ($F_{4,20} = 7.30$, $P < 0.001$; $F_{4,20} = 45.82$, $P < 0.001$; $F_{4,20} = 49.97$, $P < 0.001$; $F_{4,20} = 6.08$, $P < 0.002$, respectively). Post hoc Newman–Keuls tests revealed that each dose of muscimol significantly reduced the cumulative intake of food at 0.5, 1.0 and 2.0 h and at 4.0 h as well by 0.05, 0.5 and 1.0 nmol. However, at 24 h, the values for the saline and muscimol injected rats were identical ($F_{20,40} = 2.11$, $P > 0.05$, data not shown).
As portrayed in Fig. 3 (top), muscimol injected bilaterally into the CNA, at doses of either 0.05 or 0.5 nmol, suppressed feeding significantly at each time interval \( (F_{2,12} = 26.01, \ P < 0.001 \) at 0.5 h; \( F_{2,12} = 4.06, \ P < 0.05 \) at 1.0 h; \( F_{2,12} = 8.29, \ P < 0.01 \) at 2.0 h; and \( F_{2,12} = 17.96, \ P < 0.001 \) at 4 h). At 24 h, the intakes were not significantly different from control \( (F_{2,12} = 0.58, \ P > 0.05, \) data not shown). The magnitude of the effect of muscimol (Fig. 3, top) was substantially greater following bilateral injections during the first 2.0 h \( (0.05 \text{ nmol unilateral vs. } 0.05 \text{ nmol bilateral}; \ F_{1,8} = 17.72, \ P < 0.005 \) at 0.5 h; \( F_{1,8} = 28.63, \ P < 0.001 \) at 1.0 h; \( F_{1,8} = 35.44, \ P < 0.001 \) at 2.0 h; \( F_{1,8} = 2.76, \ P > 0.1 \) at 4.0 h; and \( F_{1,8} = 0.40, \ P > 0.1 \) at 24 h; 0.5 nmol unilateral vs. 0.5 nmol bilateral; \( F_{1,8} = 6.18, \ P < 0.05 \) at 0.5 h; \( F_{1,8} = 8.64, \ P < 0.02 \) at 1.0 h; \( F_{1,8} = 28.12, \ P < 0.001 \) at 2.0 h; \( F_{1,8} = 1.70, \ P > 0.1 \) at 4.0 h; and \( F_{1,8} = 0.66, \ P > 0.1 \) at 24 h). The difference between bilateral and unilateral injections of muscimol, however, was not dependent on dose of the drug \( (\text{dose: } F_{3,16} = 0.52, \ P > 0.05; \text{dose} \times \text{time: } F_{12,14} = 0.43, \ P > 0.1)\).

**Muscimol effects: food deprivation**

The microinjection of 0.05, 0.25, 0.5 or 1.0 nmol muscimol into the CNA of the rats deprived of food for 18 h, reduced the intake significantly \( (\text{dose: } F_{4,20} = 4.39, \ P < 0.01; \text{time: } F_{4,20} = 623.23, \ P < 0.001; \text{and dose} \times \text{time: } F_{4,80} = 4.39, \ P < 0.01)\).
time interaction: \( F_{(4,80)} = 2.77, P < 0.001 \). As shown in Fig. 3 (bottom), the ingestion of food was reduced at the 0.5, 1.0, 2.0 and 4.0 h period after the micro-injection of muscimol (\( F_{(4,20)} = 6.73, P < 0.001 \); \( F_{(4,20)} = 5.38, P < 0.005 \); \( F_{(4,20)} = 4.50, P < 0.01 \); and \( F_{(4,20)} = 3.29, P < 0.05 \), respectively). Post hoc Newman-Keuls tests showed significant differences in the consumption of food (Fig. 3, bottom) at 0.5, 1.0 and 2.0 h at each dose, and at 4.0 h after the 1.0 nmol dose of muscimol. However, the 24-h intake values were identical in the saline control and the muscimol injected groups (\( F_{(4,20)} = 0.50, P > 0.05 \), data not shown).

**Muscimol effects: reversal by bicuculline**

Bicuculline (0.1 nmol) microinjected into the CNA, induced a kindling-like effect in the rats whether they were satiated or fasted, which was characterized by gnawing, licking and sniffing. After a period of 30–45 min behavior of the animals returned to normal. As presented in Fig. 4, bicuculline suppressed feeding in the satiated rat when compared to saline-treated controls (dose: \( F_{(4,32)} = 13.51, P < 0.01 \); dose × time: \( F_{(4,32)} = 3.27, P < 0.05 \)); at the 2.0 and 4.0 h intervals (\( F_{(1,8)} = 22.90, P < 0.01 \); \( F_{(1,8)} = 42.96, P < 0.001 \), respectively).

In the fasted rat (Fig. 4), bicuculline injected into the CNA significantly reduced the cumulative intake of food (\( F_{(1,8)} = 21.38, P < 0.002 \)). Although no differences occurred at 4.0 or 24 h (\( F_{(1,8)} = 2.99, P > 0.05 \) and \( F_{(1,8)} = 0.94, P > 0.05 \), respectively), post-hoc comparisons showed a significant decrease in eating at 0.5, 1.0 and 2.0 h (\( F_{(1,8)} = 27.29, P < 0.01 \); \( F_{(1,8)} = 12.99, P < 0.01 \); and \( F_{(1,8)} = 11.76, P < 0.01 \), respectively).

As portrayed in Fig. 5 (top), pretreatment of the CNA of the satiated rat with 0.1 nmol bicuculline significantly antagonized the anorexic effect induced by either 0.05 or 0.5 nmol muscimol injected unilaterally into the same locus (\( F_{(1,8)} = 3.96, P < 0.01 \)). The combination of this GABA<sub>\lambda</sub> antagonist with muscimol attenuated the inhibitory effect of the agonist at each time interval during the first 4.0 h (0.05 nmol: \( F_{(1,8)} = 129.28, P < 0.001 \) at 0.5 h; \( F_{(1,8)} = 473.88, P < 0.001 \) at 1.0 h; \( F_{(1,8)} = 68.34, P < 0.001 \) at 2.0 h; \( F_{(1,8)} = 12.97, P < 0.01 \) at 4.0 h; 0.5 nmol: \( F_{(1,8)} = 74.56, P < 0.01 \) at 0.5 h; \( F_{(1,8)} = 56.16, P < 0.001 \) at 1.0 h; \( F_{(1,8)} = 50.62, P < 0.001 \) at 2.0 h; \( F_{(1,8)} = 6.92, P < 0.05 \) at 4.0 h. At the 24-h time period no differences were observed in the cumulative intakes of food in these groups (0.05 nmol: \( F_{(1,8)} = 3.38, P > 0.05 \); 0.5 nmol: \( F_{(1,8)} = 2.76, P > 0.05 \)).

A dose of 0.1 nmol bicuculline microinjected at the same site 5.0 min before muscimol antagonized the anorexic response, as shown in Fig. 5 (bottom), induced by either 0.05 or 0.5 nmol of the GABA agonist (0.05 nmol: \( F_{(1,8)} = 8.04, P < 0.05 \); 0.5 nmol: \( F_{(1,8)} = 6.36, P < 0.05 \)). The differences were consistent over the first 2.0 h (0.05 nmol: \( F_{(1,8)} = 7.20, P < 0.02 \) at 0.5 h; \( F_{(1,8)} = 60.85, P < 0.001 \) at 1.0 h; \( F_{(1,8)} = 16.11, P < 0.005 \) at 2.0 h; \( F_{(1,8)} = 0.01, P > 0.1 \) at 4.0 h; \( F_{(1,8)} = 0.20, P > 0.1 \) at 24 h; 0.5 nmol: \( F_{(1,8)} = 8.12, P < 0.05 \) at 0.5 h; \( F_{(1,8)} = 13.19,
P < 0.01 at 1.0 h; \( F_{4,20} = 5.44 \), \( P < 0.05 \) at 2.0 h; \( F_{4,20} = 1.49 \), \( P > 0.2 \) at 4.0 h and \( F_{4,20} = 0.08 \), \( P > 0.1 \) at 24 h).

Baclofen effects: satiated and food-deprived states

The microinjection of 0.05, 0.5, 5.0 or 10.0 nmol baclofen into the CNA of the satiated rat tended to evoke an increase in feeding, as presented in Fig. 6. However, an analysis of variance showed no significant effect of the drug on the intake of food during the 24 h period (\( F_{4,20} = 1.80 \), \( P > 0.1 \)). Post hoc comparisons revealed no significant effects at each time period (\( F_{4,20} = 1.25 \), \( P > 0.1 \) at 0.5 h; \( F_{4,20} = 0.81 \), \( P > 0.1 \) at 1.0 h; \( F_{4,20} = 0.73 \), \( P > 0.1 \) at 2.0 h; \( F_{4,20} = 0.24 \), \( P > 0.1 \) at 4.0 h), although the lowest dose of baclofen augmented intake of food significantly at the 24 h period (\( F_{4,20} = 3.21 \), \( P < 0.05 \), data not shown). In food-deprived rats, 0.5 and 5.0 nmol doses microinjected in homologous sites in the CNA failed to affect feeding (data not shown).

DISCUSSION

Historically, the amygdala has been implicated in several aspects of the regulation of feeding behavior from an anatomical and functional standpoint. In the 1970s, Fonberg postulated the existence of a morphological homology between lateral and medial areas of the amygdala with those of the hypothalamus. The present results not only support the involvement of the central portion of this structure in the mechanisms of hunger and satiety but also provide evidence that the GABA_A receptor in the amygdala comprises one of the neurochemical signals operating to control feeding.

The presence of both GABAergic neurons as well as GABA_A and GABA_B receptors has been demonstrated in anatomical areas related functionally to the mechanism for feeding, including the hypothalamus and amygdala. Our experiments show that the GABA_A receptor agonists, muscimol, micro-injected into the CNA suppresses the feeding behavior of the rat in a dose-related manner under the conditions of both satiety and food deprivation. Since this inhibitory action is blocked locally by GABA_A receptor antagonists, the specificity of this subtype of GABA receptor in the control of feeding is supported. Since, the overall daily intake of food remained unaffected by muscimol, the rats regulate their daily caloric requirement in spite of acute perturbation of the GABA system in the amygdala. Since equimolar doses of baclofen injected in the CNA tend not to alter the intake of food, this implies that GABA_B receptors are not involved in eating behavior. The suppression of food intake induced by bicuculline in the CNA in either the food deprived or satiated rat also could be explained by an impairment of sensory-motor function or by a feedback inhibition through GABA neurons in the CNA.
increase^{220} or decrease in feeding^{42}. Overall, therefore, the specific anatomical structure affected by a GABA receptor agonist or antagonist apparently determines the nature and direction of the feeding response of the rat.

Since baclofen and muscimol in the nmol doses used in the present study do not modify locomotor activity significantly, the observed reduction in the intake of food apparently is not a secondary consequence of drug-induced impairment of sensory-motor function or arousal. Rather the satiety-like response may mimic that of endogenously released GABA acting on the GABA_A type of receptor in the amygdala. A functional link between the metabolism of glucose and release of GABA within the CNA suggests that GABA could operate as a translational mechanism between a given metabolic state and the neuronal signals for the cessation of feeding^{21,20,29,33}. Thus, it is envisaged that a reciprocal system influencing messages for satiety exists between the amygdala and medial hypothalamus by way of the stria terminalis^{13,15}. GABA_A receptors on GABAergic neurons in the CNA and their caudal-ventral projections to the hypothalamus seem to form a part of the neural regulation of ingestive behavior in the rat. Further, the reason why systemically or centrally administered GABA agonist exert a dual role on food intake could be explained by its multiple action on neurons containing certain peptides and/or monoamines, which are implicated in the regulation of ingestive behavior^{7,15,25,31,47}.

Acknowledgements. The authors wish to thank Drs. J.A. Armengol, A.F. Alonso and I.M. Cwierz for their excellent technical assistance. This research was supported in part by CICYT Grant PB 88-0551 and Junta de Andalucía Grant to F.J.M., and by National Science Foundation Grant BNS 84-10663 to R.D.M. Baclofen was kindly supplied by Ciba-Geigy, Basel, Switzerland.

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