Jejunal administration of linoleic acid increases activity of neurons in the paraventricular nucleus of the hypothalamus

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INFUSIONS OF LIPIDS INTO the small intestine can suppress food intake. Under some conditions, the suppression is large enough to result in negative energy balance (4, 8, 9, 35), suggesting a potential clinical role for weight management in humans. For example, we found that jejunal infusions of the long-chain fatty acids (linoleic acid or oleic acid) reduced total caloric intake by ~15%. When infusions were administered on 21 consecutive days, body weight was reduced by 10% and carcass fat by 48% compared with controls (8). This effect depends, in part, on activation of vagal afferents, especially those in the celiac branches. Infusions of lipids into the small intestine increase celiac vagal afferent activity (23, 25). In addition, we observed that selective celiac vagotomy attenuated linoleic acid-induced suppression of food intake by ~50% (Ref. 9; but see Ref. 33). However, because total subdiaphragmatic vagotomy was no more effective in this regard than was celiac vagotomy (6), there must be a nonvagal component that also contributes to suppression of food intake.

Our laboratories were especially interested in the potential role of the paraventricular nucleus of the hypothalamus (PVN) in mediating suppression of intake produced by intraintestinal lipids because of its central role in energy balance (cf. Refs. 3, 15, 27, and 34). For example, bilateral lesions of the PVN can produce hyperphagia (7, 28, 31) and local infusions of neuroactive substances such as melanotan II (MT II) into the PVN can suppress food intake (11). Moreover, the PVN receives a variety of signals from the periphery by both vagally mediated and vagally independent routes. Consistent with this idea, Monnikes et al. (20) reported expression of c-fos in the PVN in response to duodenal infusion of a triglyceride (Intralipid), an effect that was attenuated, but not eliminated, by perivagal application of capsaicin. This dependence on vagal and nonvagal mediation parallels the results of our vagotomy studies and suggests the hypothesis that the PVN is an important site of integration responsible for the suppression of total caloric food intake that we observed after intestinal infusions of long-chain fatty acids. As an initial test of this hypothesis, we have recorded single-unit activity of PVN neurons in rats after jejunal administration of linoleic acid. Intravenous administration of CCK-8 was also examined in a preliminary attempt to characterize these PVN neurons. CCK activates vagal afferents (cf. Ref. 22) and is purported to mediate the vagal component of lipid-induced suppression of feeding (16, 19, 24).

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats were obtained from Harlan (Pratville, AL). Rats were housed in bedded, plastic cages under a 12:12-h light-dark cycle. Food and water were available on an ad libitum basis. All rats weighed between 400 and 500 g at the time of testing. All studies were approved by the Animal Care and Use Committee at the University of Alabama at Birmingham.

Apparatus. Tungsten microelectrodes (Micro Probe, Potomac, MD; 0.9–1.05 MΩ) were used for conventional extracellular recordings of single units in the PVN. Unit potentials were amplified, displayed on an oscilloscope, isolated by using an analog delay circuit (BAK DDIS-1), and saved on a computer.

General surgical procedures. Each rat initially received an intraperitoneal injection of 50 mg/kg of pentobarbital sodium. Catheters were inserted into the trachea for artificial ventilation, right femoral artery for recording of arterial blood pressure, and right femoral vein for continuous administration of anesthetic (methohexital sodium at 2.77 mg/h). The rat was paralyzed with pancuronium bromide (0.2 ml) and artificially ventilated (60 breaths/min). The head of the rat was placed in a stereotaxic apparatus, and a midline incision was made. A region of skull and dura was removed to permit electrode insertion.

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Randich, Alan, Paula C. Chandler, Hannah C. Mebane, Meredith E. Turnbach, Stephen T. Meller, Gary R. Kelm, and James E. Cox. Jejunal administration of linoleic acid increases activity of neurons in the paraventricular nucleus of the hypothalamus. Am J Physiol Regul Integr Comp Physiol 286: R166–R173, 2004; 10.1152/ajpregu.00431.2003.—The present experiment examined whether neurons located in the paraventricular nucleus of the hypothalamus (PVN) respond to intestinal infusions of long-chain fatty acids. Single-unit recordings were made of neurons located in and adjacent to the PVN during jejunal administration of linoleic acid. Jejunal administration of linoleic acid increased single-unit activity of neurons located in the PVN but did not affect activity of neurons located in adjacent tissue outside the PVN. The largest increases in neuronal activity were observed in the anterior PVN (0.9–1.3 mm posterior to bregma) compared with the posterior PVN (1.8–2.1 mm posterior to bregma). Jejunal administration of saline failed to affect activity of neurons located either inside or outside the PVN. When the same neurons were subsequently tested for their response to intravenous administration of 2 μg/kg of CCK-8, excitatory responses were more frequently observed than inhibitory responses, but both types of responses were observed regardless of whether neurons were located inside or outside the PVN. In addition, there was no strong correlation between the magnitude of the neuronal response evoked by jejunal administration of linoleic acid compared with intravenous CCK-8. These data suggest that neurons located in the anterior PVN may play a role in the mediation of suppression of food intake produced by intestinal administration of lipids.

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Intended coordinates for PVN electrode placement ranged between 0.2 and 0.5 mm lateral, 7.1 and 7.3 mm ventral to the brain surface, and ~0.9 and 2.1 mm posterior to bregma.

A midflank incision was made, and the jejunum and ileum were exteriorized from the rat. A catheter (polyethylene-100) was inserted in the jejunum 50 cm rostral to the ileocecal junction. A suture was tied in place at the entry point of the catheter, and it eliminated any possible proximal diffusion of infusate. An exit port was made at the ileocecal junction. The jejunum and ileum were flushed with saline, the tissue was then replaced in the body, and the midflank incision was closed loosely with wound clips.

Experimental procedures. The electrode was lowered to the PVN region. A single unit was isolated, and neuronal activity was monitored until baseline activity was stable. Units were tested for mechanical sensitivity by applying a Q-tip to the jejunum/ileum/mesentry and were then rated nonquantitatively as excited, inhibited, or not affected by observing neuronal activity on the monitor. The first experiment determined whether units were affected by jejunal administration of either linoleic acid or saline (control). A 5-min baseline period was recorded and then followed by a slow infusion (~1 min) of either linoleic acid (2 ml; 16 kcal) or saline (2 ml) in the jejunum. This was followed by a 1-ml air flush. This amount of linoleic acid was sufficient to coat the entire jejunum and the proximal two-thirds of the ileum, as determined in preliminary studies. Recording then continued for the next 60 min. Only one substance was tested in each rat.

We then determined whether the same neuron was affected by intravenous administration of CCK-8, thereby indicating a possible vagal afferent input. After the test of either linoleic acid or saline was completed, neuronal activity was recorded for a 100-s baseline period. Each rat then received an intravenous bolus infusion of CCK-8 (2 μg/kg followed by a 0.5-ml saline flush), and neuronal recording continued for another 10 min. Previous studies from our laboratory have demonstrated that this dose of CCK-8 produces an immediate robust response in celiac and hepatic vagal afferents that is maximal in the first 100 s after administration (cf. Ref. 22). The site of the brain recording was then electrolytically lesioned for subsequent analysis.

Data analysis. Because some neurons could not be maintained for the intended 1-h recording period, two categories of data were formed on the basis of the duration of the neuronal recording. Complete data (n = 45) refer to recordings maintained for the entire hour. Partial data (n = 59) refer to recordings maintained for at least 30 min. Partial data included the first 30 min of data for all neurons reported in the complete data set described above and the first 30 min of data from an additional 14 neurons that could not be recorded for the full hour. Data were also grouped based on whether the site of the recording was inside or outside the PVN (referred to as hit or miss, respectively) and the type of infusate (linoleic acid vs. saline).

Neuronal activity was normalized to reduce variability between recording preparations. Specifically, raw data were collected as the number of discharges occurring in 1-s bins, i.e., discharges per second. The first 5 min of each recording session were control data, reflecting spontaneous activity in the absence of any manipulation. For statistical analysis, these 5 min of data were converted to standard scores where, for each 1-s of data, \( z = \frac{(number \ of \ discharges - mean)}{standard \ deviation} \). The same transform was then applied to each of the 1-s values following the infusion, i.e., these values were normalized relative to the mean and standard deviation of the control period. If an experimental treatment had no effect, \( z \) scores calculated for the experimental period would vary around 0, just as during the control period. On the other hand, if a treatment increased or decreased activity, then observed \( z \) scores should be consistently greater than or less than 0, respectively.

For each rat, \( z \) scores for each data point for successive 5-min blocks, and group mean \( z \) scores were then calculated. Overall ANOVAs were performed on these mean \( z \) scores and were followed by post hoc comparisons when appropriate. For post hoc analyses of complete data, contrasts were performed on means on the basis of data grouped over 15-min time blocks after the initial 5 min baseline period. For post hoc analyses of the partial data, contrasts were performed on data grouped over 10-min time blocks after the initial 5-min baseline period. Differences between pairs of means were evaluated by using Holm’s procedure (12). Statistical significance was set at \( \alpha = 0.05 \) (one-tailed) in all analyses.

Additional analyses were performed for linoleic acid tests. Recording sites were grouped within the PVN according to rostral/caudal level, i.e., 0.9, 1.3, 1.8, or 2.1 mm posterior to bregma. For each unit, a mean \( z \) score was calculated for the first 30 min after infusion of linoleic acid (partial data were used to maximize sample size for each level). One-way ANOVA was performed, followed by linear trend analysis using the rostral/caudal levels as weights. In addition, the group mean for each level was compared against a null hypothesis value of 0 using a one-sample \( t \)-test with Holm’s criterion for significance of the four tests.

To determine whether CCK-8 administration evoked either a significant increase or decrease in neuronal activity, a criterion was adopted that the mean \( z \) score for the 100-s period immediately after CCK-8 administration should exceed either \( +0.2 \) (excitation) or \( -0.2 \) (inhibition). The rationale for this decision rule was that, according to the null hypothesis, the mean should equal 0 and the standard error of the mean for that 100-s period should equal 0.1, i.e., the standard deviation, 1.0, divided by the square root of 100, because there was one data point per second. In this manner, a \( z \) score of \( \pm 0.2 \) would be two standard errors above or below the null hypothesis mean. The first 100 s after CCK administration were used because previous studies have shown that the response of both celiac and hepatic vagal afferents to 2 μg/kg of CCK-8 is maximal at this time, and we were interested in possible vagal afferent influences on PVN neurons.

Histology. Each rat was overdosed with pentobarbital sodium (100 mg/kg iv) at the termination of the experiment. Electrolytic lesions of the recording sites were histologically identified in 40-μm sections stained with cresyl violet. All sites of recording were mapped by using a modified version of PVN sections that were described by Swanson et al. (29). Four sections representing −0.9, 1.3, 1.8, and 2.1 mm posterior to bregma were used.

Drugs. Linoleic acid (60% linoleic acid, 30% oleic acid, and 10% nonspecified fatty acids) and CCK-8 were purchased from Sigma Chemical.

RESULTS

A between-groups ANOVA of mean neuronal activity obtained over the 5-min baseline periods in the complete group revealed no significant between-groups differences \( (F_{3,41} = 1.10; P = 0.35) \). The mean frequency of neuronal discharges varied greatly across neurons and ranged from a minimum of 0.64 to a maximum of 17.97 discharges/s.

Figure 1 presents the group mean neuronal responses to jejunal administration of either linoleic acid or saline on the basis of whether the recording location was inside or outside (hit or miss, respectively) of the PVN. Data were collapsed over all sites of recording from 0.9 to 2.1 posterior to bregma to form group means. Both complete and partial data sets are presented. Figure 1 shows that jejunal administration of linoleic acid resulted in a substantial, time-dependent increase in responses of units located in the PVN (linoleic/hit), whereas linoleic acid had no substantial effect on neuronal activity of units located outside the PVN (linoleic/miss). Jejunal administration of saline resulted in no substantial changes in neuronal activity, regardless of whether units were located inside (saline/hit) or outside (saline/miss) of the PVN. An ANOVA was performed on the complete data by using factors of location.
(hit vs. miss), infusate (linoleic acid vs. saline), and time. The ANOVA indicated a significant effect of infusate \((F_{1,40} = 4.79; P = 0.03)\) and a location \(\times\) infusate \(\times\) time interaction \((F_{11,440} = 3.15; P = 0.0004)\). Post hoc contrasts performed on group means blocked over 15-min time periods revealed that the mean \(z\) scores for the linoleic/hit group were significantly greater than those of the saline/hit control group in every 15-min block \((t_{40} \text{ ranges from } 1.68 \text{ to } 3.28)\). In addition, mean \(z\) scores for the linoleic/hit group were significantly greater than those of the linoleic/miss group in the last three 15-min blocks \((t_{40} \text{ ranges from } 2.76 \text{ to } 3.26)\). There were no significant differences in contrasts performed on any comparisons between the linoleic/miss and saline/miss groups. Thus only linoleic acid significantly increased the activity of neurons located in the PVN, and neither linoleic acid nor saline affected neuronal activity of neurons located outside the PVN.

Two additional points are worth noting. First, the responses that were evoked by jejunal administration of linoleic acid were almost always excitatory. For example, 17/18 neurons in the linoleic/hit group had a positive mean \(z\) score when the 5-min means were averaged across the 60-min recording period, and no strong inhibitory response was observed in the remaining unit. Second, the magnitude of the effect of linoleic acid depended on the rostral/caudal level within the PVN. As seen in Fig. 2, 30-min means increased with more rostral recordings. A one-way ANOVA revealed significant differences among the four levels \((F_{3,19} = 3.60, P = 0.03)\), and linear trend analysis accounted for 90% of the between-groups sum of squares \((F_{1,19} = 9.75, P = 0.005)\). Finally, one-sample \(t\)-tests revealed significant increases at \(-0.9\) and \(-1.3\) mm \((P < 0.01)\) but not at \(-1.8\) and \(-2.1\) mm posterior to bregma.

Figure 3 shows an example of the increase in activity of a typical neuron located in the PVN in discharges per second following administration of linoleic acid. Notice that activity is stable during the baseline period and then gradually increases after jejunal administration of linoleic acid.

Figure 4 presents the recording sites of all neurons tested with either linoleic acid or saline. When possible, the neurons reported above were tested for their response to intravenous administration of 2 \(\mu g/kg\) CCK-8. We were interested to determine whether a vigorous response could be observed in the first 100 s after administration of CCK-8, which would be indicative of a vagal afferent signal. Some neurons showed vigorous excitatory responses, whereas a few showed inhibitory responses. Two typical examples of excitation and inhibition are shown in Fig. 5. However, whether a neuron responded to CCK-8 did not depend on whether the neuron was located inside vs. outside the PVN. Of all of the neurons that we were able to test with CCK and using the statistical criteria
defined in MATERIALS AND METHODS, excitatory responses were observed in 55% of the neurons inside and 53% of the neurons outside the PVN, inhibitory responses were observed in 14% of neurons inside and 37% of neurons outside the PVN, and no effect was observed in 32% of neurons inside and 10% of neurons outside the PVN. Figure 6 presents a summary of the location and responses to CCK-8 of all neurons that we were able to test.

We also examined whether there was any relationship between obtaining an excitatory response to CCK-8 and an excitatory response to linoleic acid in neurons located inside the PVN. This analysis used the mean z score in the first 100 s after administration of CCK-8 and the mean z score obtained in the final 5-min period of recording for linoleic acid. This analysis failed to reveal any strong relationship when either all complete neurons were used (r = 0.26) or just neurons located 0.9–1.3 mm caudal to bregma (r = 0.35).

Finally, nonquantitative tests for excitatory responses to a mechanical stimulus did not depend on the location of the neurons either inside or outside the PVN, although there was some suggestion that excitatory effects were more prominently obtained ~1.8 mm caudal to bregma (see Fig. 7).

DISCUSSION

In the present experiment, single-unit recordings were made of neurons located in and adjacent to the PVN from ~0.9 to 2.1 mm caudal to bregma. Jejunal administration of linoleic acid produced significant increases in the activity of neurons inside the PVN. Analyses comparing activity of PVN neurons at different levels revealed that strong excitatory responses to jejunal linoleic acid were largely confined to the anterior PVN, i.e., ~0.9 and 1.3 mm posterior to bregma, and not the posterior PVN, i.e., 1.8 and 2.1 mm posterior to bregma. In contrast, the activity of neurons in the PVN did not change after jejunal administration of saline, nor were changes in activity observed in neurons located outside the PVN in response to either linoleic acid or saline. Thus jejunal infusion of linoleic acid exerted an
excitatory effect that was specific to neurons in the anterior PVN.

The responsiveness described above may mediate the suppression of caloric intake and body weight and fat that we have observed in response to jejunal linoleic acid infusions (8). Electrolytic lesions of the PVN produce hyperphagia and obesity (7, 28, 31). Studies examining c-fos expression have indicated activation of PVN neurons after admin-

Fig. 5. Representative, untransformed tracings of increases (top) and decreases (bottom) in the activity of PVN neurons (discharges/s) produced by intravenous administration of 2 μg/kg CCK-8. Baseline activity was recorded for 100 s before start of infusion.

Fig. 6. Location and responses of all neurons tested with 2 μg/kg CCK-8. A = 0.9, B = 1.3, C = 1.8, and D = 2.1 posterior to bregma. ●, Excitation; ○, inhibition; --, no effect.
ISTRATION OF DUODENAL INTRALIPID (20) AS WELL AS AFTER ADMINISTRATION OF SUBSTANCES THOUGHT TO DECREASE FOOD INTAKE BY DIRECT ACTION ON THE PVN, SUCH AS α-MELANOCYTE STIMULATING HORMONE, COCAINE-AMPHETAMINE-REGULATED TRANSCRIPT, AND SEROTONIN (18, 21, 32).

MORE SPECIFICALLY, OUR RESULTS ARE ALSO CONSISTENT WITH A PROPOSED FUNCTION IN MEDIATING FOOD INTAKE FOR THE ANTERIOR PARVOCELLULAR SUBNUCLEUS OF THE PVN (PVNAP). SARKAR ET AL. (26) BASED THIS PROPOSAL ON A NUMBER OF DISTINCTIVE CHARACTERISTICS EXHIBITED BY THIS AREA. FOR EXAMPLE, THE PVNAP IS THE ONLY SUBDIVISION OF THE PVN TO BE STRONGLY INNERVATED BY NEURONS OF THE ARC THAT CONTAIN GALANIN-LIKE PePTIDE (30). IN ADDITION, NEURONS IN THE PVNAP THAT CONTAIN THYROTROPIN-RELEASING HORMONE RECEIVE ESPECIALLY DENSE α-MELANOCYTE STIMULATING HORMONE INNERRATION (10) AND MAY NOT PROJECT TO THE MEDIAN EminENCE, UNLIKE THE THYROTROPIN-RELEASING HORMONE-CONTAINING CELLS THAT ARE LOCATED IN THE PERIVENTRICULAR NUCLEUS AND MEDIAL PVN (13). Thus according to this hypothesis, the PVNAP has primarily nonhypothalamic functions and may be involved in feeding regulation via interactions with other central sites (26).

AN INTESTINAL LIPID LOAD CAN AFFECT ACTIVITY OF PVN NEURONS BY AT LEAST TWO FUNCTIONAL PATHWAYS. ONE INVOLVES ACTIVATION OF AFFERENT FIBERS OF THE ABDOMINAL VAGUS (23, 25). THese VAGAL AFFERENTS TERMINATE IN THE NUCLEUS OF THE SOLITARY TRACT, AND THEN INPUT IS RELAYED TO THE PVN VIA FIBERS, SOME OF WHICH ARE CATECHOLAMINERGIC. THE IMPORTANCE OF THE VAGAL AFFERENT PATHWAY FOR ACTIVATION OF PVN NEURONS AFTER INTESTINAL LIPID LOADS WAS DEMONSTRATED BY MONNIKES ET AL. (20), WHO REPORTED THAT INTESTINAL LIPID-INDUCED EXPRESSION OF c-FOS IN THE PVN WAS ATTENUATED BY ~70% BY PRIOR PERYGAL CAPSAICIN TREATMENT. NO DIRECT TEST OF VAGAL AFFERENT INVOLVEMENT WAS PERFORMED IN THE PRESENT STUDY. HOWEVER, LIPID-INDUCED INCREASES IN PVN NEURONAL ACTIVITY OCCURRED WITHIN THE FIRST 5 MIN OF JEJUNAL ADMINISTRATION, AND WE OBSERVED A SIMILAR TIME COURSE OF LIPID-INDUCED INCREASES IN CELIAC VAGAL AFFERENT ACTIVITY IN A PREVIOUS STUDY (23). IN CONTRAST, JEJUNAL INFUSIONS OF LINOLEIC ACID REQUIRED ~30 MIN TO AFFECT HEPATIC VAGAL AFFERENT ACTIVITY (22).

Fig. 7. Location and responses of neurons tested with the mechanical stimulus. A = 0.9, B = 1.3, C = 1.8, and D = 2.1 posterior to bregma. ●, Excitation; ○, inhibition; –, no effect.
released endogenous CCK to such a level) as to make it difficult to detect any additional effect of exogenous CCK-8. On the other hand, the dissimilar anatomic distributions of cells responsive to either linoleic acid or CCK-8 argue against any close correspondence between these two types of responsiveness. Most of the units affected by linoleic acid were tightly clustered within the anterior PVN with little indication of responsiveness more caudally, either within the nucleus or outside of it. In contrast, we did not observe any distinct region within or even outside the PVN where neurons uniquely responded to intravenous administration of CCK-8. Across all neurons tested with either linoleic acid or saline, 55% of neurons located in the PVN increased their activity in response to CCK-8, but 53% of neurons outside the PVN did so as well. There were also approximately equal proportions of neurons excited by CCK-8 across all sections from 0.9 to 2.1 mm caudal to bregma. On the other hand, the temporal pattern of excitation that we did observe (Fig. 5) was very similar to that provided in an example by Bagdan and Pittman (1) for a putative oxytocin-containing PVN neuron that was antidromically activated from the posterior pituitary. A small number of neurons, distributed both inside and outside the PVN, were substantially inhibited by 2 μg/kg CCK-8, and we saw virtually no evidence of inhibitory responses to linoleic acid.

In summary, therefore, PVNap may serve as an important central nervous system site, mediating suppression of food intake produced by intestinal administration of lipids. Additional studies will be required to determine input pathways to the PVN and the neurochemical identity of PVN neurons that are affected by intestinal lipids.

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REFERENCES


