Interactions Between the Lateral Hypothalamus and the Periaqueductal Gray

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Anatomical and physiological experiments were conducted to characterize the interactions between the lateral hypothalamus (LH) and the periaqueductal gray (PAG) and to determine the role of neurotensin in their interaction. Anatomical studies using injection of *Phaseolus vulgaris* leucoagglutinin into the LH showed an extensive projection to the ventromedial and the ventrolateral PAG and a less dense projection to the medial and dorsal parts of this region. Physiological experiments were performed on both deeply and lightly anesthetized animals. Electrical stimulation of the LH caused excitation of PAG cells with an onset latency of approximately 14 msec. There was a strong correlation between the response of PAG cells to electrical stimulation and injection of glutamic acid into the LH. Electrical or chemical stimulation of the LH produced an increase in tail flick latency in the lightly anesthetized animals that outlasted the stimulation period. There was a strong correlation between the response of PAG cells to electrical and chemical stimulation of the LH and their response to pressure-injected neurotensin. It is concluded that an excitatory projection from the LH to PAG exists which may involve neurotensin. This pathway may be involved in the analgesia produced by LH stimulation.

Since the original study by Reynolds (1969), there has been a significant interest in the role of the periaqueductal gray (PAG) in pain inhibition (Mayer et al., 1971; Gebhart et al., 1983; Sandkuhler and Gebhart, 1985). Behavioral and physiological studies have shown that electrical and chemical stimulation of PAG produce analgesia that is relayed in part through the rostral ventral medulla (Basbaum et al., 1978; Behbehani and Fields, 1979) and subsequently inhibits nociceptive neurons in the dorsal horn (Basbaum et al., 1977; Bennett and Mayer, 1979; Fields et al., 1983; Sandkuhler and Gebhart, 1985). Although the role of PAG in descending pain inhibition has been established, it is not known how PAG neurons are activated under natural conditions or what transmitters are involved in the activation of these cells. Anatomical studies have established that there is a significant projection from the lateral hypothalamus (LH) to the PAG (Grofova et al., 1978; Krieger et al., 1979; Beitz, 1982;

Mantyh, 1982). Therefore, it is likely that this pathway is involved in the activation of PAG.

Recently we have shown that injection of neurotensin (NT) into the PAG produces strong and long-lasting analgesia that is not reversed by naloxone (Behbehani and Pert, 1984). Since there are NT-containing cell bodies in the LH (Kahn et al., 1980; Jennes et al., 1982) and some of these cells project to the PAG (Shipley et al., 1985), it is possible that activation of the LH may cause release of NT into the PAG and thereby activate these neurons. To demonstrate the projection from LH to PAG we have used injection of *Phaseolus vulgaris* leucoagglutinin (PHAL) into the LH in order to fill the cells and their fibers in this region and have examined the morphology of the fibers originating from these cells that terminate within the PAG. In physiological studies we have compared the response of PAG cells to electrical and chemical stimulation of the LH and their response to NT.

Materials and Methods

Male Sprague-Dawley rats weighing 220–300 gm were used in the physiological experiments. Two types of preparations were used. In the first series of experiments, animals were anesthetized with 400 mg/kg chloral hydrate and were maintained in deep anesthesia with additional intravenous injections of chloral hydrate. After cannulation of the femoral vein, animals were stationed in a stereotaxic instrument. The skull over the PAG, medulla, and hypothalamus was removed, and the brain was covered with 2% agar in saline or saline-soaked Gel Foam. Single-unit recording was made from PAG neurons using a glass electrode (8–12 M Ω) filled with 2 gm% pontamine skyblue dye in 0.5 M sodium acetate glued to another glass electrode filled with 3 M NT. Tungsten stimulating electrodes with tip diameters of 8–15 μ m were placed in the LH and in the nucleus raphe magnus or its adjacent region, the nucleus magnocellularis. In nearly all experiments, a glass electrode containing 100 mM glutamic acid (monosodium L-glutamate) was glued to the stimulating electrode placed in the LH. Pressure of 1–5 psi was used to inject glutamic acid. In order to determine the conduction velocity of fibers connecting the LH to the PAG, a recording electrode was glued to a tungsten stimulating electrode and was placed into the LH and a similar electrode assembly was also placed into the PAG. The PAG region was searched until an area containing neurons responsive to LH stimulation was found. This area was stimulated while a recording was made from the LH region. Once a cell in this area was isolated, its response to PAG stimulation was determined. In particular, antidromic activation of LH neurons by PAG stimulation was tested. LH neurons were considered to be antidromically activated if they responded with a constant-latency spike to high-frequency stimulation (80–100 Hz) of PAG and if collision between an evoked antidromic spike and a spontaneously generated action potential occurred. The latency from the onset of stimulus pulse to the peak of the antidromic spike was used for the calculation of the conduction velocity.

In the second series of experiments, a lightly anesthetized preparation was used. In this series, each animal was anesthetized with 40 mg/kg

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sodium pentobarbital and the surgical procedures described above were performed. Throughout the experiment the animal was infused with methohexital at a rate of 10 to 15 mg/kg/hr. The animal's tail was placed on a specially designed heater that could raise the skin temperature to a desired degree at a rate of 5°C/sec. The LH was stimulated electrically or by injection of glutamic acid (200 mM, 0.1–1 μ l), and the influence of this stimulation on tail flick (TF) latency was examined. Before each measurement the tail temperature was brought down to 25°C. The cut-off latency was 10 sec, and if TF did not occur before this time, the current was automatically cut off and the tail was removed from the heat source. The site that inhibited TF to cut off with minimum current was selected for stimulation.

In experiments involving recording from PAG cells, this area was searched until a spontaneously firing unit was isolated. The projection of that cell to the NRM/NMC was tested by stimulation of these nuclei. The site of stimulation in the LH was chosen by localizing the point in LH that produced maximum effect on PAG neurons with minimum current intensity. In these experiments, the LH-stimulating electrode was placed in the most dorsal part of the LH and currents up to 100 μ A were used to activate the region. The electrode was advanced deeper into the LH until a point that produced maximum effect with minimum current was found. The position of the stimulating electrode was fixed, and this region of the LH was stimulated throughout the experiment. In each experiment, after characterization of the PAG cell, its response to NT was measured. NT was applied incrementally using pressures of 2–20 psi.

The anatomical data presented here are drawn from a collection of 22 observations in which PHAL injections were made in various sites within the LH area. The methods used for the injection of the lectin and subsequent tissue processing follow those of Gerfin and Sawchenko (1984). Briefly, PHAL was delivered by iontophoretic injections made in male Long-Evans rats weighing between 280 and 500 gm and anesthetized with urethane (1 gm/kg). Square-wave, positive-current pulses of 6 μ A amplitude and 5 sec duration were delivered every 10 sec, with total injection times of between 20 and 60 min. The injection pipettes were pulled from 3 mm borosilicate glass capillary tubes on a Canberra-type vertical microelectrode puller (Narashige) and filled with 2.5% PHAL in 0.05 M potassium PBS. The pipette tips were cut with iridectomy scissors to have diameters of between 10 and 50 μ m.

The animals were generally allowed to survive 14 d, although times as short as 7 and as long as 35 d were tried in a few cases. These longer or shorter survival times were not found to have a significant effect on the results. At the end of the survival period, the animals were given a lethal dose of urethane and perfused through the aorta with the following sequence of solutions: 250 ml of buffered normal saline, 250 ml of 4% freshly cracked paraformaldehyde in sodium acetate buffer, pH 6.5, and 250 ml of 4% paraformaldehyde plus 0.05% glutaraldehyde in sodium borate buffer, pH 9.5. The brains were removed and stored overnight in the borate-buffered fixative. Fifty micron sagittal or coronal Vibratome (Lancer, series 1000) sections were cut and processed for immunohistochemical detection of the PHAL according to the Gerfin and Sawchenko (1984) protocol. Incubation in goat-raised primary antisera (Vector Laboratories) was for 48 hr. Subsequent detection was with the avidin-biotin-peroxidase technique (Hsu et al., 1981) using the commercially available reagent kit (Vector Laboratories). The tissue was reacted with 3'-5'-diaminobenzidine as the chromagen.

Results

Activities of 60 neurons in chloral hydrate-anesthetized (deep anesthesia; DA) and 27 neurons in pentobarbital-anesthetized (light anesthesia; LA) animals were recorded. The majority of the neurons (45/60 DA and 17/27 LA) responded to noxious stimulation with excitation. These cells had baseline firing rates that ranged between 4 and 40 impulses/second. Noxious stimulation increased the firing rate 2- to 4-fold. Inhibitory response to noxious stimulation was observed in 15% (9/60) in DA and in 11% (3/27) in LA animals. The mean baseline firing rate of PAG in DA animals was 9.36 ± 8.71 STD and in LA animals, 10.76 ± 8.26 STD. The difference between baseline activity and the response to noxious stimulation of cells in these 2 types of preparation was not statistically significant. Since PAG neurons in these 2 types of preparations showed similar responses to LH

Table 1. Response to electrical stimulation of the LH

Response to glutamic acid injected into the LH	Excitation	Inhibition	No response	Total
Excitation	18 (8, 10)	2 (0, 2)	2 (0, 2)	22
Inhibition	2 (1, 1)	8 (3, 5)	0 (0, 0)	10
No response	5 (1, 4)	4 (3, 1)	6 (2, 4)	15
Total	25	14	8	47

Responses of PAG cells that were excited by electrical stimulation and injection of glutamic acid into the lateral hypothalamus. Chi-square test shows a significant interaction between the 2 types of stimulations of LH: $\chi^2 = 7.05$, $df = 4$, $0.1 < p < 0.25$. The numbers in parentheses show the number of neurons that were recorded in lightly anesthetized and in chloral hydrate-anesthetized preparations, respectively.

stimulation and to NT, the data obtained were pooled. Electrical stimulation of the LH with 200 μ sec constant cathodal current of 10–30 μ A produced an excitation with onset latency of 14 ± 1.7 msec STD. In order to test the possibility that this excitatory effect was due to activation of fibers of passage, 2 types of experiments were conducted. In the first, the response of LH neurons to electrical stimulation of PAG was measured and the tests for antidromic activation (constant-latency, high-frequency following and the collision) were made to establish direct projection from LH to PAG. These procedures were used in 8 separate experiments. In the second type of study, the response of PAG cells to injection of glutamic acid [which activates cell bodies without activation of fibers of passage (Zieglgänsberger and Puil, 1973)] into the LH was tested. This procedure was used in the study of 29 cells in DA and of 18 cells in LA animals. In experiments involving the collision test, the antidromically activated spike occurred with a latency that ranged between 13 and 14.5 msec, with 2 cells responding at 13 msec, 4 neurons at 13.75 msec, 1 cell at 14 msec, and 1 cell at 14.5 msec. Figure 1 shows a poststimulus histogram constructed by measuring the responses of a PAG neuron to electrical stimulation of LH at 1 Hz for 300 sweeps. As shown, LH stimulation produced an excitatory effect with a latency to maximum response of 14 msec. The inset in Figure 1 shows the response of a LH neuron (located at the same site that produced excitation of PAG cell) to electrical stimulation of the PAG. As demonstrated, the LH cell was antidromically activated by PAG stimulation with a latency of 13.75 msec, indicating a direct projection of this cell to the PAG.

Injection of glutamic acid (10–100 nl of 200 mM monosodium-L-glutamate) into the LH produced a response in PAG cells that was comparable to their response to electrical stimulation. As shown in Table 1, glutamic acid injection in the LH produced excitation in 18 cells that were also excited by electrical stimulation and caused inhibition of 8 cells that were inhibited by electrical stimulation. However, in 5 cells that were excited and 4 cells that were inhibited by electrical stimulation, glutamic acid injection produced no response. This observation indicates that some of the effect of LH stimulation on PAG cells is through activation of axons of passage, but the majority of the response is due to activation of the cell bodies in the LH. In 47 cells, the responses to electrical stimulation of LH (200 μ sec constant-current pulses with intensities of up to 40 μ A at frequencies of 100 and 200 Hz for a period of 1 sec) and to microinjection of glutamic acid into the LH were compared with

Figure 1. Poststimulus histogram constructed by recording the response of a PAG cell to electrical stimulation of the LH with constant-current cathodal pulses of 200 μ sec in duration and an intensity of 20 μ A applied at a frequency of 1 Hz for 300 sweeps. *Inset*, Response of a cell in the LH at the site that produced excitation of PAG to electrical stimulation of PAG. This cell was antidromically activated with a latency of 13.75 msec and could be back-fired by PAG stimulation at 80 Hz. Abolition of an antidromically activated spike by a spontaneous action potential indicates collision of the anti- and spontaneous orthodromic action potentials. These satisfy the criteria for antidromic activation and demonstrate a direct projection from LH to PAG.

their responses to pressure injection of NT. Table 2 summarizes the results obtained in these experiments. As indicated, the majority of the PAG cells (37/47) were excited by NT. Although in 12% of the neurons (6/47), NT and stimulation of LH produced opposite effects, there was a strong correlation between excitatory responses produced by NT injection and LH stimulation.

The effect of NT was dose dependent. Increasing the dose of NT produced progressively stronger and longer-lasting excitation of PAG neurons. Figure 2 shows this property of the NT effect and its similarity to the response of PAG cells to electrical stimulation of the LH. As indicated in Figure 2, NT injected at 2 psi for 30 sec produced an increase in the firing rate from mean of 7/sec to 12/sec that lasted for 60 sec. When the injection pressure was increased to 4 psi, NT injection increased the firing rate to 20/sec, and the effect lasted for 120 sec. Electrical stimulation of LH with a train of pulses at frequencies of 100 and

200 Hz for 1 sec produced an excitation that was comparable to the excitation produced by NT.

In 32 cells, the time course of excitation of PAG cells produced by electrical stimulation or injection of glutamic acid into the LH was similar to that of the response to NT injection. Figure 3 shows the responses of a PAG cell to electrical stimulation of LH, injection of glutamic acid into the LH, and pressure injection of NT next to the cell in the PAG. As shown, all 3 manipulations produced long-lasting excitation of this neuron. The time courses of the response of PAG cells to electrical (train stimulation, 200 μ sec pulses for 100 msec at 100 and 200 Hz) and glutamic acid stimulation of the LH were very similar to the response of these cells to NT. In the majority of cells, LH stimulation produced an excitatory response that lasted for several minutes. As indicated in Figure 3, there was a slight inhibition of the firing rate of the PAG cell immediately after injection of glutamic acid into and electrical stimulation of the

Figure 2. Responses of a PAG neuron to neurotensin (NT) and to electrical stimulation of the lateral hypothalamus (LH). Pressure injection of NT produced an excitatory and dose-dependent effect. NT at 2 psi produced an excitation that lasted for 40 sec, whereas NT applied at 4 psi produced an excitation that lasted for 210 sec. The same neuron was excited when LH was stimulated with 20 μ A pulses at 100 Hz.

Figure 3. Comparison of the responses of a PAG neuron to electrical stimulation of LH, to injection of glutamic acid into the LH, and the response to NT. *Top*, Shows the response of a PAG cell to 10 sec stimulation of LH with 20 μ A depolarizing pulses at 100 Hz. *Middle*, Response of the same cell to injection of 10–20 nl of 100 mM glutamic acid into the LH. *Bottom*, Response of the cell to NT applied with a pressure of 5 psi for 5 sec. The activity of the cell during the electrical stimulation, as indicated by a bar under each figure, does not truly represent the firing pattern of the cell because the first millisecond after each pulse was ignored by the program in order to delete the stimulus artifact. The response to glutamic acid was obtained 7 min after the response to electrical stimulation was measured. The response to NT was measured 10 min after the response to glutamic acid was measured.

LH. This type of response was observed in only 11 other cells. However, the degree of inhibition was not uniform and was not statistically significant when all neurons were considered.

In 14 LA animals, stimulation of LH inhibited TF to cut-off latency of 10 sec. This inhibition was time dependent and was related to the frequency and strength of the stimulus. In all cases, the site that produced inhibition of TF and the sites that were most effective in alteration of firing rate of PAG neurons were nearly identical. At the lowest current (<50 μ A), TF inhibition by LH stimulation occurred only during the stimulation, and TF latency returned to baseline as soon as the LH stimulation stopped. When the current intensity was increased to 80 μ A, TF inhibition outlasted the stimulation time by at least 20 sec. Injections of glutamic acid in LH produced TF inhibition that outlasted the injection period for as long as 1.5 min. The increase in the firing rate of PAG cells, however, always exceeded the duration of LH stimulation.

Stimulation of a hypothalamic area dorsal to the LH also produced analgesia as measured by inhibition of TF. However, the current intensity required to produce this effect was variable. As shown in Figure 4B, a current intensity of 160 μ A was re-

Table 2. Response to electrical stimulation of or glutamic acid injected into the LH

Response to neurotensin applied to PAG	Excitation	Inhibition	No response	Total
Excitation	32 (6, 26)	4 (1, 3)	1 (1, 0)	37
Inhibition	2 (0, 2)	2 (0, 2)	0 (0, 0)	4
No response	4 (1, 3)	1 (0, 1)	1 (1, 0)	6
Total	38	7	2	47

Responses of PAG neurons to electrical or glutamic acid stimulation of the lateral hypothalamus and to micropressure injection of NT into the PAG. Numbers in the parentheses show the number of neurons that were recorded in the lightly anesthetized and chloral hydrate-anesthetized preparations respectively. Chi-square tests show significant interaction between the response to LH stimulation and to NT: $\chi^2 = 25.42$, $df = 4$, $0.0005 < p < 0.001$.

quired to produce inhibition of TF when a region 1 mm dorsal to LH was stimulated, whereas a current intensity of 40 μ A was needed to block TF when LH was stimulated. The location of the PAG cells that were responsive to LH stimulation is shown in Figure 4A. These cells were located in the ventromedial and the ventrolateral PAG.

Injections of PHAL at all rostrocaudal levels of LH produced terminal labeling in the ipsilateral PAG. The criteria for terminal fields are the presence of axonal varicosities and varicosity-containing short branches (e.g., Fig. 5, A, B). Figure 6 shows the distribution of PHAL-labeled axons and terminals in a representative case. Labeled processes meeting the criteria for terminal fields are indicated as dotted line segments in the plots in Figure 6. These plots are at the levels corresponding to the recording sites in PAG (Fig. 4A), at the level of the decussation of the superior cerebellar peduncle (Fig. 6C), and 1 mm rostral to that point, at a rostrocaudal level that passes through the rostral oculomotor nucleus (Fig. 6B). The injection site in rostral LH is shown in Figure 6A. A high-power photomicrograph of this injection site is portrayed in Figure 4D, illustrating the complete, Golgi-like filling of the neurons that give rise to the labeled axons in PAG and other efferent targets. Another example of axon terminal labeling in PAG produced from a PHAL injection is shown in Figure 4, A and C. These 2 injection sites in the rostral LH correspond to the stimulation sites used in the physiology experiments (Fig. 4B). LH terminal fields are uniformly distributed throughout the ventral two-thirds of the ipsilateral PAG and spread into the adjacent dorsal raphe nucleus.

LH axons reach mesencephalic and brain-stem periaqueductal structures by 2 routes. After passing caudally through the medial forebrain bundle, the dorsal pathway takes a direct line dorsally through the fields of Forel to reach the regions surrounding the mesencephalic aqueduct. The ventral pathway remains in the medial forebrain bundle until it reaches the mesencephalon just dorsal and caudal to the interpeduncular nucleus. The fibers then take a dorsal and caudal course through the tegmentum to reach PAG. Fibers of the ventral pathway are particularly evident in Figure 5B as they pass through midline raphe nuclei, which they innervate, and more laterally as a separate bundle in the retrorubral field.

Using the light microscope, we estimate the diameter of the LH-PAG axons to range between 0.3 and 0.8 μ m. The lower value is close to the limit of resolution of the light microscope and so should be considered only approximate.

Figure 4. *A*, Locations within PAG of cells that responded to NT. As indicated, the majority of these neurons were situated in the lateral and the ventrolateral parts of the PAG. *B*, Regions of the LH where electrical stimulation or injection of glutamic acid produced changes in the firing rate of the PAG cells. As indicated, the threshold for tail flick inhibition was the lowest when the electrode was in the medial part of the LH, about 300 μ m dorsal to the optic tract. Symbols identify the minimum current intensity that produced analgesia. ●, 100 μ A; ◇, 60 μ A; ■, 50 μ A; ○ 40 μ A; ★, 60 μ A.

Discussion

The results of these experiments indicate that activation of the LH can produce analgesia. These results are in agreement with results obtained by Carr and Coons (1982), in which stimulation of LH inhibited escape behavior induced by electrical stimulation of nucleus gigantocellularis. Several studies (Cox and Valenstein, 1965; Rose, 1974; Carr and Uysal, 1985), including the aforementioned investigation, have suggested that LH stimulation activates neuronal networks that are involved in supraspinal modulation of pain. Our observations suggest that interactions between LH and PAG can possibly be the network involved in this action of LH.

The majority of PAG neurons recorded in this study were excited by stimulation of the lateral hypothalamus. Comparison of the responses of these cells to electrical stimulation of LH and to injection of glutamic acid in this region showed comparable response characteristics. Since glutamic acid acts on the cell bodies and does not activate axons (Zieglgänsberger and Puil, 1973), these data indicate that the effect of LH stimulation on PAG cells is mediated through activation of the cell bodies rather than axons of passage. These results are consistent with the observation of monosynaptic excitatory postsynaptic potentials in dorsal raphe neurons evoked by stimulation of LH (Park, 1985).

We have shown that anterogradely labeled LH axons form terminal fields in those portions of PAG in which excitatory LH evoked responses have been recorded. This anatomical result using the new PHAL technique confirms observations from previous tract-tracing studies that employed amino acid autoradiographic techniques (Saper et al., 1979; Hosoya and Ma-

tsushita, 1981; Berk and Finkelstein, 1982). The PHAL technique provides the additional information of axon diameter, as well as greater security in distinguishing terminal fields from axons of passage.

Of the 2 pathways taken by LH efferent fibers, the trajectory of the dorsal one is nearly linear, and so its length is well approximated by the straight line distance between stimulation and recording sites. Using the stereotaxic atlas of Paxinos and Watson (1982) as a standard for determining the Cartesian coordinates of stimulation and recording sites, the straight line distances are 7.1 and 6.4 mm for the 2 sets of stimulation sites shown in Figure 4*B*. The ventral pathway passes just rostral to the interpeduncular nucleus and has a length of 8.0 mm in the Paxinos and Watson rat. The mean latency of the excitatory response recorded in PAG and the antidromic responses evoked in LH by stimulation of PAG is 14 msec, which yields conduction velocities of 0.5, 0.5, and 0.6 m/sec for the 3 distances given. Conduction velocities of this magnitude are consistent with unmyelinated or myelinated fibers less than 1 μ m in diameter (Waxman and Bennett, 1972) and are quite consistent with the fiber sizes of 0.3–0.8 μ m seen in the PHAL-labeled material.

The responses of PAG cells to NT and to stimulation of LH were compared. NT had a predominantly excitatory effect. This is in agreement with results obtained previously in this laboratory (Behbehani and Pert, 1984) and by other investigators (Stanzione and Zieglgänsberger, 1983; Herbison et al., 1986). As indicated in Table 2, there is a strong correlation between these responses. Specifically, if the duration of the response is considered, a close resemblance exists between the time course of the response to LH stimulation and to NT. This suggests that

Figure 5. PHAL-labeled terminal fields in PAG. *A*, Dark-field photomicrograph of axons in PAG found to be filled with PHAL following an injection (shown in *D*) in rostral LH. Numerous varicosities suggestive of presynaptic terminals stud the labeled axons. The arrowheads point to 3 examples: along the course of a nonbranching axon (left arrowhead) and at the branching and end of a short terminal branch (right arrowheads). Additionally, each small bright point in the field stretching from the center to upper left of the photomicrograph is an axonal varicosity. Scale bar, 30 μ m. *B*, Bright-field photomicrograph of an axonal terminal field in PAG. The arrowhead indicates a varicosity at the end of a short axonal twig. This is the same case as shown in Figure 6. Scale bar, 10 μ m. *C*, Injection site in LH. Terminals in PAG from this injection are shown in *A*. The arrowhead points to the PHAL injection site in rostral LH. Abbreviations: CPu, caudate putamen; GP, globus pallidus; 3V, third ventricle; f, fornix. Scale bar, 1.0 mm. *D*, Magnified view of a typical PHAL injection site in LH. Individual neurons that have Golgi-like filling are evident. This is the same case that is shown in *B* and in Figure 6. Scale bar, 50 μ m.

a neurotensinergic pathway may mediate the interaction between these 2 sites. However, since a neurotensin antagonist is not currently available, this hypothesis could not be tested directly.

In the lightly anesthetized preparation, stimulation of the LH produced TF inhibition that outlasted the stimulus by as long as 2 min. This result is similar to that reported by Carstens et al. (1983) and by Cox and Valenstein (1965). The electrically induced increase in TF latency we observed is also in agreement with more recent observations by Aimone and Gebhart (1987). However, in their studies, injection of glutamic acid did not cause a change in TF latency, whereas in this study glutamic acid injection in the LH produced as strong an effect as electrical stimulation on TF latency. The reason for this discrepancy is not clear. One possible reason is that the site of injection in this study was more lateral than in those reported by Aimone and Gebhart.

It has been suggested that the analgesic effect of LH stimulation can be due to activation of fibers of passage that travel

through the medial forebrain bundle and terminate in PAG, NRM, and the dorsolateral pons. Our anatomical and glutamic acid injection experiments suggest that there is a direct projection from cells in the LH to the PAG, and it is therefore likely that activation of this pathway also contributes to the analgesia produced by stimulation of the LH. However, lesion studies (either by injection of local anesthetic or electrolytic lesioning) will be required to establish this point. The existence of such a strong interaction between LH and PAG supports the hypothesis that, under natural conditions, PAG activation may be due to activation of limbic system-LH pathway (Sandrew and Poletti, 1984).

In the LA preparation, few neurons decreased their firing rate just prior to the TF. These types of neurons have been observed by Heinricher et al. (1987) and have been characterized as Off-cells. In our experiments, these cells were strongly excited by LH stimulation as well as by NT.

Comparisons of the baseline firing rates and responses of PAG neurons showed no significant difference between LA and DA

Figure 6. Plot of axonal labeling following an injection of PHAL in LH. *A*, The portion of the injection site that contained intracellularly labeled neuronal somata is indicated by the dots and a central region (black) of dense labeling. Neurophil staining extended throughout the outlined area having diagonal striations. The short line segments indicate PHAL-labeled fibers leaving the injection site. *B* and *C*, Coronal sections through PAG that correspond to the level in which single-unit recordings were made. Terminal fields are evident in PAG ipsilateral to the injection site. Abbreviations: 3, oculomotor nucleus; 3V, third ventricle; Aq, mesencephalic aqueduct; Arc, arcuate nucleus; CM, center median nucleus; cp, cerebral peduncle; CPu, caudate-putamen; f, fornix; Hb, habenula; ic, internal capsule; IP, interpeduncular nucleus; LH, lateral hypothalamic area; Me5, mesencephalic trigeminal nucleus; ml, medial lemniscus; mlf, medial longitudinal fasciculus; SN, substantia nigra; opt, optic tract; PAG, periaqueductal gray; Rt, reticular thalamic nucleus; VL, ventral lateral nucleus; VPL, ventral posterior lateral nucleus; xscp, decussation of the superior cerebellar peduncle.

animals. This observation implies that chloral hydrate anesthesia does not profoundly affect the response characteristics of PAG cells and can be a useful anesthetic agent in studies of descending pain inhibitory pathways.

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