THE PEPTIDERGIC ORGANIZATION OF THE CAT PERIAQUEDUCTAL GRAY

I. The Distribution of Immunoreactive Enkephalin-containing Neurons and Terminals¹

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Abstract

Despite the significant contribution of the periaqueductal gray (PAG) to an endogenous pain suppression system, little is known about its neurochemical organization. Previous pharmacological and physiological studies have indicated regional variations in the effectiveness with which the midbrain PAG can generate potent analgesia in response to either opiate microinjection or electrical stimulation. There is, however, no anatomical correlate of this regional variation. As a first step toward elucidating the neural circuitry underlying the PAG's contribution to endogenous pain suppression systems, we have mapped the distribution of leucine enkephalin (ENK)-like immunoreactivity in the cat PAG. Throughout the rostral-caudal extent of the PAG, ENK-containing neurons are clustered in discrete populations. ENK terminal field staining is somewhat more diffuse; however, there are several regions where terminal staining is consistently more intense. The distribution of ENK perikarya and terminals undergoes a ventral to dorsal shift from caudal to rostral PAG. Conceivably, the clustered distribution of ENK cells and terminals contributes to the differential effectiveness of various PAG regions in generating analgesia. The ventral-dorsal shift of ENK immunoreactivity may (1) correspond to a somatotopic organization within the PAG or (2) mirror the topographic relationship of the PAG's interactions with other components of the endogenous analgesia system. In addition, the changing pattern of ENK immunoreactivity may also reflect the involvement of the PAG and of endogenous opiates in systems other than those of pain control.

Tsou and Jang (1962) implicated the central gray region surrounding the third ventricle and midbrain aqueduct in the generation of morphine analgesia. Subsequently, Reynolds (1969) reported that electrical stimulation of the midbrain periaqueductal gray (PAG) produced analgesia profound enough to perform painless abdominal surgery on rats. Further studies elucidated the properties of the analgesia elicited from the PAG by

either intracerebral opiate microinjection or electrical brain stimulation (Mayer et al., 1971; Mayer and Liebeskind, 1974; Oliveras et al., 1974, 1979; Mayer and Price, 1976; Lewis and Gebhart, 1977; Yeung et al., 1977; Gebhart and Toleikas, 1978; Bennett and Mayer, 1979).

The discovery of endogenous opiates (Hughes et al., 1975; Simantov and Snyder, 1976), the presence of considerable opiate binding sites in the PAG (Kuhar et al., 1973), and the reversal of stimulation-produced analgesia (SPA) by naloxone (Akil et al., 1976; Adams, 1976; Hosobuchi et al., 1977) further implicated the midbrain central gray as an important component of an endogenous pain suppression system. In part, this system involves connections with the medullary nucleus raphe magnus (Abols and Basbaum, 1981; Gallagher and Pert, 1978; Ruda, 1976), the axons of which descend to the spinal cord via the dorsolateral funiculus and inhibit dorsal horn nociceptors (Basbaum et al., 1976, 1977, 1978; Basbaum and Fields, 1979; Fields and Anderson, 1978).

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Although many details of this descending pain control system have been determined, the mechanism by which opiates or electrical brain stimulation initiate or modulate pain suppression *at the level of the PAG* is unknown. In effect, the PAG remains a "black box." It is not known, for example, whether intracerebrally injected opiates or naloxone act on afferents to the PAG, on PAG projection neurons, or on PAG local circuit neurons. Because the site of morphine's action may be inferred from the distribution of endogenous opiates, we examined the distribution of leucine enkephalin (ENK)-like immunoreactivity in the cat periaqueductal gray. We report the presence of discrete populations of ENK-containing neurons and terminal fields throughout the rostral-caudal extent of the cat periaqueductal gray. Preliminary results of this work have been reported previously (Moss et al., 1980, 1981a).

Materials and Methods

Ten adult cats, some pretreated 24 to 48 hr before sacrifice with a third ventricle injection of colchicine (Sigma Chemical Co., 5 μ l, 20 μ g/ μ l), were used in this study. Cats were anesthetized and perfused with 0.1 M phosphate-buffered saline (pH 7.4, 37°C) containing 0.1% heparin. This was followed immediately by a 4°C fixative solution consisting of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer containing 4% sucrose. After approximately 30 min, the animal was perfused with fresh cold buffer to wash out excess fixative. The midbrain was then serially sectioned at 100 μ m using a Vibratome or at 50 μ m using a freezing microtome, or embedded in paraffin and sectioned at 30 to 50 μ m.

ENK was localized on representative sections taken at 150- to 400- μ m intervals, using the peroxidase-antiperoxidase (PAP) method (Sternberger, 1974). In Vibratome and frozen sections antibody penetration was enhanced either by preincubation in a sodium ferricyanide methanol solution (Straus, 1976) or by the addition of 0.3% Triton X-100 to all solutions. Sections were incubated in ENK antisera for 48 hr at 4°C. Bridging antisera and PAP incubations were at least 30 min in duration at room temperature. The PAP reaction product was visualized by using 3,3'-diaminobenzidine as the chromagen.

The ENK antibody (Immunonuclear Corp.) used in this study has been shown by radioimmunoassay to have a 20 to 30% cross-reactivity with methionine enkephalin and no demonstrable cross-reactivity with β -endorphin. However, its cross-reactivity with other peptides containing a leucine enkephalin sequence (e.g., dynorphin, α -neoendorphin) has not been determined. For this reason, although the term ENK-like immunoreactivity is not consistently used throughout this report, it is implied at all times. To establish histochemical specificity, adjacent control sections were incubated in ENK antisera preabsorbed with excess ENK (Sigma) (100 μ g of ENK/ml of diluted antisera). ENK staining in these control sections was abolished. In addition, several sections were treated with ENK antisera preincubated with 1 mg of either substance P or serotonin (Sigma)/ml of diluted antisera. These pretreatments had no effect on the ENK staining.

The distribution of the ENK-labeled perikarya and

terminal fields was plotted on projection drawings of appropriate sections from several animals. The results from these projection drawings were collated and plotted on representative sections of the PAG. In this manner, variability among animals introduced by slight differences in the plane of sectioning and/or colchicine administration were minimized. The visualization of ENK perikarya was highly dependent on the presence of colchicine. Despite consistent third ventricle injections, the penetration of colchicine into the surrounding neuropil was not consistent, perhaps being influenced by cerebrospinal fluid flow and the natural boundaries formed by axonal tracts. Therefore, the comparison of labeling from several animals was essential.

The schematic drawings and nuclear designations used in this study are derived from the atlases of Berman (1968), Mehler and Nauta (1958), and Taber (1961).

Results

To facilitate the description of the distribution of ENK-like immunoreactivity, the PAG will be divided into three major rostral-caudal regions. Caudal PAG (Fig. 1) includes levels from the dorsal tegmental nucleus of Gudden to a level immediately caudal to the IV nerve nucleus. Mid-PAG (Fig. 2) extends from the level of the IV nerve nucleus to the level of the Edinger-Westphal nucleus. Finally, rostral PAG (Fig. 3) includes levels from the posterior commissure to the periventricular gray. In addition, the distribution of ENK immunoreactivity in the midbrain raphe nuclei and the dorsolateral pontine tegmentum is discussed.

Midbrain raphe nuclei (Figs. 1, 2, 4, and 7). As described previously (Moss et al., 1981b), ENK-containing neurons are found throughout the rostral-caudal extent of the raphe dorsalis (RD); their distribution parallels the cytoarchitectural boundaries of the nucleus (Taber, 1961). The densest terminal field staining is found at the peripheral borders of this nucleus. The raphe nucleus centralis superior contains both ENK neurons and dense terminal staining along its lateral borders.

Locus ceruleus (LC)/parabrachial (Pb) nuclei (Figs. 4, 5, and 6). Large ENK neurons are found in the LC dorsalis, LC pars alpha, and the subceruleus region (Leger and Hernandez-Nicaise, 1980). These cells are easily distinguished from the smaller ENK cells found in the more rostral but contiguous ventrolateral PAG. Many ENK cells are also present in the lateral Pb nucleus and in the Kolliker Fuse nucleus. In addition, several ENK cells are also found in the medial Pb nucleus. The ENK neurons in the Kolliker Fuse nucleus are comparable in size to those in the LC. In contrast, ENK cells of the Pb nuclei are smaller, similar in size to those seen in the PAG. Terminal field labeling in the dorsolateral pontine tegmentum is heaviest at the dorsal tip of the mesencephalic tract. Dense terminal labeling is also found in Pb lateralis. Despite the presence of many immunoreactive ENK perikarya in the LC and subceruleus, there is only a moderate amount of terminal field staining in these nuclei.

Caudal PAG (Figs. 1 and 7). In the caudal PAG, ENK-containing neurons are clustered predominantly in the

Figure 1. Caudal periaqueductal gray levels.

Figures 1 to 3. Drawings of representative sections through the periaqueductal gray illustrating the distribution of enkephalin-containing perikarya (dots) and terminals (cross-hatching) ranging in density from 0 to 3). CS, centralis superior; Cun, nucleus cuneiformis; DTN, dorsal tegmental nucleus of Gudden; EW, Edinger-Westphal nucleus; Hb, habenulae; LGB, lateral geniculate; LL, linearis intermedius; LR, linearis rostralis; MesV, mesencephalic tract of V; MLF, medial longitudinal fasciculus; Post C, posterior commissure; Pub, pulvinar; RD, raphe dorsalis; RN, red nucleus; VTN, ventral tegmental nucleus of Gudden; III, third nerve nucleus; IV, fourth nerve nucleus. MesV, MLF, and Hb-interpeduncular tract are indicated by diagonal hatching.

Figure 2. Mid-periaqueductal gray levels.

Figure 3. Rostral periaqueductal gray levels.

Figure 4. The distribution of enkephalin (ENK) immunoreactivity in the midbrain, just caudal to the periaqueductal gray (PAG). Compare the distribution of ENK neurons in the nucleus raphe dorsalis (RD) at this level with a more rostral level shown in Figure 7. ENK perikarya and terminals surround the dorsal tegmental nucleus (DTN). The nucleus itself is relatively free of ENK immunoreactivity. This is the same staining pattern seen at caudal PAG levels. Note the different ENK cell types in the nucleus locus ceruleus (LC). Mes V, mesencephalic tract of V; MLF, medial longitudinal fasciculus; IV, fourth ventricle. Magnification $\times 47$.

Figure 5. Enkephalin neurons in the lateral parabrachial (*Pbl*) and Kolliker Fuse (*KF*) nuclei at the same level pictured in Figure 4. Note the difference in cell size between these two enkephalin neuron populations. *BC*, brachium conjunctivum. Magnification $\times 17$.

ventrolateral PAG. ENK cells are also found arching around the perimeter of the dorsal tegmental nucleus of Gudden (DTN). The DTN itself is devoid of ENK immunoreactivity.

Although ENK terminal field staining is found throughout the caudal PAG, the staining pattern is marked by areas of denser labeling. The densest terminal staining is found in the ventrolateral PAG and in the dorsal PAG. In the dorsal PAG, the distribution of ENK-labeled terminals forms paired, vertically oriented columns.

Dense ENK terminal field staining is also present in the nucleus cuneiformis. In one animal, a small number of ENK cells were seen in the nucleus cuneiformis. This staining pattern may be an example of the incomplete penetration of colchicine into this region.

Mid-PAG (Figs. 2, 8, and 9). In contrast to the caudal PAG, the ventral region of the mid-PAG contains few ENK perikarya. Instead, the distribution of ENK neurons shifts dorsally and appears as two separate populations: one is located in the lateral PAG, the other in the dorsal PAG.

At the levels of the III and IV nerve nuclei, the lateral cluster of ENK cells is located adjacent to the aqueduct. More rostrally, at the level of the Edinger-Westphal nucleus, this population shifts outward, away from the

Figure 6. Locus ceruleus. *A*, Enkephalin neurons in the dorsal portion of the locus ceruleus. *B*, Absorption control of a section 360 μm from *A*. *Mes V*, mesencephalic tract of V. Magnification $\times 55$.

Figure 7. The distribution of immunoreactive enkephalin (ENK) in the caudal periaqueductal gray (PAG). *A* ENK neurons and terminals in the caudal ventrolateral PAG. Magnification $\times 55$. *B* ENK-containing perikarya along the midline of the nucleus raphe dorsalis. At the level of the IV nerve nucleus the "wings" of the raphe dorsalis also contain ENK cells. Magnification $\times 55$. *Aq*, aqueduct of Sylvius; *Mes V*, mesencephalic tract of V.

aqueduct. The ENK cell population in the dorsal PAG becomes prominent at the level of the III nerve nucleus where it is located adjacent to the dorsal midline. At more rostral levels this dorsal ENK neuronal cluster shifts laterally, away from the midline.

At mid-PAG levels the densest terminal field staining is located in the dorsal and the lateral PAG, paralleling the dorsal shift of the ENK cell distribution. At the level of the Edinger-Westphal nucleus, large beaded immunoreactive ENK fibers are seen coursing ventrally from the PAG, along the midline.

Rostral PAG (Figs. 3 and 10). In the rostral PAG, at the level of the posterior commissure, the ENK neuron populations remain as two separate clusters. The dorsal cluster now extends from the dorsal PAG, through the fibers of the posterior commissure and into the overlaying cap of the PAG neuropil. At this level, ENK terminal field staining is densest in the dorsal PAG and also extends into the portion of the PAG that caps the posterior commissure.

The rostrally contiguous periventricular gray contains scattered ENK neurons and light terminal field labeling; the terminal labeling is somewhat denser dorsally.

ENK cell morphology. In favorable preparations, considerable information about ENK cell morphology can be collected. Both the shape of the somata and the primary dendrite branching patterns of the cells are evident. Viewed in coronal section, the ENK cells in the

PAG are generally smaller than ENK neurons found in the surrounding brain regions, specifically, the nucleus locus ceruleus. Two cell types predominate in the locus ceruleus (Fig. 4). The larger LC ENK cells ($40\ \mu\text{m}$) (measured on Vibratome sections) are generally round to fusiform, bipolar cells; the majority of smaller LC ENK cells ($30\ \mu\text{m}$) are multipolar. In contrast, the PAG ENK cell bodies range in diameter from 15 to $30\ \mu\text{m}$. The ENK neurons in the PAG are bipolar or multipolar with round, fusiform, or triangular cell bodies. We were unable to recognize either a consistent cell morphology or an orientation with which to define all ENK-containing cells in the PAG. There are, however, some regional differences in ENK cell morphology. At the levels of the III and IV nerve nuclei, the ENK-containing perikarya in the lateral ENK cell population (Fig. 9) are generally bipolar fusiform cells, while the ENK cells located dorsally (Fig. 8, *A*, and *B*) have round or triangular cell bodies which are generally multipolar. ENK cells in the dorsal cell population at the level of the posterior commissure are more variable both in shape and branching pattern (Fig. 10, *C* and *D*).

Discussion

Although several immunohistochemical studies of ENK-like immunoreactivity in the central nervous system have included observations of labeling in the PAG,

Figure 8. The distribution of immunoreactive enkephalin (ENK) in the dorsal periaqueductal gray (PAG) at the level of the IV nerve nucleus. *A* ENK neurons form a dorsal midline column. Magnification $\times 28$. *B* High power micrograph of a pyramidal ENK cell from this same region. Magnification $\times 116$. *C* ENK terminals in the PAG form two sets of paired columns on either side of the dorsal midline. Magnification $\times 23$. *Aq*, aqueduct of Sylvius.

this region has never been the prime focus of any one study. Our report is the first detailed description of the distribution of ENK immunoreactivity in the PAG of the cat, an animal used extensively in anatomical and electrophysiological studies of endogenous pain suppression systems. Throughout the entire cat PAG, ENK-containing neurons are found clustered in discrete populations. Although ENK terminal field staining is more diffuse, there are also several regions where terminal staining is consistently more intense.

In both the rat and cat, intraventricular colchicine administration is necessary for the visualization of immunoreactive ENK cell bodies in the PAG. Early immunohistochemical studies in the rat indicated that ENK perikarya are present only in the caudal, ventrolateral PAG (Hokfelt et al., 1977a, b). Subsequent studies, however, have revealed a wider distribution (Uhl et al., 1979). There are two major differences between the distributions of ENK-containing neurons in the PAG of the rat and the cat. Although a population of ENK perikarya is

present in the lateral PAG of the rat, it is found only toward the perimeter of the PAG, away from the aqueduct at all rostral-caudal levels. This is unlike the similar cell group in the cat, which is located adjacent to the aqueduct at more caudal levels. A second difference in the pattern of ENK neuronal staining between the rat and the cat is in the dorsal PAG. The distinctive columnar pattern formed by ENK perikarya in the dorsal PAG of the cat is not seen in the rat.

Differences are also apparent in the terminal field staining patterns of the two species. ENK terminal field staining in the rat PAG is more uniform than in the cat (Elde et al., 1976; Sar et al., 1978; Uhl et al., 1979). The only concentration of terminal staining in the rat is found in the lateral PAG (Uhl et al., 1979); however, its distribution is different from that of the similar population of ENK terminals in the lateral PAG of the cat. In addition, the dense terminal field staining found in the caudal ventrolateral PAG and in the dorsal PAG of the cat is not present.

The dorsal tegmental nucleus of Gudden and the parabrachial nuclei are two regions where the distribution of ENK-like immunoreactivity in the rat and cat is similar (Sar et al., 1978; Uhl et al., 1979). In fact, dense concentrations of ENK-containing perikarya and terminals surround the borders of this nucleus not only in the cat and rat but also in fetal human tissue (Miller and Pickel, 1980).

In summary, the distribution of ENK-like immunoreactivity in the rat PAG is more uniform than that in the cat, with fewer populations of ENK neurons and terminals. In contrast to the cat, there is much less perikaryal and terminal field labeling present in the dorsal PAG of the rat. It is possible that some of the differences in the distribution of immunoreactive ENK cell bodies may be due to the variable penetration of colchicine along the entire extent of PAG neuropil. However, this cannot be true for the differences in the distribution of ENK terminal field staining and indicates species differences in the PAG pattern of ENK staining. The functional implication of this remains to be determined

but may indicate species-specific differences in the opiate-mediated circuitry within the PAG.

Cytoarchitectural studies, based on Nissl and Golgi staining (Hamilton, 1973; Liu and Hamilton, 1980), subdivided the cat PAG into three nuclear regions: nucleus medialis, which surrounds the aqueduct; nucleus lateralis, which is concentric with nucleus medialis; and nucleus dorsalis, a wedge-shaped region in the dorsal PAG. Although ENK-immunoreactive perikarya in the PAG exhibit differences in shape and in dendrite branching patterns, it was not possible to draw analogies between the previous cytoarchitectural studies and either the distribution or morphology of ENK-labeled neurons. The presence of discrete clusters of ENK neurons and terminals is, however, consistent with the conclusions of Hamilton (1973) and Liu and Hamilton (1980), namely, that the PAG is an anatomically (and one presumes functionally) heterogeneous structure.

The clustered distribution of ENK perikarya and terminals may have important functional consequences. For example, it may underlie the differential effectiveness of various PAG regions in generating analgesia in response to electrical stimulation (Liebeskind et al., 1973; Oliveras et al., 1974, 1979; Yeung et al., 1977; Lewis and Gebhart, 1977; Gebhart and Toleikas, 1978). It is also consistent with the nonuniform opiate sensitivity of the PAG. For example in the rat, where extensive mapping studies have been done (Yeung et al., 1977; Yaksh et al., 1976; Lewis and Gebhart, 1977), the caudal ventrolateral PAG is the region most sensitive to both microinjected morphine and electrical stimulation. The situation is not as well delineated in the cat, because adverse behavioral reactions are often elicited from stimulation of more rostral PAG sites (Liebeskind et al., 1973; Oliveras et al., 1974). Nevertheless, both caudal ventromedial sites (i.e., in or near the RD) (Oliveras et al., 1979) and caudal ventrolateral sites (Gebhart and Toleikas, 1978) have been reported to be the most effective regions for SPA in the cat. Both of these regions contain dense concentrations of ENK-containing neurons and terminal fields.

Although the analgesia produced by intracerebral in-

Figure 9. Enkephalin neurons in the lateral periaqueductal gray at the level of the nerve III nucleus. Magnification $\times 72$. Aq, aqueduct of Sylvius.

Figure 10. The distribution of enkephalin-containing neurons in the dorsal periaqueductal gray at the level of the posterior commissure (Post C). Neurons ventral (A) and dorsal (B) to the commissure. Magnification $\times 81$ (A) and $\times 55$ (B). C and D are a higher magnification ($\times 139$) of the cell groups marked with an asterisk in A and B, respectively. Note the variety of neuronal shapes. Aq, aqueduct of Sylvius.

jection of opiates is readily antagonized by intracerebral naloxone (Lewis and Gebhart, 1977; Yaksh et al., 1976; Yeung et al., 1977), there are conflicting reports on naloxone's ability to reverse SPA from PAG stimulation (Adams, 1976; Akil et al., 1976; Hosobuchi et al., 1977; Yaksh et al., 1978; Gebhart and Toleikas, 1978). Conceivably, this reflects differences in the location of the stimulation electrode relative to the clusters of ENK-containing neurons or terminals. Electrical stimulation in a region of ENK-containing cells is more likely to generate a naloxone-sensitive analgesia. In contrast, electrical stimulation distant to the ENK perikarya but in ENK-containing terminal regions may, by activating a postsynaptic neuron, bypass the enkephalinergic link in the analgesic pathway. It is also possible that electrical stimulation of the PAG could directly activate a parallel, nonopioid-containing pain suppression system. This would also produce an analgesia that is not naloxone sensitive.

Since SPA can result from the activation of either ENK neurons or any of the elements in their postsynaptic chain, it is clear that electrical stimulation is less selective than opiate microinjection in elucidating actual endogenous opiate pathways. On the other hand, sites of dense terminal staining would be most sensitive to pharmacological manipulation and presumably would offer a better clue to the locus of opiate action. Unfortunately, a study of this kind has not yet been done in the cat.

What is the significance of the changing pattern of clustered immunoreactive ENK cells and terminals as one proceeds through the rostral-caudal extent of the midbrain central gray? Recordings of somatosensory evoked responses in the rat indicate that there is a crude rostral-caudal somatotopic organization (Liebeskind and Mayer, 1971) to the PAG. Pharmacological studies provide further evidence for somatotopia. It has been reported that opiate microinjection into the rostral PAG suppresses pain only in the rostral half of the body; whole body analgesia is generated when morphine is injected into the caudal PAG (Yaksh et al., 1976). Microinjection of an opiate peptide analogue into the PAG produces an analgesia more potent for facial pain than for tail flick (Rosenfeld and Keresztes-Nagy, 1980). Finally, there are reports that the analgesia produced by stimulation of the PAG is restricted to only half of the body (Mayer and Liebeskind, 1974). Thus one possible functional correlate of the rostral-caudal shift in the distribution of ENK neurons and terminals in the PAG is that it corresponds to an underlying somatotopic organization; that is, a "body map" for antinociception.

A second possible role for the rostral-caudal change in the distribution of ENK-like immunoreactivity in the PAG is that it mirrors the topographic relationship of the PAG's interaction with other components of the endogenous analgesia system. For example, the ENK cells and terminals in the dorsal region of the mid-PAG overlap with the distribution of PAG cells which project to the nucleus raphe magnus (NRM) in the medulla (Abols and Basbaum, 1981). The NRM is recognized as an important link in the analgesia generated by PAG activation (Basbaum et al., 1976; Fields and Anderson,

1978). Although there are similarities in the cell shape and dendrite branching pattern between the ENK cells in the dorsal PAG and the dorsal PAG cells which project to the NRM, it is not known whether ENK neurons in the PAG project directly to the NRM. Whereas enkephalin and other opiate agonists generally have inhibitory postsynaptic effects (Nicoll et al., 1980; Pepper and Henderson, 1980), an excitatory connection between the PAG and NRM appears to mediate the production of analgesia (Bebehani and Fields, 1979). It follows, then, that activation of the NRM by the PAG ENK neurons would involve disinhibitory circuits. Alternatively, ENK cells and terminals in the dorsal PAG may modulate the activity of the cells projecting to the NRM.

Unfortunately, this study cannot determine whether ENK neurons in the PAG are local circuit neurons which act directly on PAG output neurons or on afferents to the PAG involved in the antinociceptive system, or alternatively, whether some of the ENK-containing PAG cells project to other regions, such as the nucleus locus ceruleus (Sakai et al., 1977) or the nucleus cuneiformis (Mantyh, 1981) which also contain ENK terminals. Since the densest clusters of ENK cells and terminals do not always overlap, it is possible that the PAG ENK terminals derive either from local circuit neurons whose axonal arbors extend somewhat beyond their perikarya or from distant ENK neurons that project into the PAG. We suggest that most of the analgesia-relevant ENK neurons in the PAG are inhibitory local circuit neurons that modulate a tonic inhibitory input to PAG pain suppression projection neurons. Thus the analgesia from opiate injection would result from a disinhibition of the PAG-medullary connection. The origin of the hypothesized tonic inhibition of the PAG output neurons is unknown; however, there is some suggestion that norepinephrine inputs may be involved (Basbaum et al., 1983).

It must also be stressed that the PAG contains other opiate peptides besides enkephalin, such as β -endorphin and dynorphin (Bloom et al., 1978; Goldstein and Ghazavossian, 1980; Holtt et al., 1980; Zakarian and Smyth, 1982; Gramsch et al., 1982). In addition, some neurons have been shown to contain more than one opiate receptor subtype (Egan and North, 1981). Thus opiate injection into the PAG could produce analgesia via the neuronal circuitry through which these other peptides, or some combination of them, act.

We have emphasized the possible functional subdivisions of the PAG as they relate to the topography of the descending endogenous analgesia system. It is conceivable that the PAG contributes to ascending pain control pathways. Reciprocal connections between the PAG and limbic and prefrontal cortical areas (Beitz, 1982; Hardy and Leichnetz, 1981; Mantyh, 1981; Ruda, 1976) indicate a possible involvement in the affective component of pain perception. There is some evidence (Rosenfeld and Keresztes-Nagy, 1980), however, that opiates at the level of the PAG do not activate an ascending pain suppression system.

Finally, many functions other than pain suppression have been attributed to the PAG (Skultety, 1963; Jurgens and Pratt, 1979; Sakuma and Pfaff, 1980; Johnson et al.,

1982; Lakosky and Gebhart, 1982). The rostral-caudal differences in the distribution of ENK immunoreactivity may thus reflect the involvement of the PAG and of endogenous opiates in systems other than those of endogenous pain control. To determine the systems involved, it is essential to establish the identity of the neurons upon which PAG ENK neurons act. It is also important to dissect the microcircuitry through which the PAG ENK terminals exert their effects. For example, are pre- or postsynaptic inhibitory mechanisms involved? Toward this end, we have initiated electron microscopic studies of immunoreactive ENK varicosities in the PAG (Moss et al., 1981a). Hopefully, these studies will provide insight into the mechanisms by which exogenous opiates, by mimicking the action of PAG endogenous opiates, elicit such powerful behavioral effects.

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