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Immunoreactive Dynorphin B in Sacral Primary Afferent Fibers of the Cat

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Immunocytochemical analysis of the distribution of dynorphin B terminals in the sacral spinal cord of the cat revealed a pattern of staining very similar to that produced with antisera directed against the primary afferent derived, putative neurotransmitter, vasoactive intestinal polypeptide. Labeled axons and terminals were concentrated in lamina I and V and there was dense fiber staining in the tract of Lissauer. Of particular interest was the presence of immunoreactive axons in attached dorsal rootlets.

To specifically focus on the possibility that some of the sacral primary afferent fibers are dynorphin-immunoreactive, we first tried to increase perikaryal labeling in the sacral dorsal root ganglia by topical treatment with colchicine. This did not produce immunoreactive labeling of cell bodies in the ganglia. Unilateral multiple dorsal rhizotomy (L5 to coccygeal 1), however, significantly decreased the staining of dynorphin-immunoreactive axons and terminals in the tract of Lissauer and in the dorsal horn of sacral segments ipsilateral to the deafferentation. No changes were detected in the lumbar cord. Finally, radioimmunoassay of caudal lumbar and sacral dorsal root ganglia was performed. Measurable immunoreactivity was found in all ganglia assayed, but, consistent with the histochemical analysis, sacral ganglia contained the highest concentration of immunoreactive dynorphin B. These data indicate that a significant component of the sacral spinal cord dynorphin terminal immunoreactivity derives from primary afferent fibers. Since the pattern of terminal staining is also comparable to the spinal cord terminal arborization of pelvic visceral afferents revealed by transganglionic transport of horseradish peroxidase, these data further suggest that a component of the dynorphin staining in the sacral spinal cord of the cat derives from pelvic visceral afferents.

Immunohistochemical studies of the distribution of enkephalin and dynorphin cells and terminals in the spinal cord have identified important regional differences. Of particular interest is the restricted concentration of dynorphin cells in areas associated with the processing of nociceptive information, specifically lamina I and V (Cruz and Basbaum, 1985; Khachaturian et al., 1982; Vincent et al., 1982). These observations have raised the possibility that the analgesic action of spinal narcotic administration (Yaksh and Rudy, 1976) not only reflects the synaptic organization of enkephalin neurons but of dynorphin systems as well. In fact, prolonged analgesia after intrathecal dynorphin injection has been reported (Basbaum et al., 1983; Han and Xie, 1982; Piercey et al., 1982; Przewlocki et al., 1983).

Spinal enkephalin terminals are presumed to derive largely from local spinal interneurons (Elde et al., 1976; Glazer and Basbaum, 1981). With the exception of a brief description of some immunoreactive cells in the trigeminal ganglion (Morgan et al., 1983), enkephalin terminals have not been thought to derive from primary afferents. In contrast, biochemical analysis has found measurable levels of dynorphin in dorsal root ganglia of the rat (see Botticelli et al., 1981). Dorsal rhizotomy, however, did not alter the spinal levels of dynorphin and thus the significance of this observation is not clear. More recently, Sweetnam et al. (1982) found immunoreactive dynorphin neurons in some cultured dorsal root ganglion cells.

In our immunohistochemical studies of dynorphin in the sacral cord of the cat (Cruz and Basbaum, 1985), we were impressed by the similarity between the immunoreactive pattern of dynorphin staining and that produced with antisera directed against vasoactive intestinal polypeptide (VIP). Several studies have established that the VIP immunoreactivity derives, largely, from sacral primary afferent fibers (Basbaum and Glazer, 1983; de Groat et al., 1983; Honda et al., 1983). We therefore directed our attention to the possibility that some primary afferent fibers are dynorphin-containing. In this study, we provide both anatomical and biochemical evidence that this is indeed the case and further establish that the sacral spinal cord receives a particularly dense primary afferent dynorphin input. A preliminary report of this work has been published (Cruz and Basbaum, 1983).

Materials and Methods

Adult cats were used in this study. For characterization of the immunoreactive dynorphin distribution in normal animals, the cats were anesthetized with pentobarbital and perfused intracardially with the following solutions: 500 ml, 0.1 M phosphate-buffered saline, followed by 2 liter of a fixative solution containing 4% paraformaldehyde in a phosphate buffer (pH 7.4) containing 4% sucrose. After the perfusion, the spinal cord was removed and placed in the same fixative solution for 3 hr. Appropriate tissue segments were blocked and placed either in a cryoprotectant (30% sucrose in phosphate buffer for 24 hr), after which 50 μm sections were cut on a freezing microtome, or into buffer solution. Tissue from the latter solution was cut at 50 μm on a Vibratome. Our studies concentrated on the caudal lumbar (L5–7), sacral, and coccygeal segments. Both transverse and sagittal sections were examined. For the latter, care was taken to include the attached dorsal rootlets. The sections were reacted for immunoreactive peptides according to the protocol of Sternberger (1979). In the majority of studies, we used an antiserum directed against one of the products of the dynorphin precursor, dynorphin B. The specificity of this antiserum has been characterized extensively in radioimmunoassay (RIA) (Weber and Barchas, 1983). In addition to the RIA characterization, absorption controls were used. In this case, the primary antiserum was preincubated

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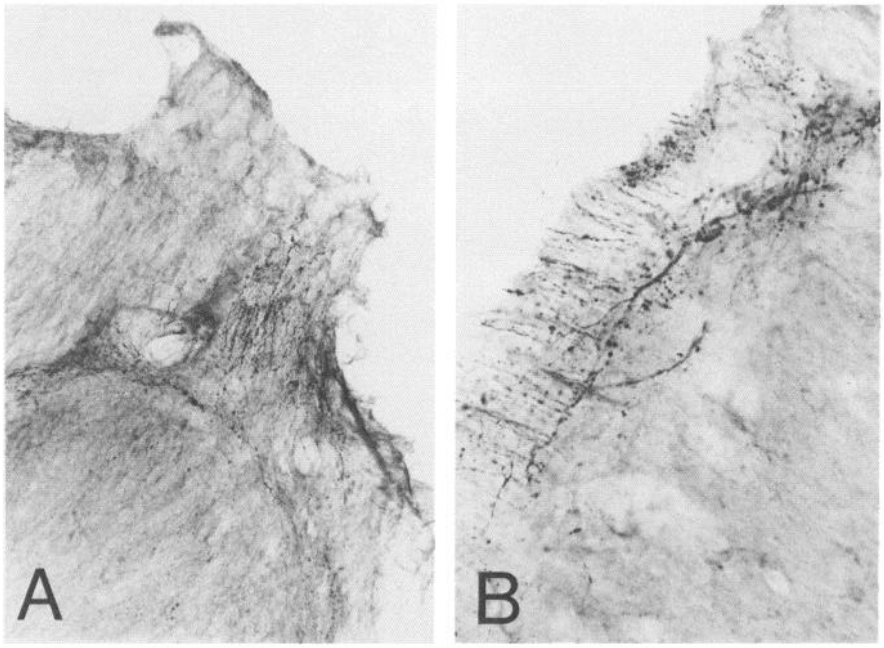

Figure 1. A, Immunoreactive dynorphin B axons can be seen extending from the cord into an attached rootlet (S2 spinal segment). The direction which the axons are coursing cannot, of course, be discerned. (See, however, Fig. 3*B*.) 260×. *B,* Immunoreactive dynorphin B fibers are concentrated in Lissauer's tract and extend to the surface of the spinal cord. This section was taken from a colchicine-treated cat. Fusiform, immunoreactive dynorphin B, marginal neuron can also be seen. 100×.

with varying concentrations of the antigen to which it was directed, or against inappropriate antigens. In all cases, staining with the dynorphin B antiserum was completely eliminated by preincubating the antiserum with 10 μM dynorphin B (Fig. 2*B*), but not with a 10-fold greater concentration of leucine enkephalin. For the deafferentation study, antisera directed against leucine enkephalin (l-enk) and Substance P (SP) were also used. The l-enk antiserum has been characterized (see Cruz and Basbaum, 1985; Glazer and Basbaum, 1981). The antiserum against Substance P was purchased from Immunonuclear Corp. We cannot comment on its biochemical characterization, however—in absorption controls, the staining with this antiserum is eliminated by incubating the primary antiserum with 10 μg synthetic SP/ml diluted antiserum.

In several cats, a variety of colchicine routes were administered to increase dorsal root ganglia levels of dynorphin immunoreactivity. In some animals, a PE-10 tubing was inserted through a small puncture in the dura overlying the cauda equina. Up to 150 μg colchicine in 10 μl saline was administered directly into the subarachnoid space. In other cases, a pledget of Gelfoam soaked in colchicine was placed over exposed sacral dorsal root ganglia. The pledget was removed after 15 min.

To examine the effect of deafferentation on the immunoreactive staining pattern in the spinal cord, two cats underwent an extensive dorsal rhizotomy. Under sterile conditions, a laminectomy over the lumbosacral cord and cauda equina was performed. The dura was incised and reflected laterally to expose the dorsal root ganglia (L5-CCI). Next, the dorsal roots of these segments were cut; in all cases, the ventral root was avoided. The overlying muscle and skin were sutured and the cats allowed to recover. Ten days after the surgical procedure, the animals were reanesthetized and perfused with the protocol described above. Transverse sections through caudal lumbar and all sacral segments were embedded in polyethylene glycol, from which adjacent 20 μm sections were stained for immunoreactive dynorphin B, l-enk, and SP.

For biochemical analysis, spinal ganglia were dissected from five animals previously perfused with saline. (These cats had previously been used in an acute electrophysiological study of tracheal parasympathetic ganglia.) The dorsal root ganglia were frozen on dry ice and extracted into acid acetone (acetone : water : 12 N HCl extraction, 40:6:1; 1 ml per ganglia). The extract was spun at 12,000 × *g* for 15 min. Aliquots of the supernatant were removed for protein determination (Lowry et al., 1951), and the rest was evaporated to dryness under a stream of nitrogen. The residue was dissolved in RIA buffer, and aliquots were assayed in an RIA for dynorphin B using the same antibody that was used for immunocytochemistry (Weber and Barchas, 1983). The RIA procedure was precisely as described (see Weber et al., 1982), with double-antibody immunoprecipitation for separating bound antibody from free antigen. All samples measured showed >10% displacement of ^{125}I-labeled tracer from the antibodies.

Results

As described previously (Cruz and Basbaum, 1985), the distribution of immunoreactive dynorphin B terminals in the spinal cord of the cat is limited both spatially and in density. At all levels of the spinal cord, dynorphin B terminals are concentrated in the marginal zone, lamina I. Fewer and more scattered fibers are found in the outer part of the substantia gelatinosa, in the region of lamina V, and around the central canal. The sacral cord is unusual in that the density of dynorphin B terminals is consistently greater. More important, the pattern of staining provided the clue that a component of the dynorphin B terminal immunoreactivity originated from primary afferent fibers.

There is a very dense cluster of labeled fibers in the tract of Lissauer (Figs. 1*B*, 2*A*); individual fibers can be traced to the surface of the cord, near the dorsal root entry zone. In samples in which a dorsal rootlet fortuitously remained attached to a transversely cut section of the sacral spinal cord, it was often possible to trace labeled dynorphin fibers from Lissauer's tract into the rootlet (Fig. 1*A*).

Two major dynorphin fiber branches appear to derive from the cluster of fibers in Lissauer's tract. The most prominent bundle courses ventrolaterally, along the lateral border of the dorsal horn (Fig. 3*B*, left). At the base of the dorsal horn, axons from this branch penetrate the spinal gray and appear to terminate in the region of laminae V–VII. Some fibers can be traced across the medial–lateral extent of the gray matter. A few terminate around the central canal, others cross just dorsal to the canal and terminate on the opposite side. In many cases, labeled fibers penetrate the ependymal layer of the central canal. The second branch of fibers that appears to arise from the region of Lissauer's tract courses medially along the marginal layer of the dorsal horn. Terminal fibers distribute throughout the marginal layer and occasionally in the outer part of the substantia gelatinosa. A few axons can be traced to the medial edge of the dorsal horn, where they turn ventrally. In the caudal sacral segments (S2 and S3), it is often possible to see collaterals of the lateral pathway coursing ventrally into the region of the sacral autonomic nucleus. Labeled fibers are very sparse in the ventral horn and are absent in the motor column of Onuf, which receives a dense enkephalin innervation (Glazer and Basbaum, 1980).

This pattern of staining is very similar to that seen with antisera directed against VIP (Basbaum and Glazer, 1983). It is also comparable to the pattern of termination of pelvic visceral afferent fibers labeled by transganglionic transport of horseradish peroxidase (Morgan et al., 1981). We, therefore, next performed experiments to more directly assess whether the dynorphin staining pattern originates in part from sacral primary afferent fibers.

Our first studies were directed at the sacral dorsal root ganglia (DRG). A variety of procedures were used to enhance dynorphin immunoreactivity in DRG cells. These included soaking the exposed DRG to colchicine, ligation of dorsal roots, or colchicine treatment of the cauda equina. Unfortunately, we never succeeded in unequivocally identifying dynorphin B immunoreactive DRG cells. There were suggestions of labeling, however; what we presumed to be background staining was generally too high to permit positive identification of individually labeled neurons.

Since we could not label DRG cells, we next examined the pattern of staining in parasagittal sections of the spinal cord. Sections cut in this plane permit a more detailed tracing of labeled axons into attached rootlets. In fact, in a single sagittal section it was often possible to examine labeled axons in several adjacent, attached rootlets and thus to compare the density of different rootlets. We found that all sacral dorsal roots contained labeled axons; however, dynorphin-immunoreactive fibers were most common in the second and third sacral dorsal roots (Fig. 3*A*). Often, labeled afferents could be traced a considerable distance within a single rootlet, into the tract of Lissauer, and then the dorsal horn. Note that Figure 3*A* contains several immunoreactive dynorphin B neurons in the marginal layer of the dorsal horn. This particular section was taken from an animal treated with colchicine in the hope of labeling immunoreactive dynorphin dorsal root ganglia cells.

As illustrated in Figure 3*B*, a more medial parasagittal section, the pattern of staining is characteristically intermittent. Patches of labeled fibers are interspersed with unlabeled regions. From analysis of adjacent sections, it was determined that the patches of staining derived from collaterals of a labeled axon bundle located dorsally, in Lissauer's tract. It was further established that, after dropping ventrally towards lamina V, the collaterals recurved dorsally and caudally. The interval between each patch varied between 36 and 216 μm: generally, the interpatch space was a multiple of 36 μm.

Having established that the pattern of labeled axons was consistent with their originating in primary afferents, we next examined the effect of dorsal rhizotomy on the immunoreactivity of the spinal cord. To this end, two cats underwent an extensive lumbosacral rhizotomy, in which dorsal roots L6 to CCI, inclusive, were cut intradurally. After a 10 d survival period, the cats were perfused with our standard 4% paraformaldehyde fixative. Lumbar and sacral cord segments were embedded in polyethylene glycol (PEG) and prepared for immunocytochemistry. In addition to examining for immunoreactive dynorphin B, we used antisera directed against SP and leucine-enkephalin. Spinal levels of SP have been shown to originate, in part, from dorsal root ganglia (Hokfelt et al., 1975), and thus the dorsal horn SP staining should decrease ipsilateral to a rhizotomy. In contrast, enkephalin immunoreactivity is presumed to originate entirely from within the CNS and thus should not change after rhizotomy.

The results from these experiments bore out our hypothesis. Figure 4 illustrates the changes produced by unilateral lumbosacral rhizotomy. As expected, there was a significant, but far from complete, decrease in immunoreactive Substance P terminal staining ipsilateral to the lesion (Fig. 4*A*). This was found over all sacral and lumbar segments examined (L6–S3). Enkephalin immunoreactivity was not noticeably changed on the deafferented side (Fig. 4*C*); if anything, there was an increase in enkephalin staining on this side. In contrast, dynorphin immunoreactivity was significantly reduced ipsilateral to the rhizotomy (Fig. 4*B*). Clear differences were found in all sacral segments; however, the difference between the two sides was somewhat less apparent in sections from the first sacral segment. Differences could not be detected in the lumbar segments examined. The most characteristic diminution in staining was in the branch of fibers that coursed along the lateral border of the gray matter. In general, there were also fewer fibers that coursed and terminated across the base of the dorsal horn. Changes in the marginal zone were more difficult to assess.

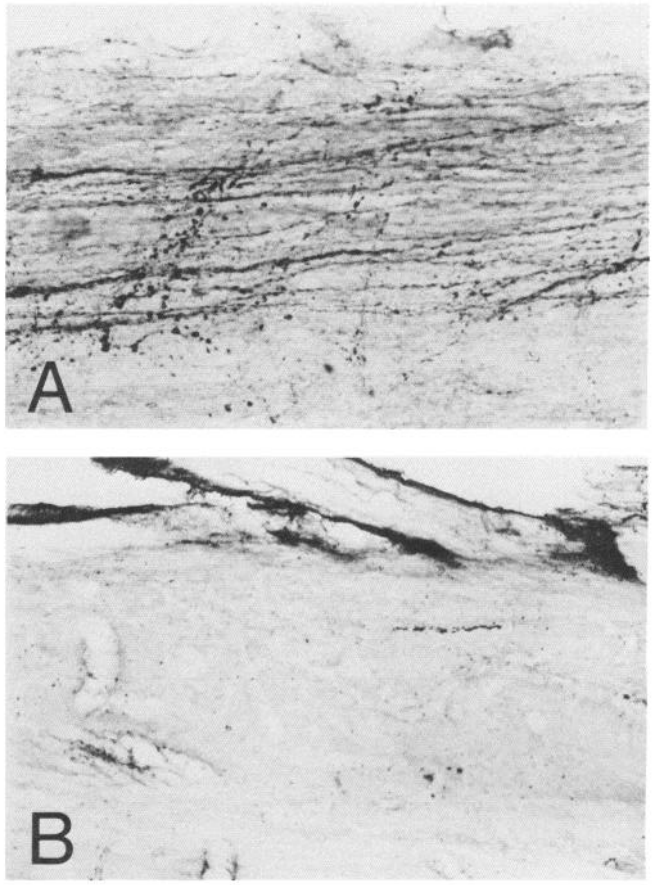

Figure 2. *A*, Parasagittal section through Lissauer's tract of the second sacral segment. Immunoreactive dynorphin B axons are found in large numbers. 260×. *B*, Absorption control. Parasagittal section through Lissauer's tract. Note absence of labeled fibers and also of immunoreactive axons in attached dorsal rootlet. (Compare Fig. 3*A*.) 67×.

Since we were unable to stain dynorphin B DRG cells immunocytochemically, we turned to a radioimmunoassay for dynorphin B on whole dorsal root ganglia. We examined all dorsal root ganglia of the cat; however, it was often difficult to get sufficient tissue to adequately assess small ganglia (e.g., thoracic). Therefore, although measurable levels of dynorphin were detected in all ganglia examined, we only report data from the lumbosacral ganglia. Consistent with the denser dynorphin B terminal staining in sacral cord, we found that sacral ganglia had the highest concentration of immunoreactive dynorphin B (Table 1). They were comparable to those previously reported on in the rat, using an antiserum directed against dynorphin A (Botticelli et al., 1981).

Discussion

The present study has established that there is a particularly high concentration of immunoreactive dynorphin B staining in

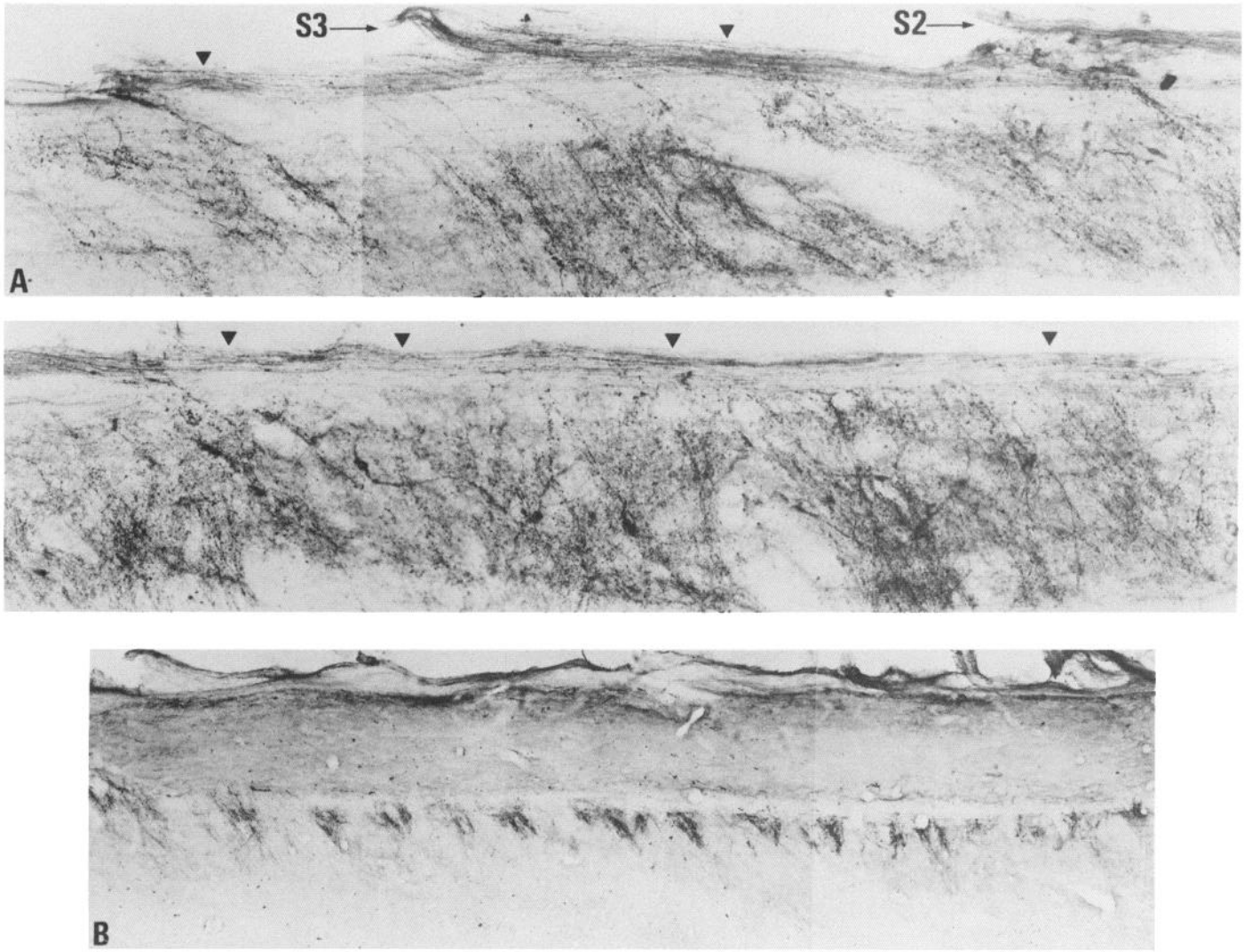

Figure 3. *A,* Photomontage of parasagittal section through the sacral spinal cord of a colchicine-treated cat. The section is cut through the lateral border of the dorsal horn, i.e., through the marginal zone as it caps the gray matter laterally. Immunoreactive dynorphin fibers can be identified in attached dorsal roots, *S2* and *S3.* The *arrowheads* illustrate labeled fibers in Lissauer's tract. Labeled axons that penetrate the marginal zone appear to arise from the overlying Lissauer tract. Many labeled marginal cells can also be seen. 100×. *B,* Photomontage of parasagittal section taken more medial to that of *A.* Discontinuous pattern of labeling is seen in the region of laminae V and VI. This pattern is also characteristic of spinal distribution of pelvic visceral afferents labeled by the transganglionic transport of horseradish peroxidase (Morgan et al., 1981) and of immunoreactive VIP in the same region of the cord (Basbaum and Glazer, 1983). 40×.

sacral segments of the spinal cord and in the attached dorsal roots of the cat. Although we were not able to label dorsal root ganglion cells, the biochemical studies indicate that sacral DRGs contain significant levels of the peptide. The reduction in immunoreactive dynorphin terminal staining in deafferented spinal cord provides evidence that the dorsal root staining was indeed in afferent fibers. Taken together, these data suggest that a population of primary afferent fibers contains dynorphin.

It is interesting that, although Botticelli et al. (1981) found measurable dynorphin A immunoreactivity in rat and guinea pig dorsal root ganglia, RIA revealed no change in the cord levels after dorsal rhizotomy. They could not conclude, therefore, that the DRG dynorphin immunoreactivity was neuronal in origin. Although there may be species differences, it is more likely that the failure of their RIA to detect spinal changes after rhizotomy in rat reflects the very restricted dorsal horn distribution of the peptide. Immunocytochemistry, although probably less sensitive than RIA, revealed the change, since the technique is best suited for identifying very localized regional changes in peptide distribution. The fact that our deafferentiation study focused on the sacral cord may also have increased the chances of demonstrating a decrease. Finally, the possibility that dynorphin A and dynorphin B are differentially processed and axonally transported by DRG cells must be considered. Immunohistochemical studies with antisera directed against dynorphin A would, therefore, be of interest.

There are several possible explanations for our failure to stain individual DRG cells. It is usually difficult to label cell bodies immunohistochemically without prior treatment with colchicine. In this study, we tried direct application of colchicine to the ganglia. This, however, significantly increased the background staining of the ganglia, making it very difficult to identify labeled cells. In our experience, the variability in staining of cat DRG cells is considerable, even for peptides known to be localized there, e.g., Substance P. It is conceivable that there is only a limited number of dynorphin B-containing DRG cells; these could readily be missed. The biochemical evidence that there is a substantial amount of peptide in the ganglia, however,

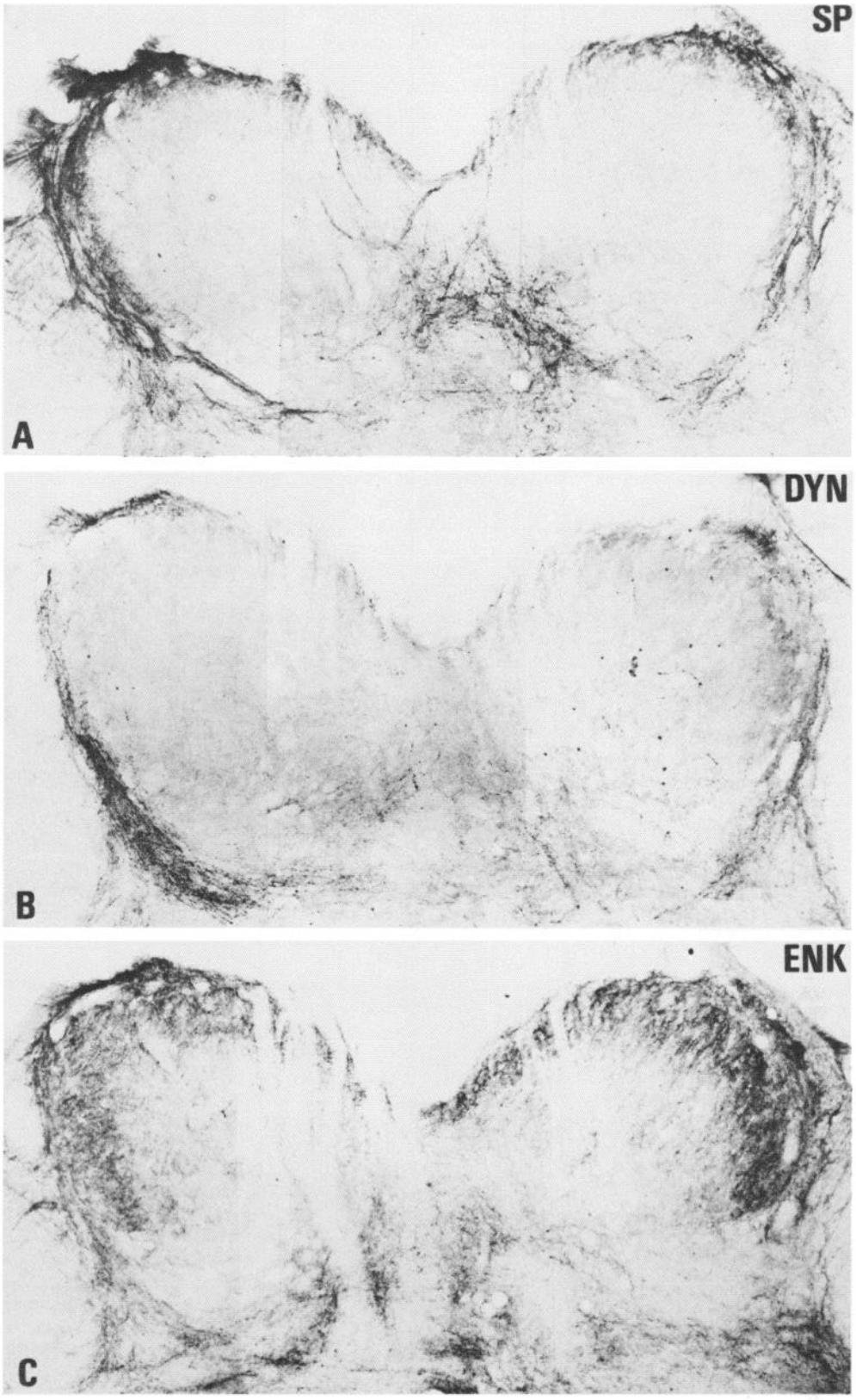

Figure 4. Immunohistochemical characterization of the effects of multiple unilateral (*right*) dorsal rhizotomy (L5–CC1) on the pattern of staining with different antisera in the first sacral segment of the cat spinal cord. *A,* Immunoreactive Substance P (SP), terminal staining is decreased approximately 50%, ipsilateral to the rhizotomy. 67×. *B,* Immunoreactive dynorphin B terminal staining is reduced to an even greater extent ipsilateral to the rhizotomy. 67×. *C,* Immunoreactive leucine enkephalin terminal staining is largely unchanged after unilateral rhizotomy. If anything, there is a slight increase ipsilateral to the rhizotomy. 67×.

suggests that the population of cells is significant. Finally, it is possible that the dynorphin in DRG cells is stored in a precursor form that is not recognized by the antiserum in immunohistochemical preparations.

The presence of immunoreactive dynorphin B in some primary afferent fiber underscores the anatomical differences between the enkephalin and dynorphin opioid peptides in the spinal cord. Functional differences are also likely. Enkephalin is found presynaptic to spinothalamic tract neurons (Ruda, 1982) and iontophoresis of enkephalin inhibits the firing of dorsal horn nociceptors (Randic and Miletic, 1978; Willcockson et al., 1984; Zieglgansberger and Tulloch, 1979). This action is presumed to

Table 1. Concentration (pmol/mg protein) of immunoreactive dynorphin B in extracts of spinal ganglia of the cat ($N = 5$)

Spinal ganglia	Dynorphin B immunoreactivity
L_5	1.02 ± 0.17
L_6	1.02 ± 0.33
L_7	0.79 ± 0.14
S_1	1.98 ± 0.76
S_2	2.19 ± 0.46
S_3	1.84 ± 0.57

underlie the analgesia produced by spinally administered narcotics and opioid peptides (Yaksh and Rudy, 1976). Several studies have reported an analgesic affect of dynorphin (Basbaum et al., 1983; Han and Xie, 1982; Piercey et al., 1982). The analgesia can be very prolonged. However, it often requires very high doses. Furthermore, there was minimal antagonistic effect of naloxone, indicating that the μ-opiate receptor, presumed to mediate morphine analgesia, was not involved. Thus different spinal circuits probably underlie the analgesic effects of the two different endogenous opioid peptides. More recently, Yaksh (1984) suggested that different nociceptive inputs, e.g., visceral vs thermal, may be selectively controlled by different endogenous opioid peptides. The presence of dynorphin afferent fibers, of course, indicates that the effect of intrathecal dynorphin not only reflects the activity within dynorphin interneuronal circuits but also the interactions between dynorphin-containing primary afferent fibers and spinal cord neurons.

It is generally assumed that all primary afferent fibers excite spinal cord interneurons. This raises the possibility that dynorphin-containing primary afferent fiber neurons exert an effect opposite to that proposed for spinal enkephalin-containing interneurons, which are probably exclusively inhibitory (Nicoll et al., 1980). Finding immunoreactive dynorphin in a population of afferent fibers and in spinal cord neurons located in laminae I and V, in fact, suggests that dynorphin may be concentrated in neurons that *transmit* nociceptive messages, in contrast to the spinal enkephalin neurons, which are traditionally associated with antinociceptive mechanisms. It is of interest, in this regard, that intracerebroventricular injection of dynorphin, or of des-Tyr dynorphin, rather than generating analgesia, reportedly antagonizes the analgesic action of morphine (Tulunay et al., 1981; Walker et al., 1982).

These data may also bear on the paradox concerning the nature of the opioid peptide input to primary afferent fibers. There is considerable evidence that a population of primary afferent fibers is opiate receptor-laden (Fields et al., 1980; Hiller et al., 1978; LaMotte et al., 1976). Jessel and Iversen (1977) demonstrated that enkephalin peptides and narcotic opiates block the potassium-evoked release of Substance P from slices of trigeminal nucleus caudalis. These observations led to the hypothesis that there is an enkephalin presynaptic control of Substance P-containing primary afferent fibers. Since neonatal capsaicin treatment destroys SP afferents (Jessel et al., 1978), decreases opiate receptor binding in the spinal cord (Gamse et al., 1979), and increases nociceptive thresholds, a corollary of the hypothesis was that the control of primary afferent nociceptive inputs to the dorsal horn involves an enkephalinergic presynaptic control.

To date, however, there is no firm electron microscopic evidence for axoaxonic synaptic relationships in which enkephalin terminals are presynaptic to primary afferent fibers (Aronin et al., 1981; Glazer and Basbaum, 1983; Hunt et al., 1980; La Motte and de Lanerolle, 1983; Sumal et al., 1982). Thus the requisite anatomical substrate for presynaptic control is missing. It has been suggested that a nonsynaptic interaction might underlie the enkephalin control (Glazer and Basbaum, 1983). Given the overlap in the distribution of dynorphin and SP terminals in the spinal dorsal horn, the possibility that dynorphin fibers, both of afferent and intraspinal origin, are the source of the input to the primary afferent opiate receptor must be considered. If primary afferent dynorphin-containing fibers provide a presynaptic input to other afferents (or to themselves), this might also generate an "autoreceptor" control mechanism.

There is preliminary evidence that dynorphin indeed acts on primary afferent fibers. Macdonald and Werz (1983) described a population of cultured dorsal root ganglion cells that are specifically sensitive to dynorphin. Dynorphin directly blocked calcium conductance in these neurons. This would have the effect of decreasing primary afferent transmitter release and would effectively exert a presynaptic inhibitory control. Mudge et al. (1979) previously reported that enkephalin reduced Ca^{++} entry into DRG cells; it is possible, however, that the doses used in the latter study were large enough to act at the receptor for which dynorphin is considered the endogenous ligand—specifically, the kappa receptor (Chavkin et al., 1982).

While all dorsal ganglia examined had measurable levels of dynorphin (in RIA), the sacral and coccygeal ganglia were consistently highest. From immunohistochemical analysis, it appeared that the second and third dorsal roots had the greatest number of labeled fibers. This differs somewhat from the pattern of VIP immunoreactivity, in which the first sacral segment receives the largest afferent input, via the S1 dorsal root. Since the pattern of termination of pelvic visceral afferent fibers in the dorsal horn strongly overlaps with that of VIP, it has been suggested that VIP may be a neurotransmitter of some pelvic visceral afferents. The similarity in the immunoreactive dynorphin B and VIP distributions, which includes the characteristic discontinuous staining seen in sagittal sections (Fig. 3*B*), is consistent with some dynorphin afferents' having a pelvic visceral origin. In fact, the largest pelvic visceral afferent projection courses via the S2 dorsal root (Morgan et al., 1981). This is similar to the dynorphin B pattern. Unfortunately, we were unaware that the methanol dehydration step in the PEG protocol would extract VIP. Thus we were unable to directly compare the patterns of VIP and dynorphin in the deafferented cord. Comparable studies in other laboratories, however, have reported a similar unilateral decrease in VIP staining. Studies directed at the peripheral origin distribution of dynorphin would help to establish the origin of the primary afferent dynorphin input. It appears likely that at least some component will be found in organs of the pelvic viscera (see Vincent et al., 1984). Another interesting possibility is that some primary afferent fibers are both VIP and dynorphin immunoreactive.

In summary, we have provided evidence that measurable levels of the opioid peptide, dynorphin B, are found in all dorsal root ganglia of the cat and that the sacral DRG contain very high levels. Immunocytochemical studies further establish that dynorphin is found in all sacral dorsal roots. Lumbosacral rhizotomies decreased the dynorphin immunoreactivity ipsilateral to the deafferentiation. These data indicate that some primary afferent fibers contain dynorphin and may use it as a neurotransmitter. Clearly, the analysis of opioid peptide contribution to spinal antinociceptive control mechanisms, and to the control of pelvic visceral organ function, must take into account the dynorphin component that derives from primary afferents, as well as the organization of enkephalin and dynorphin terminals that originate in the spinal cord. Studies directed at the peripheral origin of the spinal dynorphin and at the physiological effects of dynorphin on spinal neurons should provide important information not only about the functional significance of spinal dynorphin peptides but also about the differences between the two families of endorphins, dynorphin and enkephalin.

References

Aronin, N., M. Difiglia, A. S. Liotta, and J. B. Martin (1981) Ultrastructural localization and biochemical features of immunoreactive leu-enkephalin in monkey dorsal horn. J. Neurosci. *1:* 561–577.

Basbaum, A. I., and E. J. Glazer (1983) Immunoreactive vasoactive intestinal polypeptide is concentrated in sacral spinal cord: A possible marker for pelvic visceral afferent fibers. Somatosensory Res. *1:* 69–82.

Basbaum, A. I., D. Jacknow, J. Mulcahy, and J. Levine (1983) Studies on the contribution of different endogenous opioid peptides to the control of pain. In *Current Topics in Pain Research and Therapy,* T. Yokota and R. Dubner, eds., pp. 118–125, Elsevier, Amsterdam.

Botticelli, L. H., B. M. Cox, and A. Goldstein (1981) Immunoreactive dynorphin in mammalian spinal cord and dorsal root ganglia. Proc. Natl. Acad. Sci. USA *78:* 7783–7786.

Chavkin, D., I. F. James, and A. Goldstein (1982) Dynorphin is a specific endogenous ligand of the κ-opioid receptor. Science *215:* 413–415.

Cruz, L. J., and A. I. Basbaum (1983) Immunoreactive leucine enkephalin, methionine enkephalin-arg-gly-leu and dynorphin in the spinal cord of the cat. Neurosci. Abstr. *9:* 256.

Cruz, L. J., and A. I. Basbaum (1985) Multiple opioid peptides and the modulation of pain: Immunohistochemical analysis of dynorphin and enkephalin in the trigeminal nucleus caudalis and spinal cord of the cat. J. Comp. Neurol. *240:* 331–348.

deGroat, W. C., M. Kawatani, T. Hisamitsu, I. Lowe, C. Morgan, J. Roppolo, A. M. Booth, I. Nadelhaft, D. Kuo, and K. Thor (1983) The role of neuropeptides in the sacral autonomic reflex pathways of the cat. J. Autonom. Nerv. Syst. *7:* 339–350.

Elde, R., T. Hokfelt, O. Johansson, and L. Terenius (1976) Immunohistochemical studies using antibodies to leucine-enkephalin: Initial observations on the nervous system of the rat. Neuroscience *1:* 349–351.

Fields, H. L., P. C. Emson, B. K. Leigh, R. F. T. Gilbert, and L. L. Iversen (1980) Multiple opiate receptor sites on primary afferent fibres. Nature *284:* 351–353.

Gamse, R., P. Holzer, and F. Lembeck (1979) Indirect evidence for presynaptic location of opiate receptors on chemosensitive primary sensitive neurones. Naunyn-Schmiedeberg's Arch. Pharmacol. *308:* 281–285.

Glazer, E. J., and A. I. Basbaum (1980) Leucine enkephalin: Localization in and axoplasmic transport by sacral parasympathetic preganglionic neurons. Science *208:* 1479–1481.

Glazer, E. J. and A. I. Basbaum (1981) Immunohistochemical localization of leucine-enkephalin in the spinal cord of the cat: Enkephalin-containing marginal neurons and pain modulation. J. Comp. Neurol. *196:* 377–389.

Glazer, E. J. and A. I. Basbaum (1983) Opioid neurons and pain modulation: An ultrastructural analysis of enkephalin in cat superficial dorsal horn. Neuroscience *10:* 357–376.

Han, J. S. and C. W. Xie (1982) Dynorphin: Potent analgesic effect in spinal cord of the rat. Life Sci. *31:* 1781–1784.

Hiller, J. M., E. J. Simon, S. M. Crain, and E. R. Peterson (1978) Opiate receptors in culture of fetal mouse dorsal root ganglia (DRG) and spinal cord: Predominance in DRG neurites. Brain Res. *145:* 396–400.

Hokfelt, T., J. O. Kellerth, G. Nilsson, and B. Pernow (1975) Experimental immunohistochemical studies on the localization and distribution of substance P in cat primary sensory neurons. Brain Res. *100:* 235–252.

Honda, C. N., M. Retheyi, and P. Petrusz (1983) Preferential immunohistochemical localization of vasoactive intestinal polypeptide (VIP) in the sacral spinal cord of the cat: Light and electron microscopic observations. J. Neurosci. *3:* 2183–2196.

Hunt, S. P., J. S. Kelly, and P. C. Emson (1980) The electron microscopic localization of methionine-enkephalin within the superficial layers (I and II) of the spinal cord. Neuroscience *5:* 1871–1890.

Jessel, T. M., and L. L. Iversen (1977) Opiate analgesics inhibit substance P release from rat trigeminal nucleus. Nature *268:* 549–551.

Jessel, T. M., L. L. Iversen, and A. C. Cuello (1978) Capsaicin-induced depletion of substance P from primary sensory neurones. Brain Res. *152:* 183–188.

Khachaturian, H., S. J. Watson, M. E. Lewis, D. Coy, A. Goldstein, and H. Akil (1982) Dynorphin immunocytochemistry in the rat central nervous system. Peptides *3:* 941–954.

LaMotte, C., and N. C. DeLanerolle (1983) Ultrastructure of chemically defined neuron systems in the dorsal horn of the monkey. II: Methionine-enkephalin immunoreactivity. Brain Res. *274:* 51–63.

LaMotte, C., C. B. Pert, and S. H. Snyder (1976) Opiate receptor binding in primate spinal cord. Distribution and changes after dorsal root section. Brain Res. *112:* 407–412.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193:* 265–275.

Macdonald, R. L., and M. A. Werz (1983) Dynorphin decreases calcium conductance of mouse cultured dorsal root ganglion neurons. Neurosci. Abst. *9:* 1129.

Morgan, C., W. C. deGroat, and P. J. Jannetta (1982) Leucine enkephalin and Substance-P identified in trigeminal ganglion neurons innervating the cornea in the cat. Neurosci. Abstr. *8:* 474.

Morgan, C., I. Nadelhaft, and W. C. deGroat (1981) The distribution of visceral primary afferents from the pelvic nerve to Lissauer's Tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. J. Comp. Neurol. *201:* 415–440.

Mudge, A. W., S. E. Leeman, and G. D. Fischbach (1979) Enkephalin inhibits release of substance P from sensory neurons in culture and decreases action potential duration. Proc. Natl. Acad. Sci. USA *76:* 526–530.

Nicoll, R. A., B. E. Alger, and C. Jahr (1980) Enkephalin blocks inhibitory pathways in the vertebrate CNS. Nature *287:* 22–25.

Piercey, M. F., R. A. Lahti, L. A. Schroeder, E. J. Einspahr, and C. Barsuhn (1982) U50488H, a pure kappa receptor agonist with spinal analgesic loci in the mouse. Life Sci. *31:* 1197–1200.

Przewlocki, R., L. Stala, M. Grezcek, G. T. Shearman, B. Przewlocki, and A. Herz (1983) Analgesic effect of μ- and κ-opiate agonists and, in particular, dynorphin at the spinal level. Life Sci. *33:* 649–652.

Randic, M., and V. Miletic (1978) Depressant actions of methionine-enkephalin and somatostatin in cat dorsal horn neurones activated by noxious stimuli. Brain Res. *152:* 196–202.

Ruda, M. A. (1982) Opiates and pain pathways: Demonstration of enkephalin synapses on dorsal horn projection neurons. Science *215:* 1523–1524.

Sternberger, L. A. (1979) *Immunocytochemistry,* 2nd Ed., Wiley, New York.

Sumal, K. K., V. M. Pickel, R. J. Miller, and D. J. Reis (1982) Enkephalin-containing neurons in substantia gelatinosa of spinal trigeminal complex: Ultrastructure and synaptic interaction with primary sensory afferents. Brain Res. *248:* 223–236.

Sweetnam, P. M., J. H. Neale, J. L. Barker, and A. Goldstein (1982) Localization of immunoreactive dynorphin in neurons cultured from spinal cord and dorsal root ganglia. Proc. Natl. Acad. Sci. USA *79:* 6742–6746.

Tulunay, F. C., M. F. Jen, J. K. Chang, H. H. Loh, and H. M. Lee (1981) Possible regulatory role of dynorphin on morphine and β-endorphin-induced analgesia. J. Pharmacol. Exp. Ther. *219:* 296.

Vincent, S. R., C.-J. Dalsgaard, M. Schultzberg, T. Hökfelt, I. Christensson, and L. Terenius (1984) Dynorphin-immunoreactive neurons in the autonomic nervous system. Neuroscience *11:* 973–988.

Walker, J. M., D. Tucker, D. H. Moy, B. B. Walker, and H. Akil (1982) Des-tyrosine-dynorphin antagonizes morphine analgesia. Eur. J. Pharmacol. *85:* 121–122.

Weber, E., and J. D. Barchas (1983) Immunohistochemical distribution of dynorphin B in rat brain: Relation to dynorphin A and αneo-endorphin systems. Proc. Natl. Acad. Sci. USA *1980:* 1125–1129.

Weber, E., C. J. Evans, J. K. Chang, and J. D. Barchas (1982) Antibodies specific for α-*N*-acetyl β-endorphins: Radioimmunoassays and detection of acetylated β-endorphins in pituitary extracts. J. Neurochem. *38:* 436–447.

Willcockson, W. S., J. M. Chung, Y. Hori, K. H. Lee, and W. D. Willis (1984) Effects of iontophoretically released peptides on primate spinothalamic tract cells. J. Neurosci. *4:* 741–750.

Yaksh, T. L. (1984) Multiple spinal opiate receptor systems. Adv. Pain Res. Ther. *6:* 197–215.

Yaksh, T. L., and T. A. Rudy (1976) Analgesia mediated by a direct spinal action of narcotics. Science *192:* 1357–1358.

Zieglgansberger, W., and I. F. Tulloch (1979) The effects of methionine-enkephalin and leucine-enkephalin on spinal neurones of the cat. Brain Res. *167:* 53–64.