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Vesicular Glutamate Transporter-1 Colocalizes With Endogenous Opioid Peptides in Axon Terminals of the Rat Locus Coeruleus

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ABSTRACT

We have previously shown that a subset of axon terminals in the locus coeruleus (LC) containing methionine^5-enkephalin (ENK) forms type I (asymmetric-type) synaptic specializations that are characteristic of excitatory-type transmitters. In addition, we previously provided ultrastructural evidence showing that ENK is colocalized with glutamate using a combination of pre- and postembedding immunohistochemistry. To examine cellular substrates for interactions between glutamate and other endogenous opioid peptides in the LC, we examined the localization of the vesicular glutamate transporter 1 (VGLUT1), a transporter protein involved in the accumulation of the transmitter glutamate into synaptic vesicles, with either ENK or preprodynorphin (ppDYN). Dual-immunofluorescence and electron microscopy showed prominent coexistence of VGLUT1 and ENK in varicose processes of the LC, confirming our previous report using postembedding immunolabeling for glutamate. Likewise, VGLUT1 and ppDYN were identified in common varicose processes in the LC using confocal fluorescence microscopy. Immunoelectron microscopy using gold-silver labeling for VGLUT1 and peroxidase labeling for ppDYN established that this endogenous opioid peptide also colocalizes with glutamate transporters. The majority of these formed asymmetric-type synapses. Taken together, these results demonstrate that excitatory LC afferents are enriched with endogenous opioid peptides and are positioned to modulate LC neuronal activity dually.

Key words: norepinephrine; excitatory amino acid; dynorphin; encephalin; glutamate

The nucleus locus coeruleus (LC), located on the floor of the fourth ventricle in the rostral pons, provides extensive noradrenergic projections throughout the neuraxis. The collection and processing of salient sensory information having effects on memory, attention and arousal, cardiovascular regulation, antinociception, regulation of anxiety states and stress response have been associated with these neurons (Berridge and Waterhouse, 2003). The LC receives a major glutamatergic input from the medullary nucleus paragigantocellularis (PGi) (Ennis and Aston-Jones, 1988; Aston-Jones et al., 1991). These afferents also express endogenous opioid peptides, including methionine^5-enkephalin (ENK) (Drolet et al., 1992).

Modulation of postsynaptic glutamate receptors activates LC neurons (Oleskevich et al., 1993; Van Bockstaele et al., 2000), while the activity of LC neurons is inhibited by opioid peptides via activation of an inward rectifying

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K^+ channel and inhibition of an Na^+-dependent inward current (Christie, 1991; Alreja and Aghajanian, 1993). The release rate of glutamate in the LC is increased by noxious stimulation and morphine withdrawal (Akaoka and Aston-Jones, 1991; Singewald et al., 1995) and endogenous opioids modulate LC activity following exposure to stress (Abercrombie and Jacobs, 1988; Curtis et al., 2001).

Transport of glutamate across the plasma membrane and conversion of glutamine into glutamate by phosphate-activated glutaminase are processes that occur in multiple cell types. Therefore, vesicular storage and exocytotic release of glutamate define its role as a neurotransmitter. Glutamate is accumulated into vesicles by vesicular glutamate transporters (VGLUTs). Three isoforms of VGLUT have so far been established. Two are recognized as definitive markers of glutamatergic neurons and their axon terminals (VGLUT1, VGLUT2) (Bellocchio et al., 1998, 2000; Takamori et al., 2000; Fremeau et al., 2001, 2002; Herzog et al., 2001; Hisano et al., 2002; Hisano, 2003) and a third is expressed by cholinergic and serotonergic neurons (VGLUT3) (Gras et al., 2002; Schafer et al., 2002). VGLUT1 mRNA expression in distinct regions of rat and human brain has been confirmed by in situ hybridization studies (Ni et al., 1994, 1996; Hisano et al., 2002; Hioki et al., 2003; Hisano, 2003).

Several lines of evidence support the likelihood that opioid peptides coexist with vesicular glutamate transporters in axon terminals in the LC. First, heterogeneous synaptic vesicles, small clear and large dense core vesicles, are found in axon terminals in the LC. Neuropeptides, packaged in large dense core vesicles (Whim et al., 1997; Hokfelt et al., 2000), have been identified in axon terminals in the LC (Van Bockstaele et al., 2000). Second, previous ultrastructural evidence has indicated that a subset of ENK-containing afferents in the LC exhibits glutamate immunoreactivity using a combination of pre- and postembedding immunohistochemistry (Van Bockstaele et al., 2000). Third, dendrites in the LC that express μ-opioid receptor immunoreactivity also exhibit immunolabeling for the glutamate receptor subtypes kainate and N-methyl-D-aspartate (NMDA) (Van Bockstaele et al., 2000). Finally, axon terminals form asymmetric (excitatory-type) synapses with dendrites exhibiting opioid and excitatory amino acid receptors in the LC (Van Bockstaele et al., 2000). In the present study, we investigated whether ENK- and dynorphin-containing afferents in the LC contain VGLUT1 using dual-immunofluorescence and immunoelectron microscopy.

MATERIALS AND METHODS

The procedures used in this study were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University and conformed to National Institutes of Health guidelines. Although it is not possible to use alternatives to in vivo techniques, every attempt was made to reduce the number of animals used and minimize suffering.

Tissue Preparation

Adult male Sprague-Dawley rats (225–249 g; Harlan Sprague-Dawley, Indianapolis, IN) were used in this study. All rats were deeply anesthetized with sodium pentobarbital (60 mg/kg) and perfused transcardially through the ascending aorta with 50 ml of 3.8% acrolein (Electron Microscopy Sciences, Fort Washington, PA) and 200 ml of 2% paraformaldehyde (Electron Microscopy Sciences) in 0.1 M phosphate buffer (PB; pH 7.4). Immediately after perfusion fixation, the brains were dissected out, placed in 2% paraformaldehyde, and allowed to sit under refrigeration for 24 hr. Forty micron thick sections were then cut through the rostrocaudal extent of the LC by using a Vibratome (Technical Products, St. Louis, MO) and collected into chilled 0.1 M PB. The sections were placed for 30 min in 1% sodium borohydride in 0.1 M PB to remove reactive aldehydes and rinsed extensively in 0.1 M PB before primary antibody incubation.

Antibody Specificity

The following antisera were used. A rabbit polyclonal antibody raised against a glutathione S-transferase fusion protein containing amino acid residues 456–561 of rat VGLUT1 (Synaptic Systems, Gottingen, Germany). A mouse monoclonal antibody raised against the opioid peptide leucine^5-enkephalin (ENK; Fitzgerald, Concord, MA). A monoclonal antibody directed against tyrosine hydroxylase (TH) isolated and purified from rat P12 cells (Immunostar, Hudson, WI) and a guinea pig polyclonal antibody (Sasco, Omaha, NE) raised against amino acid residues 235–248 of rat preprodynorphin (DYN) and recognizes all dynorphin peptides. This antiserum was a generous gift from Dr. Robert Elde, University of Minnesota. Details on antibody specificity are described below.

The characterization and specificity of the antisera against ENK, preprodynorphin (ppDYN), TH, and VGLUT1 have been described previously (Van Bockstaele and Pickel, 1993; Arvidsson et al., 1995; Milner et al., 1995; Bellocchio et al., 1998). The VGLUT1 antibody was shown to recognize VGLUT1 in transfected but not control cells. Preadsorption with the GST fusion protein prevented the detection of the immunoreaction product. The ENK antibody was shown to react primarily with leucine^5-enkephalin (L-ENK) but also to cross-react slightly with methionine^5-enkephalin (M-ENK). The immunoreaction product was abolished in tissue sections through the medulla by preadsorption with L-ENK and M-ENK. The specificity of the polyclonal antibody directed against ppDYN, which recognized all dynorphin peptides, was also examined by preadsorption of the antibody with its cognate peptide.

Immunofluorescence

Dual-immunofluorescence detection was used for the detection of VGLUT1 and TH, or VGLUT1 and ENK as well as VGLUT1 and DYN in the LC region. Forty μm thick sections of the rat brain were rinsed extensively in 0.1 M tris-buffered saline (TBS; pH 7.6) followed by incubation for 30 min in 0.1 M TBS containing 0.5% bovine serum albumin (BSA) and 0.25% Triton X-100. Tissue sections were then incubated overnight in a cocktail of rabbit anti-VGLUT1 (1:1,000) and mouse anti-ENK (1:100) or anti-TH (1:1,000) or guinea pig anti-DYN (1:10,000) in 0.1 M TBS containing 0.1% bovine serum albumin (BSA) and 0.25% Triton X-100. The next day, sections were again rinsed extensively in 0.1 M TBS and subsequently incubated in secondary antibodies containing two distinct fluorophores: rhodamine isothiocyanate

(TRITC)-conjugated donkey antirabbit (1:100; Jackson Immunoresearch, West Grove, PA) for VGLUT1- or TRITC-conjugated donkey antiguinea pig (1:100; Jackson Immunoresearch) for DYN and fluorescein isothiocyanate (FITC; Jackson Immunoresearch)-conjugated donkey antimouse (1:50) for ENK or FITC-conjugated donkey antirabbit for VGLUT1 (1:100; Jackson Immunoresearch) or FITC-conjugated donkey antimouse for TH (1:100; Jackson Immunoresearch). The tissue was extensively rinsed in 0.1 M TBS for 30 min in reduced light conditions. The sections were then coverslipped with D.P.X. mounting medium (Aldrich, St. Louis, MO). A series of images were taken using a confocal microscope (z-series, Zeiss) and combined to reconstruct immunolabeling throughout the section.

Immunoelectron Microscopy

Methods for immunoelectron microscopy followed standard protocols (Chan et al., 1990). For dual-immunoelectron microscopy, sections were incubated in primary antibodies directed against VGLUT1 (1:4,000) and ENK (1:100) or ppDYN (1:16,000) for 15–18 hr at room temperature. Sections were then rinsed three times in 0.1 M phosphate buffer (PB) and incubated in the secondary antiserum at room temperature. For ENK and DYN immunohistochemistry, sections were incubated in biotinylated goat antimouse IgG (1:400) or biotinylated donkey antirabbit IgG (1:400) for 30 min, followed by 30-min incubation in avidin-biotin complex (ABC; Vector Laboratories). ENK and DYN were visualized by a 6- to 10-min reaction in 22 mg of 3,3′-diaminobenzidine (Aldrich) and 10 μl of 30% hydrogen peroxide in 100 ml of 0.1 M PBS. For VGLUT1 immunohistochemistry, the same sections were incubated in a solution of 0.01 M PBS containing 0.1% gelatin and 0.8% bovine serum albumin (BSA) for 30 min. The LC sections were then incubated in a goat antirabbit IgG conjugated to 1 nm gold particles (Amersham, Piscataway, NJ) for 2 hr at room temperature. These were rinsed in 0.01 M PBS containing the same concentrations of gelatin and BSA as described above and subsequently rinsed with 0.01 M PBS alone. Sections were then incubated in 1.25% glutaraldehyde (Electron Microscopy Sciences) in 0.01 M PBS for 10 min, followed by a wash in 0.01 M PBS. Sections were then placed in 0.2 M sodium citrate buffer, pH 7.4, followed by silver intensification of the gold particles using a silver enhancement kit (Amersham). The optimal silver-enhancement times were determined empirically for each experiment and averaged over 10–12 min for electron microscopy. Sections were then incubated in 2% osmium tetroxide (Electron Microscopy Sciences) in 0.1 M PB for 1 hr, washed in 0.1 M PB, dehydrated, and flat-embedded in Epon 812 (Electron Microscopy Sciences). Thin sections of approximately 80–100 nm were cut from the outer surface of the tissue with a diamond knife (Diatome, Fort Washington, PA) on a Leica ultramicrotome. These were collected on grids and counterstained with uranyl acetate and Reynolds lead citrate. Captured images of selected sections of tissue will be compared with captured light microscopic images of the block face before sectioning.

Controls and Data Analysis

To evaluate the possible labeling of the primary antibodies by secondary antibodies in dual-labeling experiments, some sections were processed for dual labeling with omission of one of the primary antibodies. No cross-reactivity was observed in such control experiments. Thin sections of tissue selected for electron microscopy were selected immediately adjacent to the fourth ventricle in the region known to contain catecholamine cell bodies and dendrites (Swanson, 1976). The classification of identified cellular elements was based on Peters (1991). Structures were defined as being proximal dendrites if they contained endoplasmic reticulum and were larger than 0.7 μm in diameter. Axon terminals were distinguished from unmyelinated axons based on their content of synaptic vesicles and diameter greater than 0.1 μm. A terminal was considered to be synaptic when it shows a restricted zone of parallel membrane apposition with a slight enlargement of the intercellular space and/or associated postsynaptic thickening. Asymmetric synapses were identified by the presence of thick postsynaptic densities (Gray's type I) (Gray, 1959). Symmetric synapses, on the other hand, have thin densities postsynaptically (Gray's type II) (Gray, 1959). Appositions were defined by closely spaced parallel plasma membranes of immunoreactive axons and other axon terminals or dendrites. These lack recognizable specializations but are not separated by glial processes.

Peroxidase immunoreactivity appeared as a dense homogeneous precipitate in cellular profiles. For DYN immunohistochemistry, electron-dense immunolabeling was often associated with large dense core vesicles, although numerous small vesicles were also apparent in the axoplasm. Some axon terminals, however, did not contain any detectable large dense core vesicles in single sections examined. These axon terminals were categorized as ENK- or ppDYN-immunoreactive if they contained peroxidase immunolabeling within their axoplasm or if they contained clumps of peroxidase reaction product within their axoplasm. The peroxidase-labeled axon terminals were clearly more electron-dense than their neighboring unlabeled axon terminals. Selective gold-silver-labeled profiles were identified by the presence, in single thin sections, of at least 3–4 gold particles within cellular profiles. Whenever possible, the more lightly labeled processes were confirmed by detection in at least two serial sections. A profile containing a small number of gold particles (e.g., two gold particles) that was unlabeled in adjacent thin sections was designated as lacking detectable immunoreactivity.

RESULTS

Confocal Fluorescence Microscopy of VGLUT1 and Opioid Peptides

By confocal microscopy, the LC was visualized using an antibody directed against TH and appeared as a dense cluster of neurons in the rostral pons adjacent to the fourth ventricle (Fig. 1A). Immunocytochemical labeling for VGLUT1 revealed varicose processes enriched within the LC (Fig. 1B–D). Immunocytochemical labeling for VGLUT, ppDYN, and ENK exhibited a punctate pattern of staining in the LC and densely labeled fibers in the peri-LC, which contains extensive dendrites of noradrenergic neurons. Using dual-immunofluorescence microscopy, ppDYN and ENK were found within VGLUT1-labeled processes in the LC (Fig. 2). Dual localization of VGLUT1/ppDYN and VGLUT1/ENK was more prominent

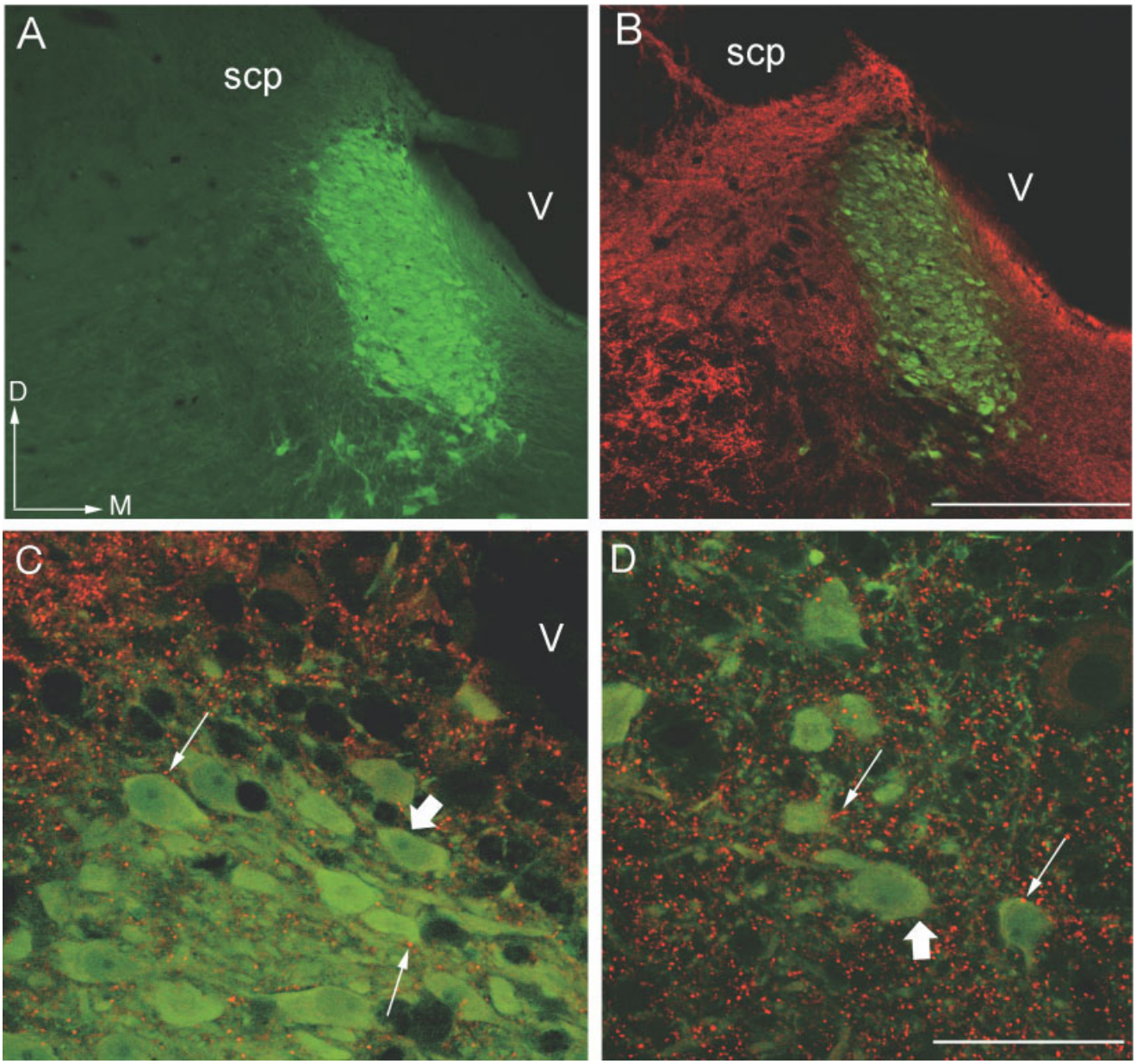

Fig. 1. **A** and **B:** Low-magnification photomicrographs of a coronal section through the LC of rat brain showing immunofluorescence labeling for VGLUT1 and TH. TH was detected using a FITC-tagged secondary antibody (green) and VGLUT1 immunoreactivity was detected using a TRITC-tagged secondary antibody (red). **C** and **D:** High-power photomicrographs showing merged images of VGLUT1 (red) and TH (green) in the LC. The dorsolateral portion of the LC is shown in C while the ventromedial aspect of the LC is shown in higher magnification in D. Thin white arrows denote VGLUT1-labeled processes apposed to TH-labeled somata. TH-labeled somata are indicated by thick white arrows. scp, superior cerebellar peduncle; V, fourth ventricle. Scale bar = 300 μm (A and B); 40 μm (C and D).

in the peri-LC region as compared to the core or somatic region (Fig. 2).

Ultrastructural Analysis of VGLUT1 and Opioid Peptides

VGLUT1 immunolabeling identified using either peroxidase or gold-silver (Fig. 3A) detection methods was localized to axon terminals that formed primarily Gray's type I (asymmetric) synaptic specializations (Fig. 3A). VGLUT1-labeled axon terminals contained heterogeneous types of synaptic vesicles (small clear and large dense core) and often contacted medium- to small-sized dendrites.

Immunoperoxidase labeling for VGLUT1 was combined with gold-silver labeling of ENK in the same section of tissue through the dorsal pons. Both markers were readily distinguishable in the same section of tissue (Fig. 3B). Peroxidase labeling for VGLUT1 appeared as an electron-dense homogeneous product confined to axon terminals

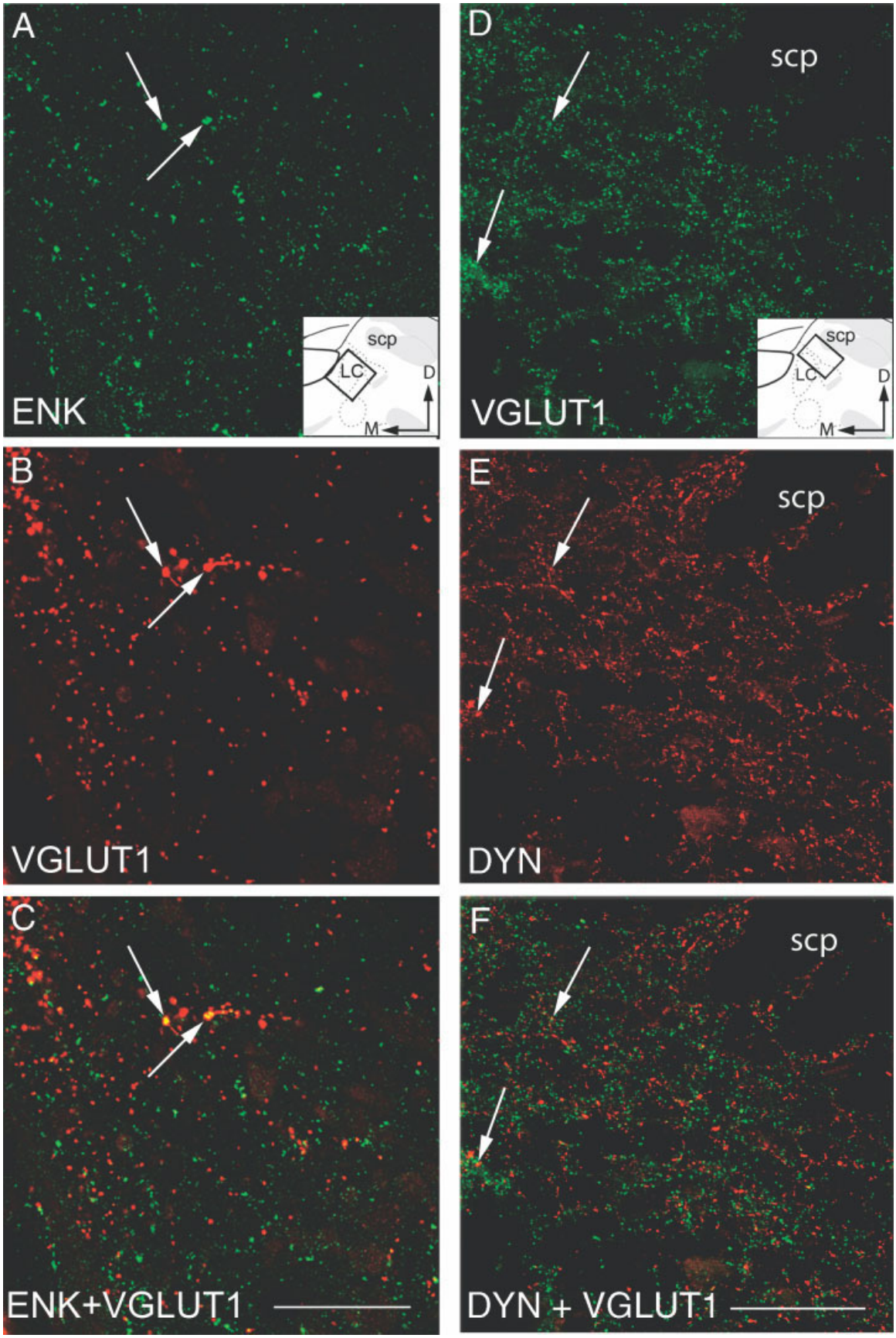

Fig. 2. Photomicrographs of coronal sections through the LC of rat brain showing dual-immunofluorescence labeling for VGLUT1/ENK and VGLUT1/ppDYN. Insets in A and D are adapted from the rat brain atlas of Swanson (2001) showing a low-magnification schematic diagram of the region sampled. In the insets, arrows indicate dorsal and medial orientation of the sections illustrated. **A–C:** Photomicrographs of the same section through the LC showing ENK immunolabeling detected using an FITC-tagged secondary antibody (A), VGLUT1 detected using a TRITC-tagged secondary antibody (B), and both antigens in the merged image (C). Processes exhibiting both VGLUT1 and ENK can be seen at thin white arrows. **D–F:** Photomicrographs showing VGLUT1-labeled processes using an FITC-tagged secondary antibody (D), ppDYN immunoreactivity detected using a TRITC-tagged secondary antibody (E), and both antigens in a merged image (F). Note that processes exhibiting both VGLUT1 and ppDYN can be identified at thin white arrows. Scale bar = 10 μm (A–C); 100 μm (D–F).

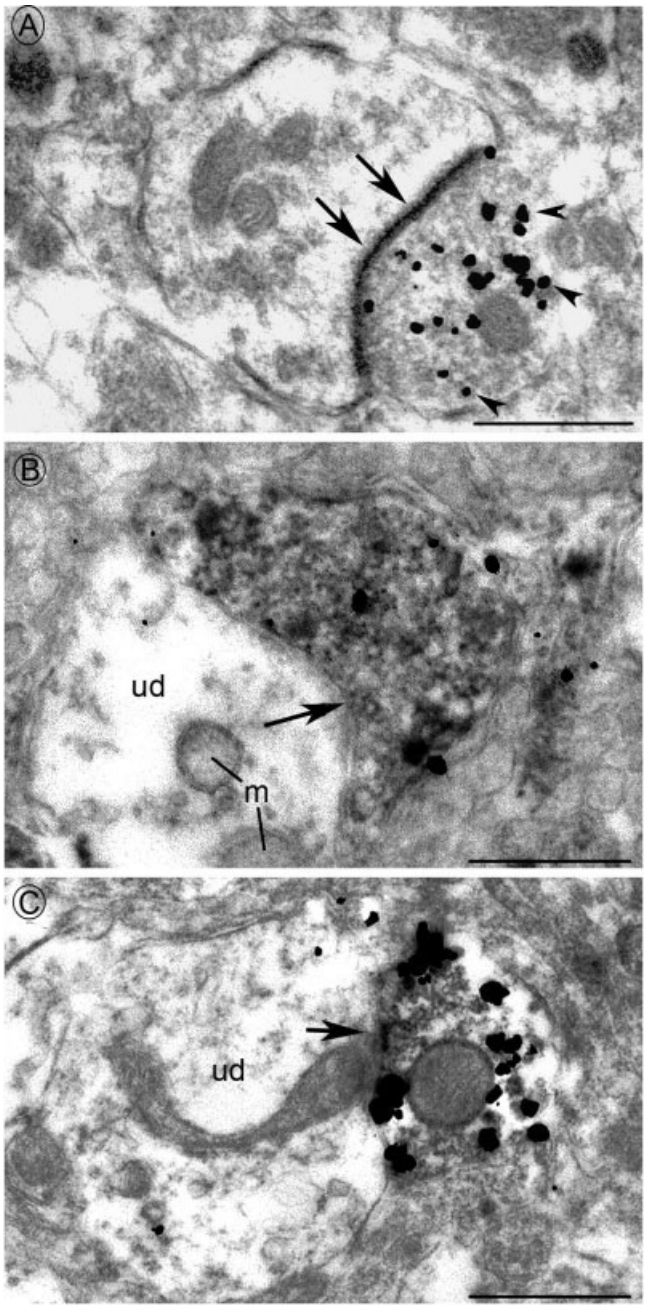

Fig. 3. **A:** Immunogold-silver labeling (arrowheads) for VGLUT1 is found in an axon terminal forming an asymmetric-type synaptic specialization (black arrow) with a dendritic process. Scale bar = 0.5 μm. **B** and **C:** Electron photomicrographs showing the coexistence of VGLUT1 with ENK (B) or ppDYN (C) in axon terminals in the rat LC. The VGLUT1/ENK axon terminal forms an asymmetric contact (black arrow) with an unlabeled dendrite. In C, gold-silver labeling indicates ppDYN immunoreactivity while peroxidase labeling shows VGLUT1 immunoreactivity. The VGLUT1/ppDYN terminal is directly apposed to an unlabeled dendrite (ud). Scale bar = 0.5 μm.

and associated with small vesicles mostly distributed throughout the axon terminal (Fig. 3C). Gold-silver labeling of ENK appeared as large irregularly shaped black deposits that were often distributed randomly within and along the perimeter of axon terminals. Separately labeled axon terminals for VGLUT1 or ENK were readily observed in similar portions of the neuropil. Of 118 axon terminals containing VGLUT1, 43% (n = 51) contained immunolabeling for ENK. When synaptic junctions were identifiable, VGLUT1/ENK dual-labeled axon terminals frequently formed asymmetric synapses.

In separate sections of tissue, gold-silver labeling of VGLUT1 was combined with immunoperoxidase labeling for ppDYN (Fig. 3C). Both markers were again readily distinguishable in the same section of tissue. Separately labeled axon terminals for VGLUT1 or ppDYN were readily observed. Axon terminals containing peroxidase labeling for ppDYN and immunogold-silver labeling for VGLUT1 were observed (Fig. 3B). Of 275 axon terminals containing VGLUT1, 48% contained immunolabeling for ppDYN (n = 133). When synaptic junctions were identifiable, VGLUT1/ppDYN dual-labeled axon terminals frequently formed asymmetric synapses (Fig. 4).

DISCUSSION

The results of this study provide ultrastructural evidence showing that opioid-containing axon terminals exhibit immunolabeling for vesicular glutamate transporters and form asymmetric-type synapses with dendrites in the LC. Such data provide a cellular substrate for the dual release of endogenous opioid peptides and glutamate from common afferents in the LC.

Methodological Considerations

The quantitative approach used in the present study has previously been discussed (Van Bockstaele and Pickel, 1993; Van Bockstaele et al., 1996). To ensure the reproducibility of the quantitative evaluation of the colocalization of neurotransmitters and types of junctions formed by immunoreactive processes, carefully controlled conditions were required. For quantification of labeled profiles in thick sections immunolabeled prior to embedding for electron microscopy, we have observed that the collection of sections from the surface of the section minimizes artifacts that may be associated with incomplete penetration of antiserum. Therefore, in the present study, the analysis of tissue sections collected at the plastic-tissue interface ensured both markers were clearly detectable in all sections used for analysis (Chan et al., 1990).

VGLUT1 Is Abundant in LC

In the LC, VGLUT1 localizes to axon terminals in the neuropil, not cell bodies or dendritic profiles. The distribution of VGLUT1 labeling in axon terminals in the LC and the high frequency of asymmetric synapses formed by VGLUT1 axon terminals with unlabeled dendrites are in agreement with other previously published studies (Bellocchio et al., 1998; Herzog et al., 2001; Kaneko and Fujiyama, 2002). Asymmetric synapses (Gray's type I synapses) are believed to mediate excitation based on the detection of enhanced populations of thickened postsynaptic densities in regions of the brain containing elevated proportions of excitatory synapses (Peters, 1991). The

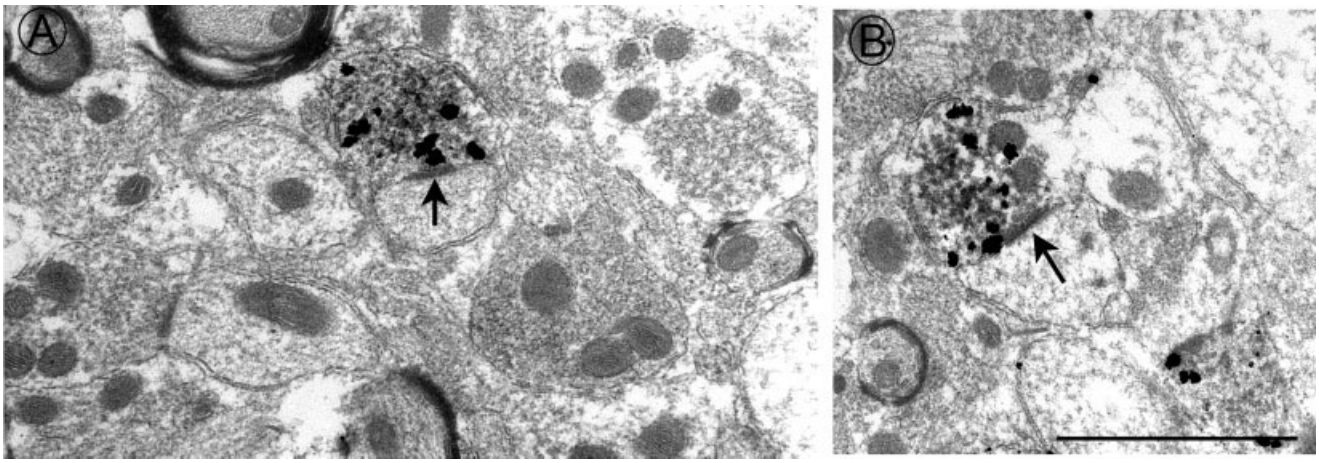

Fig. 4. **A** and **B:** Axon terminals containing peroxidase labeling for ppDYN and immunogold-silver labeling for VGLUT1 form asymmetric contacts (black arrows) with unlabeled dendrites in the LC. Scale bar = 1.0 μm.

present results indicate that a subset of ENK- and ppDYN-containing axon terminals forming asymmetric-type synaptic specializations are excitatory in nature by virtue of the fact that they coexpress vesicular glutamate transporters.

Glutamate and Opioids as Cotransmitters

In the present study, we report ultrastructural evidence for the coexistence of VGLUT1 and ENK as well as VGLUT1 and ppDYN in the same axon terminals in the LC. These findings extend previous studies from our laboratory showing that ENK-containing axon terminals in the LC contain glutamate (Van Bockstaele et al., 2000) by using a definitive marker for the glutamatergic phenotype, the localization of vesicular glutamate transporters. Potential caveats associated with the use of an antibody directed against glutamate [as conducted in our previous study (Van Bockstaele et al., 2000)] include identifying cellular sites where the compound is involved in the normal metabolism of cells (Peters et al., 1991), e.g., mitochondria, or localizing the compound that may have been taken up from the extracellular space. Therefore, using dual immunolabeling for ENK and VGLUT1 provides the unequivocal demonstration that ENK and glutamate are cotransmitters in afferents to the LC.

The functional consequences of corelease of glutamate and ENK from common afferents in the LC are not fully understood. Glutamate is released from axon terminals in the LC following noxious stimulation and withdrawal from opiates (Abercrombie and Jacobs, 1988; Akaoka and Aston-Jones, 1991; Aghajanian et al., 1994; Singewald et al., 1995). An excitatory amino acid pathway has been described between neurons in the PGi in the rostral ventral medulla and the LC (Aston-Jones et al., 1991) and a large proportion of PGi neurons projecting to the LC contain ENK and express opiod receptors (Van Bockstaele et al., 1999; Drolet et al., 1992). Stimulation of the PGi activates LC cells, a response that is blocked by excitatory amino acid receptor antagonists, while lesions of the PGi attenuate LC cell hyperactivity following precipitated opiate withdrawal (Rasmussen and Aghajanian, 1989; Rasmussen et al., 1990). Naloxone potentiates the increase in LC activity in response to stress but not in a resting state (Abercrombie and Jacobs, 1988), suggesting endogenous opioid inputs to LC are not tonically active during resting conditions but may become active during stress to exert a modulatory role on LC discharge activity. Also, termination of hypotensive stress induces opioid release in the LC, restoring basal activity and presumably protecting against adverse consequences of continued cellular activation (Curtis et al., 2001). Our previous studies have shown that, in opiate-dependent rats, medullary afferents to the LC show a decrease in ENK and ENK mRNA expression (Van Bockstaele et al., 2000), which could be a factor in the hyperactivity of LC cells following withdrawal. As excitatory amino acid release from LC afferents is increased during withdrawal (Aghajanian et al., 1994; Zhang et al., 1994), an imbalance in the release of opioid peptides from glutamatergic terminals that could restore basal activity could lead to continuous activation of LC neurons by excitatory transmitters. Future studies are needed to determine the conditions under which both transmitters are coreleased.

The present study also indicates that dynorphin and glutamate are cotransmitters in LC axon terminals. Previous light microscopy studies have shown that dynorphin peptides are localized in the LC (Zamir et al., 1983) and in situ hybridization studies have successfully demonstrated the presence of kappa opioid receptors in this region (Mansour et al., 1994). Electrophysiological studies have shown that kappa opioid receptor agonists produce a depression of excitatory postsynaptic potentials evoked by electrical stimulation of afferent inputs to the LC (McFadzean et al., 1987; Pinnock, 1992). Dynorphin release may affect LC discharge via presynaptic modulation of kappa receptors that are localized to excitatory amino acid-containing axon terminals. This could be an anatomical substrate underlying depression of excitatory postsynaptic potentials evoked by electrical stimulation of afferent inputs to the LC.

Functional Implications

The LC-norepinephrine system is activated by a variety of stressors and recent studies indicate that opioid peptides are an important modulator of this system following stress termination (Curtis et al., 2001). The present ultrastructural study extends our knowledge of afferent regulation of noradrenergic neurons by demonstrating that glutamate and opioid peptides are cotransmitters in this region. Future studies are required to address under which conditions corelease occurs.

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