GABAergic and Non-GABAergic Spiking Interneurons of Local and Intersegmental Groups in the Crayfish Terminal Abdominal Ganglion

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ABSTRACT
In the first step toward identifying the neurotransmitter released from spiking interneurons of both local and intersegmental groups in the crayfish terminal abdominal ganglion, the authors examined whether spiking local interneurons and ascending intersegmental interneurons contain the transmitter γ-aminobutyric acid (GABA). In this paper, 17 identified ascending interneurons and three spiking local interneurons were stained by intracellular injection of Lucifer yellow and subsequently treated for immunocytochemical staining against GABA. Double-labeling experiments revealed that six identified ascending interneurons are GABAergic, but no spiking local interneurons show GABA-like immunoreactivity. Four ascending interneurons with GABA-like immunoreactivity (reciprocal closing ascending neuron 5 [RC-5], reciprocal opening ascending neuron 6 [RO-6], variable-effect ascending interneuron 1 [VE-1], and no-effect ascending interneuron 4 [NE-4]) had cell bodies that formed a cluster on the ventral surface of the rostral edge of the ganglion, whereas two GABAergic interneurons (coinhibiting ascending interneuron 2 [CI-2] and NE-2) had cell bodies in a caudal region around the cell body of the seventh flexor inhibitor (FI) motor neuron. Another four rostral interneurons (RC-2, RC-3, RC-4, and NE-3) and seven caudal interneurons (CI-3, RC-7, RO-1, RO-2, RO-3, RO-4, and NE-1) had no GABA-like immunoreactivity. Because VE-1 is known to make direct inhibitory connections with other ascending interneurons, whereas RC-3 and RO-1 are known to make direct excitatory connections, the immunocytochemical results from this study are consistent with previous physiological studies. Although many spiking local interneurons (including spiking local interneuron 1 of the anterior group [sp-ant1]) made direct inhibitory connections with nonspiking local interneurons, three spiking local interneurons (sp-ant1, spiking local interneuron 6 of the medial group [sp-med6], and spiking interneuron 5 of the posterior group [sp-post]) do not show GABA-like immunoreactivity. These results suggest that the inhibitory transmitter released from spiking local interneurons is not GABA but that another substance mediates the inhibitory action of these interneurons. J. Comp. Neurol. 410:677–688.

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Arthropods offer many advantages for neuroethological studies, because many neurons in the central nervous system are identifiable as individuals, and we readily can analyze the neural basis of animal behavior at the single-cell level. To identify the neural elements, to characterize their functional properties, and to reveal their patterns of connection, it is essential to clarify the neural circuitry responsible for particular behavioral acts. Furthermore, knowledge of the transmitters released by presynaptic neurons and the properties of receptors of postsynaptic
neurons is important to gain a better understanding of the synaptic organization of a neural circuit.

The crayfish uropod motor control system has been well analyzed physiologically, morphologically, and pharmacologically. Mechanical stimulation of the tailfan of the crayfish elicits an avoidance behavior such that the animal rapidly closes the uropods on both sides and walks forward, away from the stimulus source (Nagayama et al., 1986). A great deal is known about the neural circuitry that closes the uropods (Nagayama et al., 1994). The terminal (sixth) abdominal ganglion is the neural center for performing uropod movements, but uropod motor neurons do not receive sensory inputs directly from afferents innervating hairs on the surface of the tailfan. Spiking and nonspiking local interneurons and ascending intersegmental interneurons all are intercalated in the circuitry and receive direct inputs from sensory afferents and transform this information into a uropod motor pattern (Nagayama and Sato, 1993; Nagayama, 1997). The predominant premotor elements are unilateral nonspiking local interneurons, which are divided into two major groups of anterolateral (AL) and postero-lateral (PL) types (Nagayama and Hisada, 1987) and form opposing and parallel pathways to the uropod motor neurons (Nagayama et al., 1984; Namba et al., 1994). Twenty spiking local interneurons have been identified so far (Nagayama et al., 1993b), and they act as signal inverters. They receive direct excitatory inputs from afferents and make direct inhibitory connections with nonspiking local interneurons (Nagayama, 1997). Thirty ascending interneurons also have been identified (Nagayama et al., 1993a) that act as signal followers to make direct excitatory outputs with nonspiking interneurons (Nagayama, 1997). Furthermore, they send axons to anterior ganglia and act as intersegmental coordinators with output effect on abdominal postural motor neurons (Aonuma et al., 1994).

The neurotransmitter released by sensory afferents is acetylcholine, and many interneurons have vertebrate nicotine-like receptors (Ushizawa et al., 1996). Many premotor, nonspiking local interneurons and the sensory bilateral nonspiking interneurons (LDS) are γ-aminobutyric acid (GABAergic) and make inhibitory connections with uropod motor neurons in the case of unilateral interneurons (Nagayama et al., 1997) and with ascending interneurons in the case of LDS (Nagayama et al., 1996). L-glutamate is the excitatory transmitter of some motor neurons (Aonuma et al., 1997). However, we have no information about the transmitters released from spiking interneurons of local and intersegmental groups. Because spiking local interneurons may make direct inhibitory connections with nonspiking interneurons (Nagayama, 1997) and some ascending interneurons also have a direct inhibitory output onto other ascending interneurons (Nagayama and Sato, 1993), we have examined whether these spiking interneurons contain the inhibitory transmitter GABA. The identification of transmitters released from these interneurons is important for a further understanding of neural organization underlying animal behavior. The GABAergic nature of spiking interneurons was examined by using double labeling with the intracellular stain with Lucifer yellow and immunocytochemical staining for GABA. The results show that six ascending interneurons are identified as GABAergic neurons, but no spiking local interneurons show GABA-like immunoreactivity.

### MATERIALS AND METHODS

Adult crayfish, Procambarus clarkii Girard of both genders (7–9 cm in length from rostrum to telson) were used in all experiments. They were obtained from a commercial supplier, maintained in laboratory tanks with running, fresh water, and fed weekly on a diet of chopped potato and liver.

The abdomen was isolated from the rest of the body and pinned ventral side up in cooled physiological solution (van Harreveld, 1936). The terminal (sixth) abdominal ganglion was exposed by removing the fifth sternite and peeling off the ventral aorta and connective tissue. The soft cuticle overlying uropod muscles was removed along with the appropriate portions of the protopodite and the exopodite. The spiny activity of the closer, reducer motor neurons was recorded extracellularly by using a suction electrode on the second nerve root just distal to the bifurcation to the reductor and adductor exopodite muscles (Nagayama et al., 1983). The terminal abdominal ganglion was stabilized on a silver platform and treated with protease (Sigma type XIV, Sigma, St. Louis, MO) for about 30 seconds. Intracellular recording was carried out from the left half of the terminal abdominal ganglion neuropil with glass microelectrodes filled with a 3% solution of Lucifer yellow CH in 0.1 M lithium chloride (electrode resistance ranged between 100 MΩ and 200 MΩ).

After physiological examination, each interneuron was stained by iontophoretic injection of Lucifer yellow by using 3–7 nA hyperpolarizing current pulses of 500 msec duration at 1 Hz for 15–30 minutes. The ventral ganglionic sheath of the terminal ganglion was then surgically removed, and the gross morphology of the stained neuron was confirmed by in situ observation using brief blue-violet illumination from a high-pressure mercury lamp (Aonuma et al., 1996). The nerve chain, including the last
two or three (fifth and sixth or fourth to sixth) abdominal ganglia, was isolated and fixed for 20 minutes in a primary fixative with 4% paraformaldehyde and 0.1% glutaraldehyde in Dulbecco's phosphate-buffered saline (DPBS; Sigma), pH 7.4–7.5. The tissue was then immersed in secondary fixative with 0.2% picric acid and 2% formaldehyde in DPBS at 4°C for 60 minutes. The tissue was washed for 30 minutes in 0.1 M glycine in DPBS, dehydrated to 70% ethanol (EtOH), and stored at 4°C overnight. The tissue was then dehydrated through an alcohol series to 90% EtOH for 1 hour, then rehydrated to DPBS, and preincubated in several changes of wash buffer (0.3% Triton X-100 [Sigma] and 5% goat serum [Chemicon, Temecula, CA]) in DPBS for 6 hours. The primary antibody, rabbit anti-GABA (Sigma), at a dilution of 1:750, was preincubated with dried crayfish nerve and muscle powder (Mulloney and Hall, 1990) for 6 hours at 4°C in wash buffer (0.3% Triton X-100 in DPBS). The solution was centrifuged for 10 minutes, and the supernatant was collected and used. The tissue was incubated in the primary antibody solution for about 90 hours at 4°C on a rotator and then washed in several changes of wash buffer for 6 hours. Anti-rabbit immunoglobulin G (IgG) indocarbocyanine (Cy-3; Chemicon) at a dilution of 1:50 was used as the secondary antibody. The tissue was incubated in the secondary antibody solution for about 40 hours at 4°C on a rotator, then washed in several changes of wash buffer for 5 hours. The tissue was then dehydrated in an alcohol series and cleared in methyl salicylate.

Fluorescence was detected by using Olympus fluorescence microscopes (Olympus, Tokyo, Japan). The light employed for excitation was passed through 450–490 nm (for Lucifer yellow) and 510–560 nm (for Cy-3) bandpass excitation filters, and the resulting fluorescence passed through LP 520 (for Lucifer yellow) and LP 590 (for Cy-3) barrier filters attached to the fluorescence microscope. The fluorescent images were recorded in the same focal plane as negative images by using a cooled CCD video camera (Imagepoint; Photometrics, Hamamatu, Japan) to store on an IBM-compatible computer as files of TIF format with a parallel interface for later image analysis. Thirty ascending intersegmental and 20 spiking local interneurons have

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**Fig. 1.** γ-Aminobutyric acid (GABA)-like immunoreactivity of variable-effect ascending interneuron 1 (VE-1). A: Gross morphology of VE-1 viewed from the ventral side. Anterior is at the top. An arrow indicates the most posterior secondary neurite of VE-1 that crosses the midline. B: Cell body of the interneuron labeled by intracellular injection with Lucifer yellow. C: Distribution of cell bodies with GABA-like immunoreactivity in the same focal plane shown in B. The cell body of VE-1 shown in B is indicated by an arrowhead in C. Scale bars = 100 µm in A; 50 µm in B,C.
been identified so far both physiologically and morphologi-
cally (Nagayama et al., 1993a,b, 1994). The interneurons
in this study, therefore, were identified according to crite-
rion described in those previous reports. Data are based on
74 double-labeling experiments of intracellular stains and
immunocytochemical staining against GABA from 17 as-
cending and three spiking local interneurons. Other types
of interneurons that were encountered only once are not
described in this study.

RESULTS

Ascending intersegmental interneurons

Ascending intersegmental interneurons are spiking in-
terneurons that have cell bodies in the ventral cortex of the
terminal abdominal ganglion and send axons to anterior
ganglia. Based on the position of cell bodies, they are
classified structurally into rostral, medial, and caudal
groups (Sigvardt et al., 1982). They are further classified
physiologically into six groups [coactivating (CA), cohibit-
ing (CI), reciprocal closing (RC), reciprocal opening (RO),
variable-effect (VE), and no-effect (NE) groups] based on
their output effects on the uropod motor neurons
(Nagayama et al., 1993a, 1994).

Rostral cell body group. Eight ascending interneu-
rons that have rostral cell bodies (RC-2, RC-3, RC-4, RC-5,
RO-6, VE-1, NE-3, and NE-4) were identified by intracellu-
lar injection of Lucifer yellow and subsequently treated with
immunocytochemical staining against GABA. Four of them
(RC-5, RO-6, VE-1, and NE-4) showed GABA-like immuno-
reactivity (Table 1). Figure 1A shows an example of the
gross morphology of an ascending interneuron, VE-1. The
cell body, which has a diameter of ≈40 µm, is located in the
most rostral portion of the terminal ganglion (Fig. 1A,B).
The primary neurite runs from the cell body posteromedial-
ly, turns medially, and crosses the midline to give rise to
an axon anteriorly. The main secondary neurites project
posterolaterally, and the most posterior secondary neurite
crosses the midline (Fig. 1A, arrow). In four preparations,
VE-1 was identified by intracellular injection of Lucifer
yellow and subsequently treated with immunocytochemi-
cal staining against GABA. In all preparations, the cell
body of VE-1 showed GABA-like immunoreactivity. For
example, the cell body of VE-1 of one crayfish (Fig. 1B) was

![Fig. 2. GABA-like immunoreactivity of no-effect ascending inter-
neuron 4 (NE-4). A: Gross morphology of NE-4 viewed from the ventral
side. Anterior is at the top. B: Cell body of NE-4 labeled by intracellu-
lar injection with Lucifer yellow. C: Distribution of cell bodies with
GABA-like immunoreactivity in the same focal plane shown in B. The
cell body of NE-4 shown in B is indicated by an arrowhead in C. Scale
bars = 100 µm in A; 50 µm in B,C.](image)
stained by an antibody against GABA (Fig. 1C, arrowhead). From three to five GABA-like-immunoreactive cell bodies also were located around the cell body of VE-1. The array and position of cell bodies were slightly variable, but GABA-like-immunoreactive cell bodies always were packed together along the anterior ganglionic edge (Fig. 1C).

Ascending interneuron NE-4 has a cell body with a diameter of \(<50 \mu m\) and thick, posteriorly and laterally projecting secondary neurites (Fig. 2A). The cell body of NE-4 is located in the most rostral portion of the ganglion close to the cell body of VE-1 (cf. Fig. 1A). Several GABA-like-immunoreactive cell bodies form a cluster in the most anterolateral edge of the ganglion (Fig. 2C), and the cell body of NE-4 is found in this cluster of cell bodies (cf. Fig. 2B and Fig. 2C, arrowhead). Identified ascending interneurons, RC-5 and RO-6, also have GABA-like-immunoreactive cell bodies in this rostral portion of the ganglion in or around the cluster of GABA-like-immunoreactive cell bodies (see Fig. 7).

Each of the ascending interneurons, RC-2, RC-3, RC-4, and NE-3, was examined three or more times. None of these interneurons, however, showed significant GABA-like immunoreactivity. For example, Figure 3A shows the gross morphology of RC-3. The cell body, which has a diameter of 40–50 \(\mu m\), is located in the rostral portion of the ganglion slightly medial from the anterior edge (Fig. 3B), and the primary neurite exits from the cell body laterally then turns medially to cross the midline. Two main secondary neurites project posteriorly and laterally to give rise numerous fine branches. Although about ten GABA-like-immunoreactive cell bodies are labeled around the cell body of RC-3 (Fig. 3C), the cell body of RC-3 does not show GABA-like immunoreactivity (Fig. 3D). The remaining three interneurons, RC-2, RC-4, and NE-3, also have cell bodies around the cluster of rostral GABA-like-immunoreactive cell bodies, but none of them show GABA-like immunoreactivity.
Medial cell body group. In only one preparation, RC-6, with a medial cell body, was identified by intracellular injection of Lucifer yellow and subsequent treatment with immunocytochemical staining against GABA. The cell body of RC-6 does not show GABA-like immunoreactivity (not shown).

Caudal cell body group. Nine ascending interneurons of the caudal cell body group (CI-2, CI-3, RC-7, RO-1, RO-2, RO-3, RO-4, NE-1, and NE-2) were identified by intracellular injection of Lucifer yellow and subsequently treated for immunocytochemical staining against GABA. Only two out of nine interneurons, CI-2 and NE-2, showed GABA-like immunoreactivity (Table 1). For example, Figure 4A shows the gross morphology of NE-2. The cell body of NE-2 is located in the caudal region of the ganglion at the level that nerve root 4 enters the ganglion. The primary neurite runs anteriorly to give off two main neurites on the soma side and crosses the midline to give rise to another secondary neurite laterally on the axon side. The cell body, which has a diameter of ≈40 µm (Fig. 4B), is labeled by the antibody against GABA (Fig. 4C, arrowhead). The cell body of NE-2 is located just above the cell body of seventh flexor inhibitor motor neuron (FI-7; Fig. 4C, asterisk). Two or three GABA-immunoreactive cell bodies also are observed around the cell body of FI-7. Interneuron CI-2 was examined in nine preparations. Five of these were strongly labeled by the GABA antibody, whereas three of them showed very weak immunoreactivity, and the remaining one showed no reactivity. GABA-like immunoreactive cell bodies of CI-2 were located just beside the cell body of FI-7 (not shown).

The remaining seven caudal interneurons showed no GABA-like immunoreactivity. For example, Figure 5A shows the gross morphology of RC-7. One of its characteristic features is its secondary neurites, which mainly project laterally. Several cell bodies with smaller diameters located around the midline show GABA-like immunoreactivity (Fig. 5B). The cell body of NE-2, which is located in the more lateral region of the ganglion at the level that nerve root 4 enters the ganglion (Fig. 5C), is not labeled by the GABA antibody (cf. Fig. 5B). Interneuron NE-1 was identified by its large cell body located in the caudal region of the ganglion and its thick and short secondary neurites and anteriorly projecting thick axon (Fig. 6A). The cell body, which has a diameter of ≈80 µm (Fig. 6B), is located beside the cell body of FI-7 (Fig. 6C, asterisk) but does not show GABA-like immunoreactivity (Fig. 6D). Figure 7 summarizes the position of the cell bodies and axons of both GABA-like-immunoreactive and nonimmunoreactive ascending interneurons. Interneurons with large-diameter cell bodies (Fig. 7A) and with large-diameter axons (Fig. 7B) do not show GABA-like immunoreactivity.
Spiking local interneurons

Spiking local interneurons have no axon-like process, and they are wholly confined within the terminal abdominal ganglion. Twenty spiking local interneurons have been identified so far both physiologically and morphologically, and they are divided into anterior, medial, and posterior groups based on the position of cell bodies (Nagayama et al., 1993b, 1994). All interneurons have small-diameter cell bodies (15–30 µm) and fine arborization in both the ventral and dorsal neuropil. The main branches of both the anterior (sp-ant) and the posterior (sp-pos) spiking local interneurons usually extend on the side either contralateral or ipsilateral to the cell body (see, e.g., Fig. 8A), whereas interneurons of the medial group (sp-med) have profuse bilateral branches (see, e.g., Fig. 9A). In this paper, three spiking local interneurons (sp-ant1, sp-med6, and sp-pos5) were identified at least twice by intracellular injection with Lucifer yellow and subsequently treated for immunocytochemical staining against GABA immunoreactivity. However, no interneurons showed GABA-like immunoreactivity (Table 1). For example, sp-ant1 has a cell body in an anteromedial portion of the ganglion and main branches projecting posteriorly and laterally on the side contralateral to the cell body (Fig. 8A). Although Nagayama (1997) showed that sp-ant1 made direct inhibitory connection with premotor nonspiking local interneurons, the cell body (~25 µm) of sp-ant1 (Fig. 8B) does not show GABA-like immunoreactivity (Fig. 8C). No GABAergic cell bodies were found around the cell body of sp-ant1, though many cell bodies showed GABA-like immunoreactivity in a more posterior area (Fig. 8D). Figure 9 shows another example of double labeling of a spiking local interneuron. The bilateral spiking local interneuron, sp-med6, has its cell body in the medial portion of the ganglion near the midline and asymmetric profusing branches on both sides (Fig. 9A). The Lucifer yellow-filled cell body of sp-med6 is not labeled by the GABA antibody (Fig. 9B–D). Three spiking local interneurons, sp-ant2, sp-ant4, and sp-med4, were identified once each by double-labeling experiments, and none of them had GABA-like-immunoreactive cell bodies (not shown).

DISCUSSION

Many ascending and spiking local interneurons in the crayfish terminal abdominal ganglion have been identified (Nagayama et al., 1993a,b), and their output profiles in the local circuitry for uropod motor control have been characterized physiologically (Nagayama and Sato, 1993; Nagayama, 1997). This study demonstrates that six identi-
fied ascending interneurons are GABAergic, but no spiking local interneurons show GABA-like immunoreactivity by using a combination of intracellular staining and immunocytochemical staining for GABA. Thus, this study provides neurochemical proof that inhibitory transmitters are released from these interneurons.

**Distribution of GABAergic ascending interneurons**

The cell bodies of four GABAergic ascending interneurons (RC-5, RO-6, VE-1, and NE-4) are located in the anterolateral area of the terminal abdominal ganglion occupied by five to ten GABA-like-immunoreactive cell bodies that form a cluster along the rostral edge of the ganglion. Two caudal interneurons (CI-2 and NE-2) also have GABA-like-immunoreactive cell bodies that are located around the cell body of the seventh flexor inhibitor motor neuron (FI-7). With the exception of VE-1, in which the axon ran through the most ventral area of the connective, the axonal projections of GABAergic ascending interneurons were limited to a medial area of the abdominal connective, mainly in area 79 and at the boundary between areas 82 and 83, as divided by Wiersma and Hughes (1961). No GABAergic ascending interneurons ran to the ventrolateral area (the boundary between areas 84 and 85 of Wiersma and Hughes, 1961) of the connective (Fig. 7B). Kirk and Govind (1990) have shown axonal projections of intersegmental interneurons with GABA-like immunoreactivity in the abdominal connective. Although a cluster of GABAergic axons also is found in the lateral edge of the connective, its immunoreactive axonal profiles are consistent with the results presented here. Because no ascending interneurons send their axons into the lateral area of the connective (area 81 in Wiersma and Hughes, 1961), immunoreactive axonal profiles in a lateral area could be descending interneurons that originate from more anterior ganglia.

Nagayama and Sato (1993) have indicated that VE-1 make direct inhibitory connections with other ascending interneurons. Spikes of VE-1, for example, were followed by inhibitory postsynaptic potentials of a short latency (≤1 msec) in NE-3. Furthermore, RC-3, RO-1, and RO-6 make direct excitatory connections with either ascending or nonspiking interneurons (Nagayama and Sato, 1993; Nagayama, 1997). Because GABA is known as an inhibitory transmitter, and pressure injection of GABA into the neuropil causes hyperpolarizing responses in many interneurons (Miyata et al., 1997), the results of this study (that VE-1 showed GABA-like immunoreactivity whereas RC-3 and RO-1 did not) are consistent with previous physiological findings. Interneuron RO-6 showed GABA-like immunoreactivity in this study, but previous physiological experiments have indicated an excitatory output (Nagayama and Sato, 1993). One possible reason for this contradiction...
would be that RO-5 was mistaken for RO-6, because the basic structure of both interneurons is similar, with wide-ranging, bilateral arborizations (Nagayama et al., 1993a), and insufficient staining of an interneuron may have led to a false identification in our previous study (Nagayama and Sato, 1993). Therefore, a physiological reexamination of the output connections of RO-6 is need to clarify this point.

Ascending interneurons make exclusively excitatory connections with nonspiking interneurons (Nagayama, 1997), whereas they make either excitatory or inhibitory connections with other ascending interneurons (Nagayama and Sato, 1993). Because many ascending interneurons are not GABAergic, although parts of them are GABAergic, there would be two functional types of ascending interneurons. Only non-GABAergic ascending interneurons make synapses directly with nonspiking interneurons to enhance the premotor activity of nonspiking interneurons. What is the role of GABAergic ascending interneurons in the local circuitry of the uropod motor control system? Many ascending interneurons receive inhibitory mechanosensory inputs indirectly from the contralateral side to their main branches (Nagayama et al., 1993a; Miyata et al., 1997). Furthermore, some interneurons receive inhibitory proprioceptive inputs indirectly from the exopodite and endopodite chordotonal afferents (Aonuma et al., manuscript in preparation). Chordotonal afferents also evoke primary afferent depolarization in the mechanosensory afferents from receptors on the uropods (Newland et al., 1996).

Because these inhibitory synaptic transmission is mediated, at least in part, by GABA, these GABAergic interneurons could participate in these inhibitory pathways.

**What is the inhibitory transmitter of spiking local interneurons?**

Spiking local interneurons, especially those in the anterior and posterior cell body (sp-ant and sp-pos) groups, act as signal inverters in the local circuit for uropod motor control system by receiving direct excitatory inputs from hair afferents and making direct inhibitory output connections with nonspiking interneurons (Nagayama, 1997). However, no spiking local interneurons, including sp-ant1, that make direct inhibitory connections with nonspiking interneurons showed GABA-like immunoreactivity (Table 1). This result is in contrast with the spiking local neurons of the locust metathoracic ganglion. A midline group of spiking local interneurons are known to be GABAergic (Watson and Burrows, 1987) and have a function similar to that of crayfish spiking local interneurons, acting as signal inverters by receiving exteroceptive and proprioceptive inputs from the hind leg afferents and by making inhibitory output connections with various classes of central neurons in the metathoracic ganglion (Burrows, 1992). Double-labeling experiments in this paper indicate that the inhibitory transmitter released from crayfish spiking local interneurons is not GABA. Besides GABA, L-glutamate and histamine also are known to act as inhibi-
tory transmitters in the crayfish central nervous system (El Manira and Clarac, 1994; Pearlstein et al., 1994, 1997; Sherff and Mulloney, 1996). Further pharmacological and immunocytochemical analyses, therefore, are indispensable to identify the inhibitory transmitter of crayfish spiking local interneurons.

**GABAergic neurons in the crayfish terminal ganglion**

In the crayfish terminal abdominal ganglion, about 10% of the total number of central neurons are thought to be GABAergic (Mulloney and Hall, 1990; Nagayama et al., 1996). Because the terminal ganglion contains about 650 cell bodies (Reichert et al., 1982; Kondoh and Hisada, 1986), less than 35 pairs of central neurons are estimated to be GABAergic neurons. Three inhibitory motor neurons have been identified: Two are flexor inhibitor motor neurons (Otsuka et al., 1967), and the other is a uropod common inhibitory motor neuron (Nagayama, 1999). Two further unidentified motor neurons have axons with GABA-like immunoreactivity that enter nerve root 3 (Mulloney and Hall, 1990). Nonspiking local interneurons are divided into bilateral and unilateral groups. Bilateral nonspiking local interneuron LDS is a GABAergic neuron (Nagayama et al., 1996). Unilateral nonspiking interneurons are divided further into PL and AL types. From 6 to 11 GABA-like-immunoreactive cell bodies of PL interneurons of three identified sets pack together, forming a cluster at the posterolateral edge of the terminal ganglion, and at least seven AL interneurons also are GABAergic (Nagayama et al., 1997). If the remaining GABAergic neurons are ascending interneurons, then they number about ten. Therefore, the results of this study, showing that only 7 of 21 identified ascending interneurons had GABA-like immunoreactivity, are consistent with the previous estimate.

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**LITERATURE CITED**


