Distribution of GABAergic and Glycinergic Premotor Neurons Projecting to the Facial and Hypoglossal Nuclei in the Rat

YUN-QING LI, MASAHIKO TAKADA, TAKESHI KANEKO, AND NOBORU MIZUNO*
Department of Morphological Brain Science, Faculty of Medicine, Kyoto University, Kyoto 606-01, Japan

ABSTRACT
The distribution of inhibitory premotor neurons for the facial and hypoglossal nuclei was examined in the lower brainstem of the rat. A retrograde axonal tracing method with the fluorescent tracer, tetramethylrhodamine dextran amine (TMR-DA), was combined with immunofluorescence histochemistry for glutamic acid decarboxylase (GAD), i.e., the enzyme involved in gamma-aminobutyric acid synthesis, or glycine. In the rats injected with TMR-DA unilaterally into the facial or hypoglossal nucleus, the distribution of TMR-DA-labeled neurons showing GAD-like immunoreactivity (GAD/TMR-DA neurons) was essentially the same as that of TMR-DA-labeled neurons displaying glycine-like immunoreactivity (Gly/TMR-DA neurons). The distributions of GAD/TMR-DA and Gly/TMR-DA neurons in the rats injected with TMR-DA into the facial nucleus were also similar to those in the rats injected with TMR-DA into the hypoglossal nucleus. These neurons were seen most frequently in the lateral aspect of the pontine reticular formation, the supratrigeminal region, the dorsal aspect of the lateral reticular formation of the medulla oblongata, and the reticular regions around the raphe magnus nucleus and the gigantocellular reticular nucleus pars alpha, bilaterally with a slight dominance on the side ipsilateral to the injection site. A number of GAD/TMR-DA and Gly/TMR-DA neurons were also seen in the oral and interpolar subnuclei of the spinal trigeminal nucleus, bilaterally with a slight ipsilateral dominance. In the rats injected with TMR-DA into the facial nucleus, GAD/TMR-DA and Gly/TMR-DA neurons were also seen in the oral and interpolar subnuclei of the spinal trigeminal nucleus, bilaterally with a slight ipsilateral dominance. In the rats injected with TMR-DA into the facial nuclei, GAD/TMR-DA and Gly/TMR-DA neurons were also seen in the oral and interpolar subnuclei of the spinal trigeminal nucleus, bilaterally with a slight ipsilateral dominance. A large part of these inhibitory premotor neurons for the facial and hypoglossal nuclei and the excitatory ones may constitute premotor neuron pools common to the orofacial motor nuclei implicated in the control of integrated orofacial movements. J. Comp. Neurol. 378:283–294, 1997.

Indexing terms: face; tongue; reticular formation; brainstem; immunohistochemistry

The distribution of premotor neurons, which send their axons to the facial and hypoglossal nuclei, have been examined by retrograde axonal tracing in the rat (Borke et al., 1983; Hinrichsen and Watson, 1983; Travers and Norgren, 1983; Grzanna et al., 1987; Isokawa-Akesson and Komisaruk, 1987; Alves, 1990; Alves et al., 1992; Manaker et al., 1992; Manaker and Tischler, 1993; Li et al., 1993a; Mogoseanu et al., 1994; Dobbins and Feldman, 1995; Ugolini, 1995; Yang et al., 1995) and in the cat (Tanaka et al., 1978; Takeuchi et al., 1979; Itoh et al., 1983; Takada et al., 1984a,b; Fort et al., 1989; May et al., 1990; Ono et al., 1994). Both excitatory and inhibitory neurons must be contained in these premotor neurons projecting to the facial and hypoglossal nuclei. In fact, many GABAergic and glycineergic afferent fibers are distributed within the facial and hypoglossal nuclei (Mugnaini and Oertel, 1985; Alves et al., 1988; Fort et al., 1990). The distribution of inhibitory premotor neurons projecting to the facial and hypoglossal nuclei, however, have not been investigated

Contract grant sponsor: Ministry of Education, Science, Sports, and Culture of Japan; Contract grant numbers 0827906, 08458245.
Dr. Yun-Qing Li is on leave from the Department of Anatomy, The Fourth Military Medical University, Xi'an, People's Republic of China.
*Correspondence to: Dr. Noboru Mizuno, Department of Morphological Brain Science, Faculty of Medicine, Kyoto University, Kyoto 606-01, Japan.
E-mail: mizuno@mbs.med.kyoto-u.ac.jp
Received 3 June 1996; Revised 19 August 1996; Accepted 25 September 1996

© 1997 WILEY-LISS, INC.
systematically. In our previous study (Li et al., 1996), the distributions of gamma-aminobutyric acid (GABA)-ergic and glycergic premotor neurons projecting to the trigeminal motor nucleus were examined in the rat by a double-labeling method that combined retrograde axonal tracing with immunofluorescence histochemistry. Thus, the present study was attempted primarily to examine the distribution of GABAergic and glycergic premotor neurons projecting to the facial and hypoglossal nuclei in the rat. A retrograde axonal tracing method with the fluorescent tracer, tetramethylrhodamine dextran amine (TMR-DA), was combined with immunofluorescence histochemistry for glutamic acid decarboxylase (GAD), i.e., the enzyme involved in GABA synthesis, or glycine. Transplanted TMR-DA was immunoreacted with a guinea pig anti-TMR-DA IgG (Kaneko et al., 1996). GAD or glycine was immunoreacted with a sheep anti-GAD serum or a rabbit anti-glycine serum, respectively; the fluorescence dye used for visualization of GAD or glycine was fluorescein isothiocyanate (FITC) or dichlorotriazinyl aminofluorescein (DTAF), respectively.

MATERIALS AND METHODS

Adult male Wistar rats (Oriental BioService, Kyoto, Japan) weighing 300–400 g were used in the present study. In 18 rats anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight), single injections of TMR-DA (MW 3,000, D-3308; Molecular Probes, Eugen, OR) were made stereotaxically into the facial or hypoglossal nuclei for retrograde axonal tracing (see Kaneko et al., 1996). An approximate volume of 0.05–0.1 µl of a 10% solution of TMR-DA dissolved in 0.1 M phosphate buffer (PB; pH 7.4) was injected by pressure through a glass micropipette (internal tip diameter = 50–60 µm) attached to a 1-µl Hamilton microsyringe. Each injection was made slowly over 20 minutes, and then the glass micropipette used for the TMR-DA injection was kept in place for additional 20 minutes. After 3 days, the rats were again anesthetized and then injected stereotaxically with a volume of 10 µl of 1% colchicine (Sigma, St. Louis, MO) dissolved in 0.9% saline into the lateral ventricle. Subsequently, the rats were allowed to survive for 36–48 hours, then anesthetized deeply with an overdose of sodium pentobarbital, and perfused transcardially with a volume of 100 ml of 0.9% saline, followed by a volume of 500 ml of 0.1 M PB (pH 7.4) containing 4% paraformaldehyde, 0.25% glutaraldehyde, and 0.2% picric acid. After the perfusion, the brains were removed immediately, placed in 0.1 M PB (pH 7.4) containing 4% paraformaldehyde and 0.2% picric acid for 4–6 hours at 4°C and then saturated with 30% sucrose in 0.1 M PB (pH 7.4) at 4°C. Subsequently, the brainstems were cut serially into frontal sections 40-µm thick on a freezing microtome. The sections were collected serially into three dishes containing 0.01 M phosphate buffered saline (PBS; pH 7.3); each dish contained every third section.

The sections in the first and the second dishes were rinsed in 0.01 M PBS (pH 7.3) and then processed for GAD and glycine immunofluorescence histochemistry, respectively. The incubation medium used for dilution of the primary and the secondary antibodies was 0.1 M phosphate-buffered saline (PBS) containing 2% normal donkey serum (NDS), 0.5% Triton X-100, 0.05% sodium azide (NaN₃), and 0.25% Triton X-100 (pH 7.4) containing 4% paraformaldehyde and 0.2% picric acid. The brains were removed immediately, placed in 0.1 M PB (pH 7.3) containing 4% paraformaldehyde and 0.2% picric acid for 4–6 hours at 4°C and then saturated with 30% sucrose in 0.1 M PB (pH 7.4) at 4°C. Subsequently, the brainstems were cut serially into frontal sections 40-µm thick on a freezing microtome. The sections were collected serially into three dishes containing 0.01 M phosphate buffered saline (PBS; pH 7.3); each dish contained every third section.

After immunofluorescence staining, the sections were mounted onto clean glass slides, air dried at room tempera-
ture, and overlapped with PBS containing 50% glycerol and 2.5% triethylenediamine. Neuronal cell bodies labeled retrogradely with TMR-DA, GAD-like immunoreactive cell bodies labeled with FITC, and glycine-like immunoreactive ones labeled with DTAF were observed with an epifluorescence microscope (Axioskop; Zeiss, Oberkochen, Germany) under appropriate filters for TMR-DA (excitation = 540–552 nm; emission ≥ 590 nm) and for FITC and DTAF (excitation = 450–490 nm; emission = 515–565 nm). The sections in the first dish were mounted onto gelatin-coated glass slides and stained with 1% cresyl violet. A number of cresyl violet-stained sections through lower brainstem levels were selected, and then large projection drawings (×40) were prepared. In the sections stained by immunofluorescence histochemistry, the sites of tracer injection and the locations of neuronal cell bodies labeled with TMR-DA were identified under the epifluorescence microscope (×10) and charted on the projection drawings of the adjacent cresyl violet-stained sections as exactly as possible by referring to large blood vessels and other prominent landmarks in the sections. Neuronal cell bodies double-labeled with TMR-DA/FITC or TMR-DA/DTAF were then identified among the cell bodies single-labeled with TMR-DA at a higher magnification (×20) to record them in the projection drawings. Subsequently, the data were reconstructed onto tracings of sets of serial sections (Figs. 3, 4, 6, 8); in each rat, the locations of neuronal cell bodies double-labeled with TMR-DA/FITC and TMR-DA/DTAF were reconstructed separately from single-labeled ones in a single set of projection drawings of a series of serial sections (Figs. 6, 8). The nonmenbrane and boundaries of the structures in the lower brainstem were basically taken from the atlas of Paxinos and Watson (1986); the brainstem reticular formation was divided into the lateral and medial parts according to Holstege and Kuypers (1977).

To observe the morphological features of GAD-like and glycine-like immunoreactive neurons under brightfield illumination, immunohistochemical staining for GAD and glycine was also performed according to the ABC method (Hsu et al., 1981). Two rats underwent the colchicine treatment and transcardial perfusion. The brainstems were cut serially into frontal sections 40-μm thick on the freezing microtome and collected into three dishes containing 0.01 M PBS (pH 7.3). The sections in the first and the second dishes were processed for GAD and glycine immunohistochemistry, respectively. Briefly, for GAD immunohistochemistry, the sections were incubated overnight with NDS-PBS containing the sheep antiserum against GAD (see Oertel et al., 1981) diluted at 1:3,000 and then with biotinylated donkey anti-sheep IgG (Chemicon) diluted at 10 μg/ml in NDS-PBS for 4 hours. For glycine immunohistochemistry, the sections were incubated overnight with NDS-PBS containing the rabbit antiserum against glycine (Chemicon) diluted at 1:2,000 and then with 10 μg/ml of biotinylated goat anti-rabbit IgG (Vector) for 4 hours. These sections processed for GAD or glycine immunohistochemistry were then incubated with ABC (Vector) diluted at 1:200 in 0.01 M PBS (pH 7.3) containing 0.3% Triton X-100 for 2 hours. Subsequently, the sections were placed for 20–30 minutes in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.04% diaminobenzidine tetrahydrochloride (Dojin, Kumamoto, J papan) and 0.001% H2O2. All immunohistochemical procedures were carried out at room temperature; the sections were rinsed three times (10 minutes each) in 0.01 M PBS (pH 7.3) between each step of the immunostaining.

The sections in the third dish were mounted onto gelatin-coated glass slides and then stained with 1% cresyl violet. These sections were used to examine the anatomical sites of distribution of GAD-like and glycine-like immunoreactive cells in the adjacent sections stained immunohistochemically.

In control immunostaining for GAD, the anti-GAD serum was replaced with a preimmune sheep serum diluted at 1:1,000. In control immunostaining for glycine, the antiglycine serum was preabsorbed with an excess amount of glycine or glycine-bovine serum albumin (BSA) conjugate. The glycine–BSA conjugate was made as follows: 2 mg (30.3 nmol) of BSA and 9.1 μl (910 nmol) of 0.1 M glycine were mixed in 1 ml of 0.1 M PB (pH 7.4) and conjugated by incubation for 2 hours at room temperature after an addition of 25 μl of 5% glutaraldehyde. The conjugate was dialyzed against PBS before use. The antiglycine serum diluted at 1:1,000 in NDS-PBS was incubated with 0.1–0.5 M glycine or 0.2–1 mg BSA-equivalent/ml glycine–BSA conjugate for 2 hours at room temperature before immunostaining.

**RESULTS**

**Distribution of GAD-like and glycine-like immunoreactive neurons in the lower brainstem**

Immunohistochemical staining for GAD and glycine was done by the ABC method (Hsu et al., 1981). A considerable number of neurons with GAD-like immunoreactivity and those with glycine-like immunoreactivity were widely scattered in the lower brainstem (Fig. 1a,c), as was observed in our previous study (Li et al., 1996). Briefly, they were seen most frequently in the reticular formation of the pons and medulla oblongata, the supratrigeminal region, the paralemniscal zone, the superior olivary complex, and the prepositus hypoglossi nucleus. A number of GAD-like immunoreactive neurons were also distributed in the nuclei of the cranial nerves, such as the oculomotor nucleus, the trigeminal motor nucleus, the sensory trigeminal nuclei, the cochlear nuclei, the vestibular nuclei, and the nucleus of the solitary tract. The dorsal column nuclei also contained GAD-like immunoreactive neurons. Glycine-like immunoreactive neurons were particularly marked in the superior olivary complex, the nuclei of the trapezoid body, the cochlear nuclei, the vestibular nuclei, and the prepositus hypoglossi nucleus, although they were distributed in virtually all nuclei of the lower brainstem. The GAD-like or glycine-like immunoreactive neurons were oval, triangular, or fusiform in shape in most of them, the longest soma diameter was 8–25 μm for GAD and 8–30 μm for glycine. However, in some regions, particularly in the regions around the gigantocellular reticular nucleus pars alpha, a number of fusiform neurons with GAD-like immunoreactivity had larger soma diameters reaching up to 35 μm.

The existence of many fine axonal components showing GAD-like or glycine-like immunoreactivity was also confirmed in the facial and hypoglossal nuclei (data not shown).

In control immunostaining, when the anti-GAD serum was replaced with preimmune sheep serum diluted at 1:1,000, no immunoreactivity was found in the sections (Fig. 1b). When the antiglycine serum diluted at
1:1,000 in NDS-PBS was preincubated with 0.5 M glycine or 0.2 mg/ml glycine–BSA conjugate, no glycine-like immunoreactivity was found in the sections (Fig. 1d). Distribution of neurons labeled retrogradely with TMR-DA injected into the facial or hypoglossal nucleus

In the present study, the intense deposition area of TMR-DA at the center of the injection site and the diffusion area surrounding the center of the injection site were considered as the maximum probable sites of termination for neurons labeled retrogradely with the injected TMR-DA. The sites of TMR-DA injection were successfully centered in the facial or hypoglossal nucleus in 12 rats (Fig. 2). The injection sites in six rats of each group were not identical, and the number of TMR-DA-labeled neurons differed according to the extent of the injection site. However, the lower brainstem areas containing the TMR-DA-labeled neuronal cell bodies were virtually identical among the rats of each group. Therefore, to avoid unnecessary repetition, only the typical findings observed in a representative rat of each group will be described; in these representative rats (R6 and R10), the number of TMR-DA-labeled neurons was largest in each group.

After TMR-DA injection into the facial nucleus (Fig. 2a), many TMR-DA-labeled neuronal cell bodies were seen in the tegmental regions of the lower brainstem (Fig. 3). At midbrain levels, many labeled neurons were seen in the medial aspect of the pretectal area and the paralemniscal zone close to the dorso medial border of the medial lemniscus, bilaterally with an apparent predominance on the side contralateral to the injection site. Labeled neuronal cell bodies were also distributed in the periculomotor regions, including the nucleus of Edinger–Westphal, the nucleus of Darkschewitsch, the interstitial nucleus of Cajal, the ventral part of the periaqueductual gray, the dorsal raphe nucleus, and the oculomotor nucleus, and in the mesencephalic reticular formation, bilaterally with an ipsilateral dominance. Labeled neurons within the oculomotor nucleus were smaller than the oculomotor motoneurons. A small number of neurons in the red nucleus and the deep part of the superior colliculus also were labeled contralaterally to Fig. 1. Glutamic acid decarboxylase (GAD)-like (a) and glycine-like (c) immunoreactivities in the lateral part of the medullary reticular formation. When the anti-GAD serum was replaced with preimmune sheep serum, no immunoreactivity was seen in the lateral part of the medullary reticular formation (b). When the antglycine serum was preabsorbed with an excess amount of glycine, no glycine-like immunoreactivity was seen in the lateral part of the medullary reticular formation (d). Scale bar = 50 µm.

Fig. 2. The sites of tetramethylrhodamine dextran amine (TMR-DA) injection in the facial nucleus of a rat (R6; a) and in the hypoglossal nucleus of another rat (R10; b). Scale bars = 250 µm.
the injection site; few, if any, labeled neurons were found in these regions ipsilateral to the injection site. At pontine levels, many labeled neurons were seen in the parabrachial region, including the medial and lateral parabrachial nuclei and the nucleus of Kölliker–Fuse, the supratrigeminal region, the lateral part of the pontine reticular formation, and the ventral part of the reticular formation around the raphe magnus nucleus, bilaterally with a slight dominance on the side ipsilateral to the injection site. At levels of the medulla oblongata, labeled neuronal cell bodies were encountered most frequently in the lateral and dorsal parts of the reticular formation and the ventral parts of the reticular formation around the raphe magnus nucleus and the gigantocellular reticular nucleus pars alpha, bilaterally with a slight ipsilateral dominance. Only a few labeled neurons were distributed in the medial part of the reticular formation. A number of labeled neurons were also seen in the sensory trigeminal nuclei, especially in the oral and interpolar subnuclei of the spinal trigeminal nucleus ipsilateral to the injection site. A small number of labeled neuronal cell bodies were also distributed in the intermediate zone of the upper and middle cervical segments of the spinal cord.

After TMR-DA injection into the hypoglossal nucleus (Fig. 2b), many labeled neuronal cell bodies were seen in the pontine and medullary reticular formation, bilaterally...
with a slight dominance on the side ipsilateral to the injection site (Fig. 4). The pattern of distribution of these labeled neuronal cell bodies in the pontine and medullary reticular formation was essentially the same as that observed after TMR-DA injection into the facial nucleus. At mesencephalic levels, however, only a small number of labeled neurons were scattered in the mesencephalic reticular formation, the periaqueductal gray, and the dorsal raphe nucleus. No labeled neurons were found in the pretectal area or the red nucleus. Few, if any, labeled neurons were observed in the deep part of the superior colliculus or the paralemniscal zone. At pontine levels, many labeled neurons were seen in the parabrachial region, especially in the nucleus of Kölliker–Fuse ipsilateral to the injection site. A number of labeled neurons were seen in the sensory trigeminal nuclei and the intermediate zone of the upper and middle cervical segments of the spinal cord.

**GAD-like and glycine-like immunoreactive neurons labeled retrogradely with TMR-DA injected into the facial or hypoglossal nucleus**

In the rats injected with TMR-DA into the facial nucleus (Fig. 2a), some of TMR-DA-labeled neurons had GAD-like or glycine-like immunoreactivity (Fig. 5). Both TMR-DA-labeled neurons showing GAD-like immunoreactivity (GAD/TMR-DA neurons) and TMR-DA-labeled neurons displaying glycine-like immunoreactivity (Gly/TMR-DA neurons) were found mainly in the paralemniscal zone, the lateral part of the pontine reticular formation, the suprar-
trigeminal region, the dorsal part of the lateral reticular formation of the medulla oblongata, the ventral parts of the medullary reticular formation around the raphe magnus nucleus and the gigantocellular reticular nucleus pars alpha, and the oral and interpolar subnuclei of the spinal trigeminal nucleus (Fig. 6). In the paralemniscal zone, GAD/TMR-DA neurons were seen bilaterally with an apparent dominance on the side contralateral to the injection site. In the other regions, GAD/TMR-DA neurons were seen bilaterally with a slight dominance on the side ipsilateral to the injection site (Fig. 7). Some of TMR-DA-labeled neurons observed in the rats injected with TMR-DA into the hypoglossal nucleus (Fig. 2b) also showed GAD-like or glycine-like immunoreactivity (Fig. 7), although the numbers of these GAD/TMR-DA and Gly/TMR-DA neurons were smaller than those found in the rats injected with TMR-DA into the facial nucleus (compare Fig. 8 with Fig. 6). Both of these GAD/TMR-DA and Gly/TMR-DA neurons were found mainly in the dorsal part of the lateral reticular formation of the medulla oblongata and in the supratrigeminal region, the lateral part of the pontine reticular formation, the reticular regions around the raphe magnus nucleus and the gigantocellular reticular nucleus pars alpha, and the oral and interpolar subnuclei of the spinal trigeminal nucleus, bilaterally with a slight dominance on the side ipsilateral to the injection site (Fig. 8).

**DISCUSSION**

In the present study, the distribution of premotor neurons for the facial and hypoglossal nuclei in the lower brainstem was examined in the rat by the retrograde labeling method with TMR-DA. Fluorochrome-conjugated dextran amines, including TMR-DA, were initially introduced as fluorescent anterograde tracers in the central nervous system. However, TMR-DA can also be used as a very sensitive retrograde tracer (Kaneko et al., 1996).

The facial and hypoglossal nuclei of the rat are composed of a number of subnuclei (Krammer et al., 1979; Watson et al., 1982; Hinrichsen and Watson, 1984; Friau and Herbert, 1985; Klein and Rhoades, 1985; Semba and Egger, 1986; Uemura-Sumi et al., 1988; Tsai et al., 1993; Aldes, 1995; Dobbins and Feldman, 1995), and the locations of premotor neurons projecting to different subdivisions are more or less different from each other (Hinrichsen and Watson, 1983; Travers and Norgren, 1983; Isokawa-Akesson and Konisaruk, 1987; Dobbins and Feldman,
In the present study, however, the TMR-DA injections were attempted to cover the entire extent of the facial or hypoglossal nucleus. The present findings concerning the distribution of premotor neurons for the facial and hypoglossal nuclei were compatible with almost all data reported previously in the rat (Borke et al., 1983; Hinrichsen and Watson, 1983; Isokawa-Akesson and Komisaruk, 1987; Aldes, 1990; Ter Horst et al., 1991; Aldes et al., 1992; Manaker et al., 1992; Manaker and Tischler, 1993; Li et al., 1993a–c; Mogoseanu et al., 1994; Dobbins and Feldman, 1995; Ugolini, 1995; Yang et al., 1995) and cat (Tanaka et al., 1978; Takeuchi et al., 1979; Itoh et al., 1983; Takada et al., 1984a,b; May et al., 1990; Ono et al., 1994).

The present study has also shown that some of the neuronal cell bodies labeled retrogradely with TMR-DA injected into the facial or hypoglossal nucleus are GAD-like or glycine-like immunoreactive. The areas of the lower brainstem containing GAD-like immunoreactive TMR-DA-labeled neurons (GAD/TMR-DA neurons) greatly overlapped the areas containing glycine-like immunoreactive TMR-DA-labeled neurons (Gly/TMR-DA neurons). Thus, both GAD-like and glycine-like immunoreactivities may coexist in some inhibitory premotor neurons projecting to the facial or hypoglossal nucleus (see Todd and Sullivan, 1990; Maxwell et al., 1995; Davanger, 1996; Lahouiji et al., 1996; Moore et al., 1996; Popratillo et al., 1996; Todd et al., 1995).
1996). At least some of the GAD/TMR-DA and Gly/TMR-DA neurons observed in the present study were GABAergic and glycinergic inhibitory premotor neurons participating in synaptic actions on facial and hypoglossal motoneurons, although local interneurons within and around the facial and hypoglossal nuclei might also be postsynaptic targets for some of the GAD/TMR-DA and Gly/TMR-DA neurons.

In the rats injected with TMR-DA into the facial nucleus, a significant number of GAD/TMR-DA and Gly/TMR-DA neurons were seen in the paralemniscal zone contralateral to the injection site, suggesting that both GABAergic and glycinergic neurons in the paralemniscal zone project directly to the facial nucleus. The paralemniscal zone, a mesencephalic tegmental zone along the dorsomedial border of the medial lemniscus, was identified in the cat as a major site of origin of mesencephalic projection fibers to the facial nucleus (Henkel and Edwards, 1978). This zone receives afferent fibers from the superior colliculus (Henkel and Edwards, 1978; Stein et al., 1984; see also Graybiel, 1977) and periaqueductal gray (Henkel, 1981) and sends projection fibers to the medial part of the facial nucleus bilaterally with a clear contralateral dominance (Henkel and Edwards, 1978; Takeuchi et al., 1979). The medial part of the facial nucleus where the projection fibers from the paralemniscal zone terminate, contains motoneurons innervating the pinna muscles (Henkel and Edwards, 1978; Kume et al., 1978). Such direct projections from the paralemniscal zone to the facial nucleus have also been observed in the rat (Hinrichsen and Watson, 1983; Isokawa-Akesson and Komisgruck, 1987) and in the oppossum (Panneton and Martin, 1978, 1979, 1983). More recently, both monosynaptic excitatory and inhibitory postsynaptic potentials were recorded from motoneurons innervating the extrinsic and intrinsic pinna muscles by electrical stimulation of the contralateral paralemniscal zone in the cat (May et al., 1990). In the rat, 10.9% of GABA-like immunoreactive neurons in the paralemniscal zone were reported to be retrogradely labeled with horseradish peroxidase injected contralaterally into the medial part of the facial nucleus (Chen et al., 1995). The present study has confirmed and extended these previous observations, indicating that not only GABAergic but also glycinergic neurons in the paralemniscal zone send inhibitory projection fibers to the facial nucleus.

In the rats injected with TMR-DA into the facial nucleus, the pretectal area contralateral to the injection site also contained a cluster of TMR-DA-labeled neurons in its medial part. However, no TMR-DA-labeled neurons in the pretectal area showed either GAD-like or glycinergic immunoreactivity. It has been assumed that the pretectofacial fibers arising from the pretectal olivary nucleus in the cat innervate the orbicularis oculi muscle and constitute a link in the arc of the visually triggered blink reflex (e.g., dazzle

---

Fig. 7. Sections containing neurons labeled with TMR-DA injected into the hypoglossal nucleus (a,b) are immunostained for GAD (a') or glycine (b'). Double arrowheads in a and a' point to GAD-like immunoreactive neuron labeled with TMR-DA in the dorsolateral part of the medullary reticular formation. Double arrowheads in b and b' indicate glycine-like immunoreactive neuron labeled with TMR-DA in the dorsolateral part of the medullary reticular formation. Arrows in a and b indicate neurons single-labeled with TMR-DA. Arrowheads in a' and b' indicate TMR-DA-negative neurons showing GAD-like (a') or glycine-like (b') immunoreactivity. Asterisks in a and a' indicate the same blood vessel. Scale bar = 25 µm.
ormenacereflex;Itohetal.,1983). Thus, it is interesting to note that the paralemniscal zone sending projection fibers to the facial motoneurons that innervate the pinna muscles contains inhibitory premotor neurons for the facial nucleus, whereas the pretectal area sending projection fibers to the facial motoneurons innervating the orbicularis oculi muscle contains no inhibitory premotor neurons for the facial nucleus.

The lateral part of the pontomedullary reticular formation, especially the parvicellular reticular formation, contains many premotor neurons for the facial nucleus (Holstege and Kuypers, 1977; Holstege et al., 1977; Takeuchi et al., 1979; Paneton and Martin, 1983; Takada et al., 1984a; Travers and Norgren, 1983; Isokawa-Akesson and Komisaruk, 1987; Ter Horst et al., 1991; Li et al., 1993a–c; Mogoseanu et al., 1994). In the present study, neuronal cell bodies that were labeled retrogradely with TMR-DA injected into the facial nucleus most frequently showed GAD-like or glycine-like immunoreactivity in the lateral part of the reticular formation in the pons and medulla oblongata.

Hypoglossal motoneurons receive indirect inputs from the trigeminal, glossopharyngeal, and superior laryngeal nerves and from the brain areas, including the cerebral cortex and the brainstem regions with pattern/rhythm generators for respiration, mastication, and deglutition (for review, see Lowe, 1981; Travers and Jackson, 1992; Ono et al., 1994); the premotor neurons mediating these inputs to hypoglossal motoneurons are located mainly in the lateral part of the medullary reticular formation (Hostege and Kuypers, 1977; Holstege et al., 1977; Borke et al., 1983; Travers and Norgren, 1983; Takada et al., 1984b; Borke and Nau, 1985; Ter Holstege et al., 1991; Ono et al., 1994). Direct excitatory projections from the giganto-
cellular reticular nucleus to the hypoglossal nucleus (Yang et al., 1995) and direct serotoninergic projections from the raphe nuclei to the hypoglossal nucleus (Li et al., 1993c; Manaker and Tischler, 1993) have also been reported in the rat. In the present study, a number of GAD/TMR-DA and Gly/TMR-DA neurons were observed in these regions of the rats injected with TMR-DA into the hypoglossal nucleus.

In summary, in rats injected with TMR-DA into the facial nucleus and those injected with TMR-DA into the hypoglossal nucleus, GAD/TMR-DA and Gly/TMR-DA neurons were observed mainly in the lateral parts of the pontomedullary reticular formation, especially in the supratrigeminal region and the parvicellular reticular formation and in the medullary reticular formation around the raphe magnus nucleus and the gigantocellular reticular nucleus pars alpha; these neurons were distributed bilaterally with a slight dominance on the side ipsilateral to the TMR-DA injection. The supratrigeminal region is a premotor neuron pool for the trigeminal motor nucleus; in particular, it contains inhibitory neurons projecting to jaw-closing motoneurons (Goldberg and Nakamura, 1968; Kidokoro et al., 1968; Mizuno et al., 1978, 1983; Ohta and Moriyama, 1986; Li et al., 1996). The patterns of distribution of GAD/TMR-DA and Gly/TMR-DA neurons in the parvicellular reticular formation and the regions around the raphe magnus nucleus and the gigantocellular reticular nucleus pars alpha were also similar to those of GAD-like and glycine-like immunoreactive neurons projecting to the trigeminal motor nucleus (compare the present data with those of the previous studies; for rat: Li et al., 1996; for guinea pig: Turman and Chandler, 1994). Thus, it was assumed that GABAergic and glycineergic inhibitory neurons with excitatory neurons in these regions might constitute premotor neuron pools common to the orofacial motor nuclei.

ACKNOWLEDGMENTS

We are grateful for the photographic help of Mr. Akira Uesugi. We also express our gratitude for the support of Drs. Satoru Fukuchi, Ritsu Hayashi, Sozaburo Hayashi, Mizuho Katsurada, Yutaka Kitani, Toshikiko Kuroda, Keiko Kumagai, Hiroshi Matsubara, Hiroshi Matsushima, Chisato Minakuchi, Goempri Niiwa, Hajime Oda, Masahiko Ohbayashi, Sei-ichi Ohbayashi, Hiroyasu Ohtsuka, Shigeo Tamaki, Eizo Watanabe, Kazuo Yoshino, and Toshiaki Ohbayashi, Sei-ichi Ohbayashi, Hiroyasu Ohtsuka, Shigeo Tamaki, Eizo Watanabe, Kazuo Yoshino, and Toshiaki Ohbayashi.

LITERATURE CITED


