LOCALIZATION OF GASTRIN-RELEASING PEPTIDE (GRP)-LIKE IMMUNOREACTIVITY IN THE BRAIN OF THE JAPANESE QUAIL, COTURNIX COTURNIX

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ABSTRACT
Localization of the gastrin-releasing peptide (GRP)-like immunoreactivity in the brain of the Japanese quail pretreated with colchicine was investigated using anti-porcine GRP serum and anti-bombesin serum. In the hypothalamus, numerous neurons exhibiting GRP-immunoreactivity were detected in the suprachiasmatic nucleus. In the extrahypothalamus, GRP neurons were widely distributed in the telencephalon (hyperstriatum accessorium, hyperstriatum ventrale, neostriatum dorsolaterale, neostriatum caudale, archistriatum and lobus parolfactorius), and also in the mesencephalon (caudal part of the stratum cellulare internum, ectomamillary nucleus, formatio reticularis medialis mesencephali and substantia grisea centralis). The GRP-immunoreactive fibers were seen in the nucleus of stria terminalis of telencephalon and in the nucleus infundibularis of the hypothalamus, where they were in close contact with the unlabeled perikarya. With the anti-bombesin serum, neurons and fibers were not immunostained throughout the brain.

Gastrin-releasing peptide (GRP) is a heptacosa-peptide originally isolated from porcine gastrointestinal tract (15). Studies on immunohistochemistry and radioimmunoassay have revealed the widespread distribution of GRP-like immunoreactivity not only in the gastrointestinal tract of mammals, but also in the central nervous system (14, 22, 30). Recently, Roth et al. (22) have demonstrated using GRP₁₋₁7 antisera absorbed by substance P that GRP-neurons are distributed in the hypothalamus, especially suprachiasmatic nucleus and parvocellular portion of the paraventricular nucleus, and in the ventral regions of the medulla oblongata of the rat. However, GRP in the nerve cells in the trigeminal and spinal ganglion of the rat has been shown immunohistochemically to coexist with substance P, which shares a common carboxyterminal dipeptide amide-Leu-Met-NH₂ with porcine GRP (11).

On the other hand, GRP has the same heptapeptide sequence as bombesin in the C-terminal region. Bombesin-like immunoreactivity has been reported in various organs, including the brain, of mammals (6, 10, 26, 27). Although there have been several reports that bombesin located in the hypothalamic areas mediates functions of the adrenal medulla via the sympathetic stimulation and the release of the prolactin (PRL) and growth hormone (GH) (5, 21), the physiological role of entire GRP in the central nervous system is still unknown.

The immunohistochemical localization of porcine GRP-like substances in the forebrain of the Japanese quail has been briefly reported (17). This communication deals with the localization of GRP-like immunoreactivity in the quail brain.
in detail using both anti-GRP and anti-bombesin antisera.

MATERIALS AND METHODS

Brains from ten Japanese quails of both sexes were used in this study. All animals received intraventricular injection of colchicine (80 μg/100 g body weight) 12 h prior to sacrifice. They were sacrificed by cardiac perfusion with physiological saline, followed by Bouin’s fixative without acetic acid. Brains were removed and immersed in the same fixative for 6-8 h, and then embedded in paraffin. Serial frontal and sagittal sections were made at 4-6 μm in thickness. Sections were deparaffinized with xylene-ethanol series, washed in 0.02 M phosphate-buffered saline (PBS, pH 7.4), and subsequently immunostained by the unlabeled antibody method using soluble peroxidase-anti-peroxidase (PAP) complex, as reported previously (28). The anti-porcineGRP serum raised in a rabbit (R-6902, Yanaihara) was characterized previously (30), and used at 1:1,500 dilution. After incubation with anti-GRP serum, a goat anti-rabbit IgG antiserum (1:600) and rabbit PAP (1:250, Polysciences) were used.

For the detection of bombesin-immunoreactivity, another immunostaining was performed by means of both indirect and unlabeled-antibody methods, using guinea pig anti-bombesin serum (GP-3303, Yanaihara), goat F(ab)’, of antiguinea pig IgG conjugated with horseradish peroxidase (Japan ImmunoRes. Co.), or rabbit anti-guinea pig IgG (1:80, Miles Lab.) and guinea pig PAP (1:50-100, Jackson ImmunoRes. Lab.). The anti-bombesin serum has been previously characterized by Yanaihara et al. (29).

To test the specificity of immunoreaction, serial adjacent sections were immunostained with several anti-GRP sera to which excess amount (100 μg/ml diluted antiserum) of synthetic GRP1-27, GRP14-27, bombesin or substance P was added. Additionally, rabbit anti-substance P serum (R-2404, Yanaihara) at 1:1,000 dilution was applied to the serial sections in order to compare the P-like immunoreactivity with the substance P-like immunoreactivity.

RESULTS

Immunoreactive neurons and fibers against the anti-GRP serum were widely seen throughout the brain. When sections were incubated with anti-GRP antiserum preabsorbed with synthetic GRP1-27, GRP14-27 or bombesin, the GRP-like immunoreactivity in the neuronal elements was completely blocked. In contrast, the immunoreactivity was not interfered by the addition of excess amount of synthetic substance P. Immunohistochemical studies on the adjacent serial sections using the anti-substance P serum and anti-GRP serum revealed that the distribution of the anti-substance P-immunopositive neurons differed from that of GRP-immunopositive neurons. Neuronal elements immunoreactive with both GRP and substance P antisera were not observed. After intraventricular administration of colchicine, the GRP- and substance P-immunopositive neurons were increased in number, stained intensely, and sometimes found in several areas undetected under the normal condition.

Any immunoreactive products against anti-bombesin serum were not observed in the quail brain by means of the indirect or PAP methods.

In the colchicine-treated quails, many GRP-like immunoreactive perikarya were widely distributed in the cortical area of the striatum, i.e., cortical layer of the hyperstriatum accessorium and hyperstriatum ventrale, neostriatum dorsolaterale and neostriatum caudale, and in the lobus parolfactorius (Fig. 1). A few immunoreactive perikarya were also observed in the archistriatum. The perikarya were spindle or multipolar in shape, and protruded the immunopositive processes in several directions. The fibers projected from these perikarya gathered in the lamina frontalis superior and extended ventromedially to the tractus strio-hypothalamicus medialis (Fig. 2). GRP-immunopositive fibers were also seen in the nucleus of stria terminalis, where they enclosed or intimately contacted with the immunonegative perikarya, and extended caudo-laterally along the stria terminalis (Figs. 3 and 4).

Some perikarya and fibers were stained with
Figs. 1-4
Fig. 5 a–c: Frontal sections through the rostral (a), medial (b) and caudal (c) parts of the supra-chiasmatic nucleus, showing the distributions of GRP-immunoreactive perikarya and fibers. OC, optic chiasm; VIII, third ventricle. $\times 188$
the anti-GRP serum in the ventral floor of the anterior part of the hypothalamus (Fig. 5, a–c). These were oval or polygonal, parvocellular neurons and distributed mainly in the anterior portion of the suprachiasmatic nucleus, which was placed at each lateral angle of the third ventricle just dorsal to the optic chiasm (Fig. 5, a–c). GRP-like immunoreactive fibers were detected mainly in the neuropil of this nucleus.

Many GRP-immunopositive fibers showing dot-like appearance were also found in the neuropil of the nucleus infundibularis (Figs. 6 and 7). These fibers were in close contacts with unlabeled parvocellular neurons in this nucleus.

GRP-immunoreactive perikarya were also observed in several groups in the brainstem. Some perikarya were small to medium in size and densely distributed in the caudal portion of the stratum cellulare internum (Fig. 8). Some were distributed on the lateral side of the descending fibers of the nervus oculomotorius. Others were found in the ectomammillary nucleus, formatio reticularis medialis mesencephali and substantia grisea centralis (Fig. 9). These cell bodies were polygonal in shape and small to medium in size (Fig. 10). Occasionally a few immunoreactive cells were distributed in the occipito-mesencephalic tract.

**DISCUSSION**

The present study extends our previous report on the distribution of GRP-immunoreactivity in the brain of the Japanese quail pretreated with colchicine (17). Recently, cross-reaction or coexistence of GRP and substance P in some neuronal structures has been reported in the rat (11, 22). The previous radioimmunoassay data (30) and/or our observation (17) have confirmed that the GRP antiserum used in this study does not cross-react with substance P. In the present study, no neurons were immunostained simultaneously with GRP antiserum and substance P antiserum; GRP-like immunoreactivity was not blocked when GRP-antiserum was used in the presence of excess amount of substance P. Thus, it is concluded that the neuronal system containing GRP-like immunoreactivity is distinct from the system containing substance P-like immunoreactivity in the quail brain.

Bombesin has a striking similarity with porcine GRP in the C-terminal 7 amino acid residues (15). Although bombesin blocked the GRP-immunoreaction in the present study, the immunoreactivity against the specific bombesin antiserum was not detected throughout the brain. The anti-bombesin serum, GP-3303, reacts only with bombesin, but not with GRP; the antiserum recognizes mainly the Asn-Glu sequence at the position 6 and 7 in the bombesin molecule, where GRP has different amino acid residues (29). Therefore, the immunoreaction against anti-GRP serum seems to demonstrate the presence of GRP-like substances in the quail brain.

In the present study, GRP-immunoreactive neurons were newly detected in the suprachiasmatic region of the rostral hypothalamus. In birds, the term of the suprachiasmatic nucleus was initially used by Crosby and Showers (8) in *Passer domesticus*. In the passerine bird, parvocellular nucleus placed at each lateral angle of the third ventricle just dorsal to the optic chiasm is identified as the suprachiasmatic nucleus (16, 18). However, the area of the suprachiasmatic nucleus varies to some extent among the species of birds. Baylé et al. (1) used the term of nucleus anterior medialis hypothalami, instead of the suprachiasmatic nucleus, in their stereotaxic atlas of the brain of the Japanese quail. Kuenzel and van Tienhoven (13) have proposed that the suprachiasmatic nucleus was identical with the nucleus anterior medialis hypothalami described by Baylé et al. (1). In the present study, the area occupied by neuronal elements containing immunoreactive GRP corresponds to the suprachiasmatic nucleus.

Numerous neurons and fibers exhibiting GRP/bombesin-like immunoreactivity have been demonstrated in the suprachiasmatic nucleus of the colchicine-treated rat (19, 22). As in mammals, the present study demonstrated numerous GRP-positive cell bodies in the suprachiasmatic nucleus of the colchicine-treated quail. The suprachiasmatic nucleus of the white-crowned sparrow has been reported ultrastructurally to consist of parvocellular secretory neurons (16). Suprachiasmatic nucleus in the mammalian hypothalamus contains several neuropeptides and amine, e.g. vasopressin, VIP and serotonin (7, 12, 23–25), which may relate to the multiple function of the suprachiasmatic nucleus in mammals. In the quail brain, however, immunoreactive GRP was the only neuropeptide demonstrated in this nucleus.

The present study has demonstrated the GRP-immunoreactive fibers occurring in a close topographical relationship with the immuno-negative parvocellular neurons in the nucleus infundibularis and nucleus of stria terminalis. Although the nature of these cell bodies is unknown, the infundibular nucleus and nucleus of stria terminalis are shown to contain several kinds of
Figs. 6-10
perikarya containing different neuropeptides; somatostatin, Met-enkephalin, substance P and VIP (2–4, 9, 17, 28). It is possible that a functional correlation exists between these peptidergic neurons and GRP fibers. However, further study is required to establish the definitive synaptic contact and functional relationship between the GRP-positive fibers and the perikarya of unknown origin.

In an extension of our previous study (17), a widespread distribution of GRP-immunoreactive perikarya in the quail telencephalon was demonstrated in the present study. In the pigeon, substance P-containing neurons in the paleostriatal complex project their fibers to the midbrain tegmentum (20). The presence of the GRP-immunoreactive fiber pathways in the lamina frontalis superior and tractus striohypothalamicus medialis suggests the projection of GRP-fibers from the GRP neurons in the telencephalon to the hypothalamus and archistriatum. The present study also demonstrated numerous perikarya exhibiting GRP-immunoreactivity in the several areas of the mesencephalon. The ubiquitous distribution of GRP neurons suggests a functional role of GRP in the quail brain.

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