0270-6474/83/0310-2021$02.00/0

The Journal of Neuroscience
Vol. 3, No. 10, pp. 2021–2029
October 1983

IMMUNOHISTOCHEMICAL LOCALIZATION OF BOMBESIN/GASTRIN-RELEASING PEPTIDE AND SUBSTANCE P IN PRIMARY SENSORY NEURONS

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Received September 28, 1982; Revised April 1, 1983; Accepted April 28, 1983

Abstract

The existence of bombesin/gastrin-releasing peptide-like immunoreactivity (BN-GRP-LI) in rat sensory ganglia and spinal cord was confirmed using immunocytochemistry, gel filtration chromatography, and high performance liquid chromatography combined with radioimmunoassay. Immunohistochemical studies showed that in the spinal sensory ganglia of the rat about 5% of the neurons exhibited BN-GRP-LI, whereas about 20% of the neurons exhibited substance P-like immunoreactivity (SP-LI). The two immunoreactivities were found in different cells, but both were located in small ganglion cells. In the posterior horn of the spinal cord, BN-GRP-LI and SP-LI were located in the superficial layers, and this distribution was different from that of Met^5-enkephalin-like immunoreactivity.

The results are in agreement with the concept that there is a primary sensory pathway from the sensory ganglia to the spinal cord which contains BN-GRP-LI and that these neurons are separate from those containing substance P. In extracts prepared from spinal ganglia, two molecular weight forms of BN-GRP-LI were found using gel filtration chromatography. The high molecular weight form coeluted with porcine GRP and the low molecular weight form was smaller than bombesin. The low molecular weight BN-GRP-LI extracted from spinal cord was more hydrophilic than bombesin or ranatensin.

The tetradecapeptide bombesin was isolated from frog skin by Anastasi et al. (1971) and, subsequently, bombesin/gastrin-releasing peptide-like immunoreactivity (BN-GRP-LI) was found in the gastrointestinal tract (Polak et al., 1976; McDonald et al., 1978; Dockray et al., 1979), lung (Wharton et al., 1978), sympathetic ganglia (Schultzberg, 1980), brain (Brown et al., 1978; Villareal and Brown, 1978; Moody et al., 1981a), and spinal cord (Moody et al., 1981b) of several mammalian species. The location of BN-GRP-LI in the rat brain and spinal cord using a rabbit antiserum against bombesin has been reported (Panula et al., 1982). In the posterior horn of the spinal cord the BN-GRP-LI was found in a dense plexus of fibers and terminals of layers I and II of Rexed (1952), and this immunoreactivity decreased after rhizotomy indicating that it could originate from primary sensory neurons. Since the distribution of substance P-like immunoreactivity (SP-LI) has been reported to be similar (e.g., see Ljungdahl et al., 1978) and the source of this immunoreactivity is mostly axons of small sensory ganglion cells (Hokfelt et al., 1975b, c, 1976), this study was undertaken to elucidate whether SP-LI and BN-GRP-LI are located in the same cells. Since it has been suggested that the mammalian counterpart of amphibian bombesin is gastrin-releasing peptide (GRP), a 27-amino acid peptide (McDonald et al., 1979), we tested its cross-reactivity with our antiserum and partially characterized the immunoreactive material found in tissue extracts using gel filtration chromatography and high performance liquid chromatography (HPLC).

Materials and Methods

Preparation and properties of the antisera

For the production of substance P antiserum, 5 mg of substance P (Peninsula Laboratories, San Carlos, CA) and 5 mg of hemocyanin were dissolved in 1 ml of water. Fifty microliters of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (100 mg/ml) were added to this solution, and the reaction mixture was kept at room temperature at pH 5.0 to 6.0 for 10 hr. The peptide-hemocyanin conjugate was then dialyzed against water and lyophilized. After dissolving in saline, the conjugate was emulsified in Freund's complete adjuvant and 1 ml of the

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emulsion containing 500 μg of the conjugate was injected intradermally into the backs of rabbits. Subsequent injections contained 250 μg of the conjugate. Immunization was repeated at 2-week intervals, and 1 week after the fifth injection the rabbits were bled. The antiserum that gave best staining in immunohistochemical procedure was characterized and used in this study for both immunohistochemistry and radioimmunoassay.

Substance P was iodinated using a modified chloramine T method. Talc powder (25 mg) was added to 5 nmol of Tyr^8-substance P (Peninsula Laboratories) in 100 μl of 0.25 M potassium phosphate buffer (pH 7.4), and the mixture was vortexed. Ten millicuries of $Na^{125}I$ (10 μl) were added followed by 20 μl of chloramine T (1 mg/ml of H_2O). The reaction mixture was vortexed for 40 sec, diluted with 5 ml of 0.05 M potassium phosphate buffer (pH 7.4), and centrifuged. The pellet was rewashed with 5 ml of 0.05 M potassium phosphate buffer (pH 7.4). The ^{125}I-labeled substance P was eluted from the pellet by 1 ml of 1 N CH_3COOH containing 0.1% bovine serum albumin (BSA) and purified chromatographically with a Bio-Gel P-2 column (100 to 200 mesh) developed with 1 N CH_3COOH containing 0.1% BSA.

The inhibition of [^{125}I]Tyr-substance P binding to the substance P antiserum by eledoisin, somatostatin, ranatensin, and bombesin (all from Peninsula Laboratories) using a standard radioimmunoassay procedure is shown in Figure 1*A*. The standard peptide was incubated with antiserum and [^{125}I]Tyr-substance P (about 12,000 cpm) in 0.5 ml of 0.2 M Tris buffer, pH 7.4, containing 0.1% BSA and 0.06% dextran at 4°C for at least 16 hr. Separation of bound and free antigen was carried out by addition of 0.2 ml of charcoal slurry (1.5% charcoal) precoated with dextran (0.15%) in 0.9% saline, followed by centrifugation. The antiserum was used at a final dilution of 1:5000 in the radioimmunoassay mixture. The cross-reactivity of substance P antiserum with eledoisin was about 10% and with bombesin, ranatensin, and somatostatin less than 0.1% (Fig. 1*A*). Thus, the C-terminal tripeptide is the minimum requirement for binding to this antiserum.

The production and specificity of the bombesin antiserum has been described elsewhere (Panula et al., 1982). The cross-reactivity of this antiserum with ranatensin is about 10% and with substance P and eledoisin less than 0.1%. To test whether the bombesin antiserum detects GRP-like peptides, the cross-reactivity of the antiserum with GRP (1–27) and GRP (14–27) was studied (Fig. 1*B*). The antiserum recognized both GRP (1–27) and GRP (14–27). The conditions of this radioimmunoassay were similar to those described for substance P and have been described in detail (Panula et al., 1982). The specificity of the Met^5-enkephalin antiserum used in this study has been described previously (Yang et al., 1979). The cross-reactivity of this antiserum with Leu^5-enkephalin is about 3%, with the tetrapeptide Gly-Gly-Phe-Met about 1%, and with the tetrapeptide Tyr-Gly-Gly-Phe less than 0.1%. This antiserum does not cross-react with α- and β-endorphin, but it detects N-terminally extended high molecular weight enkephalin-like material.

Tissue extraction and chromatography

Rats were decapitated, and the spinal cord and spinal sensory ganglia were removed and homogenized in 1 N CH_3COOH, 0.02 N HCl, 0.1% β-mercaptoethanol in a polypropylene tube with a Teflon pestle. The homogenate was boiled and centrifuged. The supernatant was passed through a Sep-Pak (C-18) cartridge, washed with H_2O, and eluted with 60% acetonitrile containing 0.1% trifluoroacetic acid. The eluate was lyophilized, resuspended in 1 N CH_3COOH, and then fractionated by gel filtration chromatography on a Bio-Gel P-30 column (0.9 × 60 cm, 100 to 200 mesh) eluted with 1 N CH_3COOH at a flow rate of 0.1 ml/min. Forty 1-ml fractions were collected and analyzed for immunoreactivity by radioimmunoassay. Fractions containing immunoreactive material were pooled, lyophilized, and further chromatographed on a reverse phase HPLC column (Bio-Sil ODS 10, 250 × 4 mm). This column was eluted with a linear gradient of acetonitrile from 20% to 50% in 0.1% trifluoroacetic acid during 60 min at a flow rate of 1 ml/min. The fractions were collected every minute, lyophilized, and radioimmunoassayed. The marker peptides were monitored spectrophotometrically at 220 nm.

Immunohistochemical procedures

Adult male Sprague-Dawley rats were perfused through the left ventricle with physiological saline followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) under sodium pentobarbital (Nembutal) anesthesia. The spinal cords and spinal sensory ganglia were removed and immersed in the same fixative for 2 hr. They were then washed in 0.1 M sodium phosphate buffer, pH 7.4, containing 15% sucrose, for at least 24 hr. Consecutive 8-μm-thick cryostat sections were cut and collected on gelatin-coated slides and then processed for immunohistochemistry. Serial sections were incubated with substance P, bombesin, and Met^5-enkephalin antisera and preabsorbed antisera as well as with preimmune serum.

The peroxidase-antiperoxidase (PAP) method of Sternberger et al. (1970) and the indirect immunofluorescence method of Coons (1958) were used. The optimal dilutions of the antisera were: substance P, 1:1000; bombesin, 1:1000; and Met^5-enkephalin, 1:2000. The sections were first incubated with normal swine serum diluted 1:5 with phosphate-buffered saline (PBS; pH 7.4) containing 0.25% Triton X-100 at room temperature for 20 min to diminish the background staining. The optimally diluted primary antisera were then applied and diluted in PBS containing Triton X-100 (0.25%), and incubation was carried out at 4°C for 48 hr for the PAP and immunofluorescence methods. The sections were then washed with PBS containing Triton X-100 twice for 20 min. For immunofluorescence, they were then incubated with rhodamine isothiocyanate-conjugated swine anti-rabbit immunoglobulins (DAKO, Copenhagen, Denmark) diluted 1:40 at room temperature for 30 min, washed with PBS without Triton X-100, and mounted with glycerol/PBS (1:2 v/v) for examination under a Leitz Ortholux II incident light fluorescence microscope. The cells were

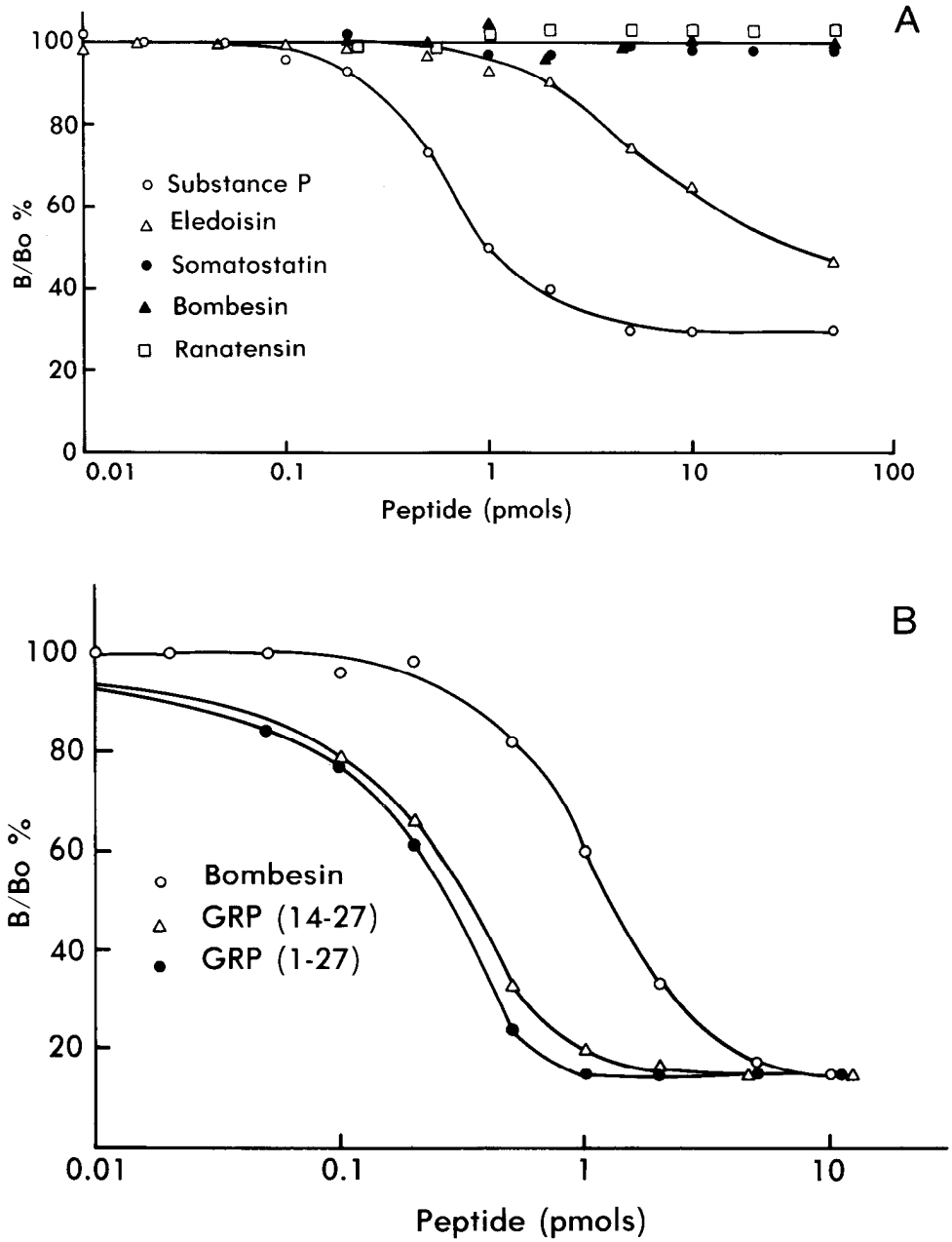


Figure 1. A, Radioimmunoassay specificity of the substance P antiserum from the rabbit used in this study. Competitive displacement of [^{125}I]Tyr^{8}-substance P bound to the antiserum by substance P, eledoisin, somatostatin, bombesin, and ranatensin indicates about 10% cross-reactivity with eledoisin as compared to substance P. The results are expressed as percentage of sample reacted without competitors (% *B/Bo*). Detailed conditions of the radioimmunoassay are given in the text. *B*, Radioimmunoassay specificity of the bombesin antiserum. Competitive displacement of antibody-bound [^{125}I]Tyr^{4}-bombesin by bombesin, GRP (14–27), and GRP (1–27) shows that the antiserum recognizes GRP (1–27) and GRP (14–27) with higher potency than does bombesin. The results are expressed as percentage of sample reacted without competitors.

counted by both fluorescence microscopy and phase contrast optics. For the PAP method, the sections were similarly washed after incubation with the peptide antisera, then incubated with swine anti-rabbit immunoglobulins (DAKO) diluted 1:100 with PBS containing Triton X-100 (0.25%) for 30 min at room temperature. They were then washed again with PBS/Triton X-100 and incubated with a soluble complex of horseradish peroxidase/rabbit antihorseradish peroxidase (DAKO) diluted 1:100 with PBS/Triton X-100 for 30 min at room temperature. The sections were then washed twice in 0.05 M Tris-HCl buffer (pH 7.6) and reacted with 3,3′-diami-

nobenzidine tetrahydrochloride (DAB; 50 mg/100 ml; Sigma Chemical Co., St. Louis, MO) and 0.003% hydrogen peroxide in 0.05 M Tris-HCl buffer (pH 7.6) for 8 min, washed with the same buffer, air dried, and embedded in Permount for examination under brightfield illumination.

Immunohistochemical controls. Because substance P and bombesin share the C-terminal dipeptide amide, it was necessary to perform an extensive series of control incubations to establish the specificity of the immunostaining. For preabsorption of the antisera, 1 to 1000 μg of bombesin or substance P was added per milliliter of diluted antisera 24 hr before applying to the sections. At 1 μg/ml, bombesin decreased the bombesin immunostaining and 5 μg/ml abolished it completely, whereas substance P did not abolish the staining at any concentration. The staining with substance P antiserum was diminished by substance P (2 μg/ml) but not by bombesin. Staining with the Met^5-enkephalin antiserum was diminished by 5 μg/ml and abolished by 10 μg/ml of Met^5-enkephalin but not by substance P or bombesin. Since other high molecular weight enkephalin-like peptides also cross-react with this antiserum, the immunostaining revealed by this study should be referred to as Met^5-enkephalin-like immunoreactivity (ME-LI). Staining with preimmune serum gave no staining in any structures where the specific staining could be absorbed with the respective peptide. Omission of each secondary antiserum abolished all staining. Incubation of the sections with DAB and hydrogen peroxide indicated that endogenous peroxidase did not interfere with the staining. There was a slight nonspecific staining of the meninges.

Results

Immunocytochemistry of the spinal cord. In the posterior horn of the spinal cord the BN-GRP-LI was found in layers I and II, where varicose fibers and terminal-like structures exhibited staining (Figs. 2 and 6). The staining was strong in Lissauer's tract, whereas only scattered fibers were observed in layer III and other deeper layers of the posterior horn. Some immunoreactive fibers were found in the posterior part of funiculus lateralis (Figs. 2 and 6). No BN-GRP-LI cell bodies were seen in the posterior horn.

Layers I and II of the spinal cord also contained intense SP-LI with scattered fibers in deeper layers (Fig. 4). The density of immunoreactive fibers was greater than in adjacent bombesin-stained sections. The poste rior part of the lateral funiculus showed an accumulation of SP-LI fibers, whereas adjacent sections stained for BN-GRP-LI showed only little immunoreactivity in this location. No immunoreactive cell bodies were found. Both SP-LI and BN-GRP-LI were completely abolished when antisera preabsorbed with the appropriate peptide were used (Figs. 3 and 5). The distribution of BN-GRP-LI was different from that of ME-LI (Figs. 6 and 7). The most intense staining for ME-LI was found in layers I and II, but immunoreactive fibers extended to deeper layers as well. The posterior part of the lateral funiculus also contained fibers exhibiting strong ME-LI, thus resembling SP-LI (Fig. 7).

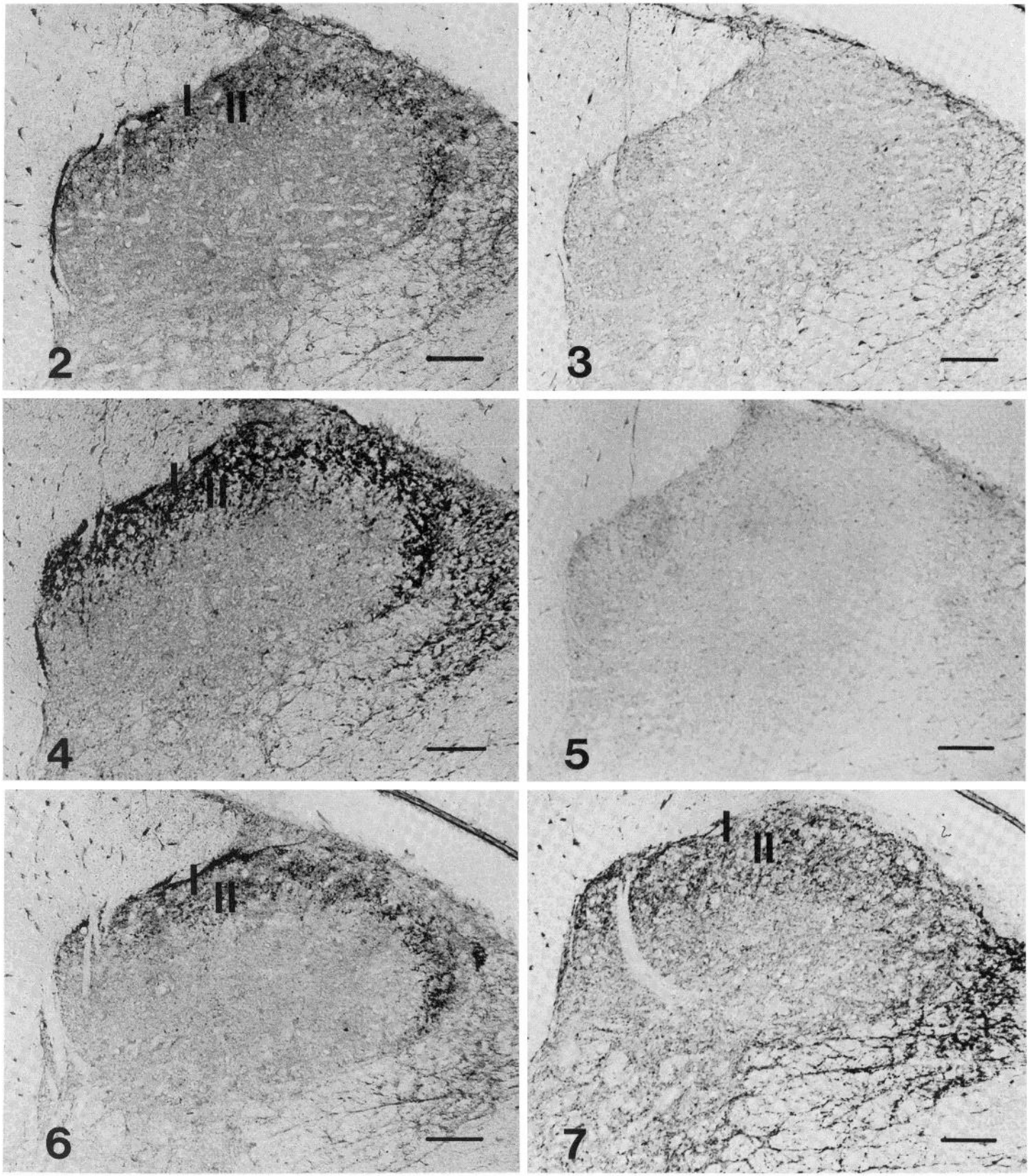
Immunocytochemistry of the sensory ganglia. About 20% of spinal sensory ganglion cells exhibited SP-LI, whereas only about 5% showed BN-GRP-LI (Figs. 8 and 9). The intensity of the fluorescence varied, probably because of different amounts of peptides in the cells and variation in the thickness of the fractions of the cells in the sections. When consecutive sections were stained, different cells exhibited SP-LI and BN-GRP-LI (Figs. 10 to 13), but no difference was found in the morphology of the cells that contained the two peptides. When cells exhibiting SP-LI were in focus, the fluorescence was granular and the nuclei were nonfluorescent (Fig. 14). The cells exhibiting SP-LI were rounded or slightly polygonal in shape, the diameter was usually < 40 μm, and many cells sent out a thin immunofluorescent process. BN-GRP-LI was also granular and the cell nucleus was less fluorescent (Fig. 15). However, fibers exhibiting BN-GRP-LI were not nearly as numerous as those exhibiting SP-LI. The large cells in the ganglia did not exhibit BN-GRP-LI or SP-LI.

Characterization of BN-GRP-LI with gel filtration. Two peaks of BN-GRP-LI were detected in spinal ganglia extracts filtered through a Bio-Gel P-30 column. A high molecular weight peak of immunoreactivity coeluted with synthetic porcine GRP (1–27) (Fig. 16*A*). A major low molecular weight immunoreactive peak eluted after synthetic bombesin, indicating that the molecular weight of this immunoreactive material is probably smaller than that of bombesin. The low molecular weight form was the main immunoreactivity detected by gel filtration chromatography in extracts from spinal cord and, again, the elution volume of this material was different from that of bombesin (Fig. 16*B*). A small peak of high molecular weight BN-GRP-LI corresponding to the elution position of GRP (1–27) was detected in the spinal cord (data not shown). Further characterization of this material is currently under study. When the low molecular weight immunoreactive peak from the spinal cord was subjected to reverse phase HPLC, two major immunoreactive peaks eluted before bombesin, substance P, or ranatensin. Thus, the low molecular weight BN-GRP-LI in the spinal cord appeared to be more hydrophilic than did bombesin. Since multiple forms of BN-GRP-LI were found in tissues, it is appropriate to call this immunoreactivity BN-GRP-LI until the peptides have been positively identified.

Discussion

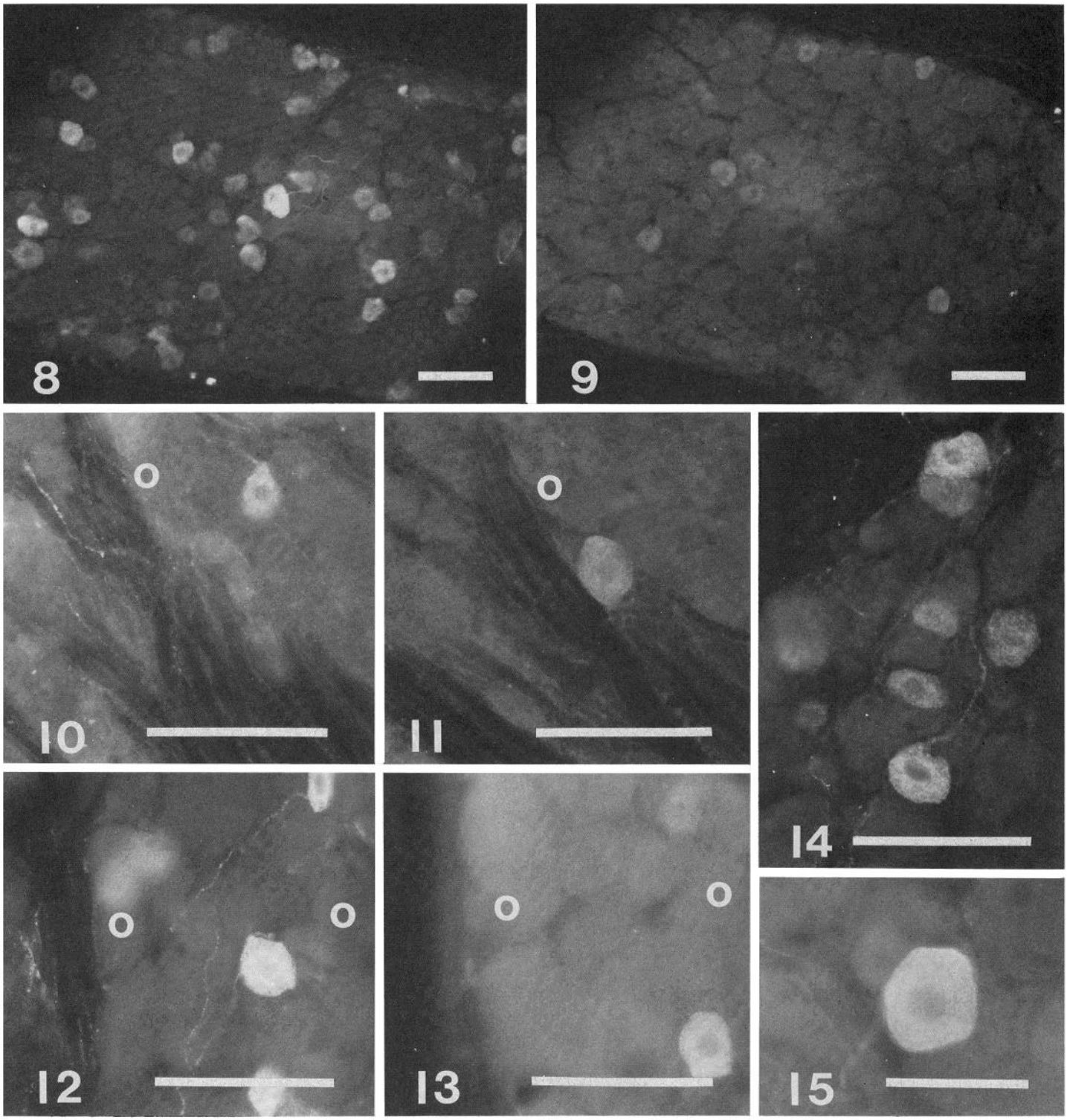
The extensive number of absorption controls carried out in this study confirms that it is possible to selectively localize BN-GRP-LI and SP-LI. The presence of the two peptides in different cells provides further evidence that the antisera recognize different endogenous peptides. Furthermore, the characterization of the BN-GRP-LI using gel filtration and HPLC shows that it differs from substance P.

The results indicate that BN-GRP-LI-containing fibers in the spinal cord partly derive from spinal sensory ganglion neurons where the peptide is stored in cells which are morphologically similar to those containing substance P. However, no evidence for colocalization of BN-GRP-LI and SP-LI was found. BN-GRP-LI in the



Figures 2 to 5. A series of consecutive 8-μm sections of the posterior horn of the cervical spinal cord stained with (Fig. 2) bombesin antiserum, (Fig. 3) bombesin antiserum preabsorbed with 10 μg/ml of bombesin, (Fig. 4) substance P-antiserum, and (Fig. 5) substance P antiserum preabsorbed with 10 μg/ml of substance P. Both peptides show the most intense staining in layers I and II (*I* and *II* in the figures), and only little immunoreactivity is found in deeper layers. Substance P immunoreactivity extends further into layer III in contrast to bombesin immunoreactivity, and the dorsal part of funiculus lateralis exhibits more SP-LI than BN-GRP-LI. Both reactions are completely abolished by preabsorption with the appropriate peptide. *Scale bar* = 100 μm.

Figures 6 and 7. Sections of the cervical spinal cord stained with bombesin antiserum (Fig. 6) and methionine-enkephalin antiserum (Fig. 7). Layers I and II exhibit the strongest immunoreactivity to both peptides but BN-GRP-LI is almost completely confined to this location whereas scattered fibers and terminals in deeper layers exhibit ME-LI. The dorsal part of funiculus lateralis exhibits strong ME-LI but little BN-GRP-LI. *Scale bar* = 100 μm.



Figures 8 and 9. Consecutive sections of spinal sensory ganglia showing rhodamine immunofluorescence for substance P (Fig. 8) and bombesin (Fig. 9). A large number of small cells exhibit SP-LI whereas considerably fewer cells of similar size show BN-GRP-LI. *Scale bar* = 100 μm.

Figures 10 and 11. Immunofluorescence of consecutive sections of spinal ganglia demonstrate SP-LI (Fig. 10) and BN-GRP-LI (Fig. 11). The same unlabeled cells are marked with *white circles.* Different cells appear to exhibit SP-LI and BN-GRP-LI. *Scale bar* = 100 μm.

Figures 12 and 13. Another pair of consecutive spinal ganglion sections exhibit SP-LI (Fig. 12) and BN-GRP-LI (Fig. 13). The same unlabeled cells are marked with *white circles.* Different cells show immunoreactivity. *Scale bar* = 100 μm.

Figure 14. Substance P-immunoreactive cells in the spinal ganglion were found or polygonal neurons with granular cytoplasmic immunofluorescence and nonfluorescent nuclei. *Scale bar* = 100 μm.

Figure 15. The morphology of cells exhibiting BN-GRP-LI was similar to that of SP-immunoreactive cells. Processes like the one in this picture (*lower left*, not in focus) were seen only occasionally. *Scale bar* = 50 μm.

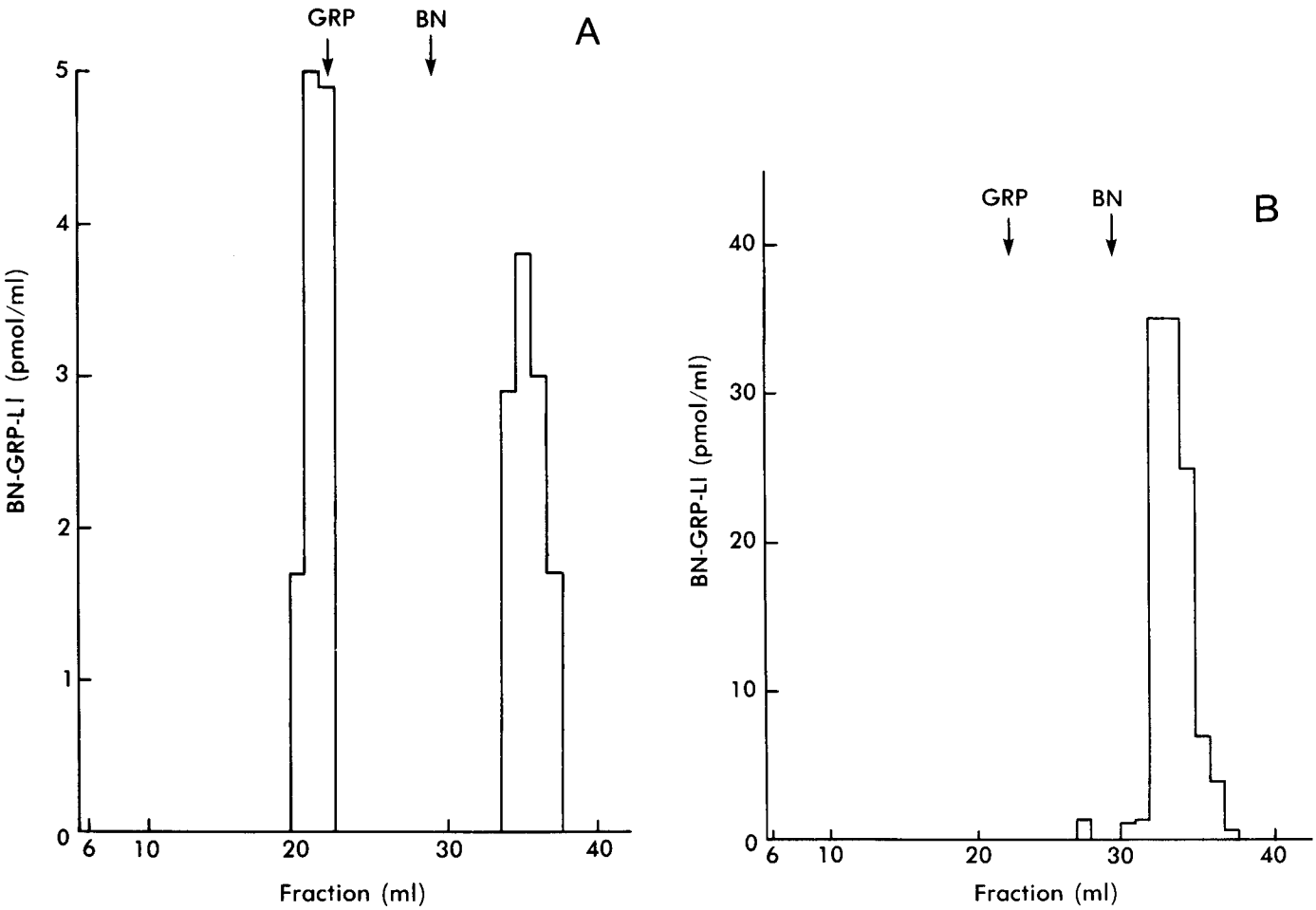


Figure 16. Bio-Gel P-30 column chromatography of extracts from rat (*A*) spinal ganglia and (*B*) whole spinal cord. Tissues from six animals were pooled for extraction. The extracts were applied to a Bio-Gel P-30 column, 0.9 × 60 cm (100 to 200 mesh) eluted with 1 N Ch_3COOH at a flow rate of 0.1 ml/min. Fractions (1 ml) were lyophilized and redissolved in H_2O. Aliquots were then radioimmunoassayed as described in the text. The results are expressed as picomoles of BN-GRP-LI per fraction. The elution positions of synthetic porcine GRP (1–27) and bombesin are indicated (*GRP* and *BN*, respectively).

spinal cord has a dual origin: the immunoreactivity in the posterior horn decreases after rhizotomy whereas the fibers and terminals in the anterior horn persist after the operation (Panula et al., 1982).

The primary sensory neurons contain a variety of peptides, including substance P (Hellauer, 1953; Lembeck, 1953; Pernow, 1953; Takahashi et al., 1974; Hokfelt et al., 1975b, c), somatostatin (Hokfelt et al., 1975a, 1976), vasoactive intestinal polypeptide (Lundberg et al., 1978), and gastrin/cholecystokinin (Lundberg et al., 1978). Substance P and somatostatin are stored in different cells with similar morphology and together they comprise about 25% of all sensory ganglion cells (Hokfelt et al., 1976). It is evident that both substance P and somatostatin (Hokfelt et al., 1976) as well as BN-GRP-LI are contained in small ganglion cells, classified as B cells by Andres (1961). Vasoactive intestinal peptide can be found in both small and large cells (Lundberg et al., 1978). In the ganglion, fibers with BN-GRP-LI were scarce, a characteristic that has been described for somatostatin (Hokfelt et al., 1976). The greater number of ganglion fibers with SP-LI could be due to the higher concentration of substance P in the fibers or to the greater sensitivity of substance P antiserum. The number of cells exhibiting BN-GRP-LI was approximately the same as that of somatostatin-containing cells (Hokfelt et al., 1976), but it is not known whether the two peptides are stored in the same or different neurons. It must also be emphasized that because of the possible lower sensitivity of the bombesin antiserum the actual number of cells containing the BN-GRP-LI may be higher than revealed by this study.

The substance P in the spinal cord is derived from sensory ganglia and from spinal cord interneurons (Hokfelt et al., 1975b, c; Takahashi and Otsuka, 1975; Stine et al., 1982) and the origin of somatostatin in the posterior horn is similar to that of substance P (Hokfelt et al., 1976; Hunt et al., 1981; Stine et al., 1982). In addition, part of the somatostatin in the spinal cord comes from ascending and descending intraspinal projections (Stine et al., 1982). The distribution of BN-GRP-LI in the posterior horn resembles that of substance P more closely than that of somatostatin (Hokfelt et al., 1976), and it is likely that there are spinal interneurons which contain BN-GRP-LI because the immunoreactivity does not disappear totally from the spinal cord after rhizotomy (Panula et al., 1982). Ascending or descending projections may also contain BN-GRP-LI.

The densest accumulation of BN-GRP-LI fibers was found in layer II which also exhibited strong ME-LI. The interactions of substance P and enkephalins in the substantia gelatinosa have been described, and it is possible that an axoaxonic presynaptic inhibition of substance P-containing fibers by enkephalin-containing axons mediates the opiate effect on nociception since opiates inhibit the release of substance P from slices of trigeminal nucleus (Jessell and Iversen, 1977). However, there is no morphological support for this kind of direct interaction at the electron microscopic level. The distribution of ME-LI in the posterior horn revealed by this study is in agreement with earlier reports (Elde et al., 1976; Sar et al., 1978), and from the light microscopic distribution it can be concluded that an interaction between bombesin- and enkephalin-containing neurons might occur in this region.

Little is known about the physiological role of bombesin-like peptides in the central nervous system. Bombesin appears to raise the pain threshold when injected into the periaqueductal gray of the rat (Pert et al., 1980) and it can be released in a Ca^{2+}-dependent manner with depolarizing concentrations of K^+ and veratridine from slices of rat hypothalamus and spinal cord (Moody et al., 1980, 1981b). These findings support the view that bombesin might act as a neurotransmitter or neuromodulator. Capsaicin releases substance P but not bombesin from slices of rat spinal cord, indicating that the two peptides are stored in different populations of vesicles (Moody et al., 1981). This is in agreement with our finding of these two peptides in different ganglion cells. A number of interneurons containing different peptides and γ-aminobutyric acid are located in the posterior horn. The relations between these various cells have been partly elucidated (Hunt et al., 1981). Our studies are now focused on determining the location of possible BN-GRP-LI-containing spinal interneurons and the relations of bombesin-immunoreactive sensory neurons with other sensory cells which do not store substance P.

Previous biochemical studies have shown that mammalian BN-GRP-LI exists in two molecular forms, one of which coelutes with bombesin in gel filtration column, while the other one appears to be a larger molecule (Walsh et al., 1979). The larger molecular weight form appears to be GRP (1–27) in porcine nonantral gastric tissue (McDonald et al., 1979). It is possible that this peptide also exists in rat sensory ganglia, since in our studies some immunoreactive material in gel filtration column coelutes with GRP (1–27). A low molecular weight BN-GRP-LI from rat spinal cord has been reported to coelute with synthetic bombesin in reverse phase HPLC (Moody et al., 1981b). In our study, the low molecular weight BN-GRP-LI from spinal cord seems to differ from synthetic bombesin chromatographically. Recently, a bombesin-like peptide from human cerebrospinal fluid was found to coelute with bombesin nonapeptide by gel filtration (Yamada et al., 1981). We are now testing the elution positions of different bombesin-like peptides in our chromatographic systems in order to identify the immunoreactive peptides and studying the possibility that the high molecular weight BN-GRP-LI in the sensory ganglion might be a precursor of the smaller peptide.

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