Title
A quantitative ultrastructural analysis of synaptic connections in the rat carotid body

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A QUANTITATIVE ULTRASTRUCTURAL ANALYSIS OF SYNAPTIC CONNECTIONS IN THE RAT CAROTID BODY

by

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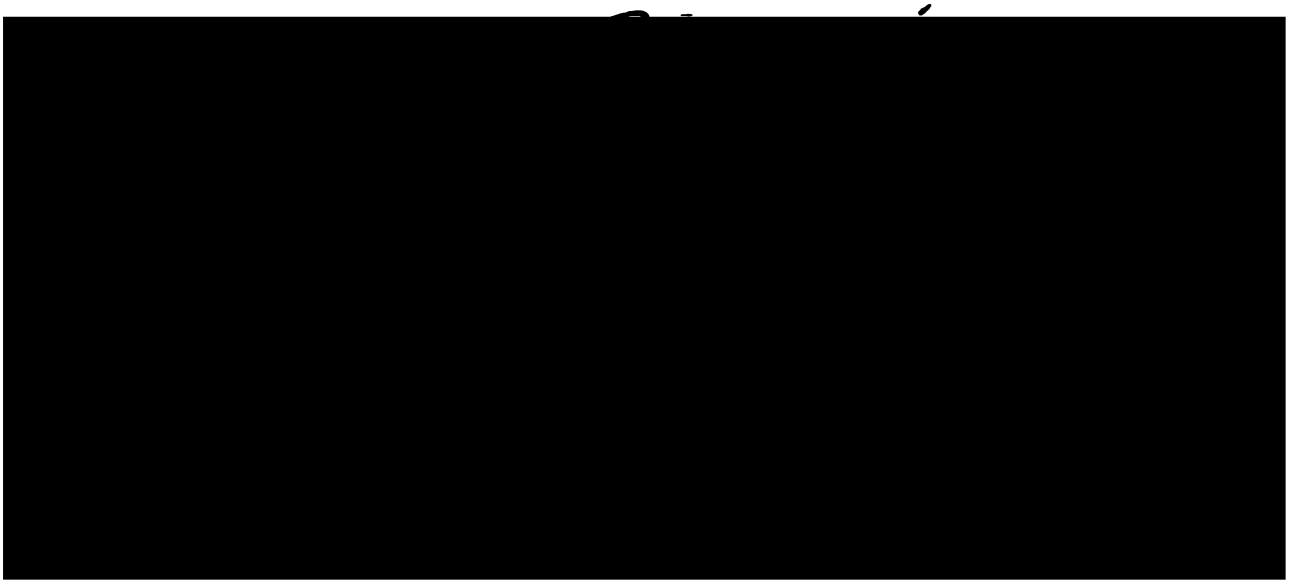

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ABSTRACT

In a quantitative ultrastructural analysis of synaptic connections in the rat carotid body, we found that two types of nerves end on (Type I) glomus cells. More than 95% are afferent axons which leave the carotid body in the carotid sinus nerve and have their cell bodies in the sensory (petrosal) ganglion of the glossopharyngeal nerve. Less than 5% are (preganglionic) efferent axons from the cervical sympathetic trunk which enter the carotid body with axons from the superior cervical sympathetic ganglion. We found no efferent axons from the glossopharyngeal nerve which end on glomus cells.

Afferent and efferent nerve endings can be distinguished by morphological criteria, although both types contain many synaptic vesicles and few large dense-cored vesicles. Synaptic vesicles in afferent nerve endings are 15% larger but only 40% as abundant as those in efferent nerve endings. Large dense-cored vesicles in afferent nerve endings are only 20% as abundant as those in efferent nerve endings.

Both afferent and efferent nerves are connected to glomus cells by synapses. Some regions of afferent nerve endings are presynaptic to glomus cells, some are postsynaptic, and some form reciprocal synapses. Both glomus cell bodies and their processes synapse on afferent nerve endings. Where glomus cell bodies are presynaptic, synaptic vesicles and large dense-cored vesicles accumulate in equal concentration in the glomus cell at the synaptic junction; but where glomus cell processes are presynaptic, synaptic vesicles predominate. This feature makes some processes resemble nerve endings, although, unlike nerve endings, all processes contain ribosomes. Many afferent nerve endings have

synaptic connections with several glomus cells, and some glomus cells probably contact more than one afferent nerve ending. Efferent nerve endings are presynaptic to glomus cells but are not in synaptic contact with afferent nerve endings. Although some glomus cells are connected synaptically to both afferent and efferent nerves, most probably are innervated only by afferent nerves.

In contrast to glomus cells, parasympathetic ganglion cells in the carotid body are innervated by (preganglionic) efferent axons from the glossopharyngeal nerve. Axons from these ganglion cells innervate blood vessels. Parasympathetic vascular nerve endings contain synaptic vesicles without dense cores. Vascular nerve endings which contain synaptic vesicles with dense cores (presumably noradrenergic) arise from sympathetic ganglion cells, most of which are in the superior cervical sympathetic ganglion. A few are in the carotid body.

We postulate that afferent nerve endings, connected by reciprocal synapses to glomus cells, are chemoreceptors sensitive to hypoxia, that glomus cells are interneurons which modulate the sensitivity of afferent nerve endings, and that autonomic ganglion cells regulate blood flow in the carotid body through their vascular nerve endings.

INTRODUCTION

This paper is a description of the ultrastructure of synaptic connections in the rat carotid body. The carotid body is a vascular chemoreceptor, stimulated by a fall in oxygen tension or a rise in carbon dioxide tension or hydrogen ion concentration in the blood flowing through it. Though Heymans discovered the chemoreceptive function of the carotid body in 1930 (41), the identity of the receptors and the mechanism by which they are stimulated have remained unknown. Previous light and electron microscopic studies (reviewed in 10 and 47) have shown that the carotid body is composed of small cells surrounded by nerves and a complicated anastomosing system of blood vessels. Most of these small cells are Type I cells (hereafter called glomus cells) which are a conspicuous feature of the carotid body. Glomus cells are enveloped by Type II cells (hereafter called sheath cells). Anatomical studies of the innervation of glomus cells have yielded conflicting results. In 1928 de Castro (25) concluded from his light microscopic studies that nerves innervating glomus cells are afferent and have their cell bodies in the sensory (petrosal) ganglion of the glossopharyngeal nerve. He postulated that the carotid body is a sense organ, that glomus cells are receptor cells, and that these cells initiate firing of adjacent afferent nerves, which in turn relay a signal to the brain.

This hypothesis was not questioned seriously for several decades, until electron microscopic studies revealed that nerves ending on glomus cells contained vesicles having the configuration and dimensions of synaptic vesicles. Initially these synaptic vesicles were held of

minor significance, because endings of some other afferent nerves contain vesicles (8, 61, 84). However, as techniques improved for preserving the ultrastructure of the carotid body, accumulations of synaptic vesicles were recognized as a conspicuous feature of some, if not all, nerves ending on glomus cells, an appearance which led to the suggestion that these nerves were efferent rather than afferent (2, 16).

In 1969 Biscoe, Lall, and Sampson reported that these nerves degenerate slowly after the glossopharyngeal nerve is cut central to the petrosal ganglion (11). From these results, they concluded that nerve endings on glomus cells belonged not to afferent axons whose cell bodies were in the petrosal ganglion, but to efferent axons whose cell bodies were in the brain stem, and that chemoreceptive nerves did not end on glomus cells but ended elsewhere in the carotid body. Hess and Zapata (39) repeated these experiments and obtained results which support the original observations of de Castro, not those of Biscoe's group. Meanwhile other investigators suggested that glomus cells are innervated both by afferent and efferent nerves (3,6,43,47); this suggestion awaits experimental confirmation.

Thus several questions remain unanswered: Are glomus cells innervated by afferent nerves, efferent nerves, or both? Are glomus cells receptor cells, or do they have another function? If glomus cells are not receptor cells, what are the chemoreceptors? Bearing these questions in mind, we began an ultrastructural study of the rat carotid body with the following objectives: 1) to determine whether glomus cells are innervated by afferent nerves, efferent nerves, or both; 2) to determine the source, proportion, structural characteristics, and synaptic connections of each type of nerve;

3) to identify the types of ganglion cells in the carotid body and the sources and structural characteristics of nerves ending on them; and 4) to determine the sources of nerves innervating blood vessels and the structural characteristics of their endings. Although the structure of the rat carotid body has been examined in several previous light and electron microscopic studies (9,18,42,46), the ultrastructure of its nerves and synaptic connections has not been investigated heretofore. We chose the rat carotid body because we believed its small size and closely packed glomus cells would facilitate our quantitative analyses, and because synapses between its glomus cells and nerves have ultrastructural features identical to those of synapses in other parts of the nervous system. We believe that the rat carotid body is not unique - that carotid bodies of other species have synaptic connections similar to those in the rat (see Discussion).

Our experiments revealed that more than 95% of nerve endings on glomus cells are from afferent axons, and that afferent nerve endings form reciprocal synapses with glomus cells. Efferent axons from the glossopharyngeal nerve end on ganglion cells, not on glomus cells. These data together with those from our neurophysiologic studies (57,58) support the hypothesis that afferent nerve endings are chemoreceptors sensitive to hypoxia. Reciprocal synapses between these endings and glomus cells complete an inhibitory feedback loop through which glomus cells adjust the sensitivity of chemoreceptive nerves.

METHODS

I. Preparation of Carotid Bodies for Electron Microscopy

We anesthetized 200-250 gm female rats of the Longs-Evans strain by injecting sodium methohexital (70 mg/kg) intraperitoneally, ventilated them mechanically with oxygen for about 30 minutes, then perfused them with fixative. The respirator delivered gas at a stroke volume of 2 ml and at a rate of 60 per minute via a tracheal cannula. To perfuse the rats with fixative, we inserted a cannula into the ascending aorta through an incision in the left ventricle and then cut the right atrium. Less than 10 seconds elapsed between incising the left ventricle and beginning the perfusion. We used two aldehyde fixatives sequentially. The first (fixative A) was our modification of the fixative introduced by Peracchia (67) containing glutaraldehyde and hydrogen peroxide; the second (fixative B) was identical to the first, except that it lacked hydrogen peroxide.[1] The concentrations of the ingredients of these fixatives, their pH, and their osmolarity are listed in Table I.[2] Both fixatives were filtered and perfused at room temperature.

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[1] We have used glutaraldehyde from three sources: Ladd Research Industries, Inc., Burlington, Vermont (70% concentration, highly purified); Fisher Scientific Co., Fair Lawn, New Jersey (50% concentration, biological grade); and Fisher Scientific (biological grade) purified by vacuum distillation in our laboratory. In our experiments, glutaraldehyde from Fisher Scientific, either biological grade or redistilled, produced fixation superior to that of purified glutaraldehyde from Ladd.

[2] We prepared fixative A in two steps. First, we mixed all ingredients except the aldehyde and peroxide. Second, within 5 minutes of the perfusion, we mixed the aldehyde (as 20% glutaraldehyde) with the peroxide (as 30% H_2O_2) and then combined them with the buffered mixture.

Table I: Ingredients of Fixatives.

	Fixative A	Fixative B
Glutaraldehyde	3%	3%
Hydrogen peroxide	60 mM	--
Cacodylate buffer	60 mM	60 mM
Calcium chloride	2 mM	2 mM
Sucrose	30 mM	30 mM
Polyvinylpyrrolidone (MW 40,000)	1 mM	1 mM
pH	7.1	7.1
Osmolarity (mOsm/L)	520	580

We perfused approximately 150 ml of fixative A over a 5 minute period, at a pressure of 150 torr for the first 1 minute and a pressure of 100 torr for the next 4 minutes. We followed fixative A with B, perfusing 50 ml over 5 minutes. Starting to dissect no sooner than 1 hour after perfusion, we removed the carotid bodies from the rats. The total duration of fixation in the aldehyde was 2 to 24 hours (under 4 hours was best). We treated the carotid bodies with 2% osmium tetroxide in 100 mM cacodylate buffer pH 7.2 for 2 hours, then with 2% uranyl acetate in 60 mM maleate buffer pH 5.0 for 1.5 hours (44). We did not wash the tissue with buffer after the aldehyde, osmium, or uranyl acetate. We dehydrated the carotid bodies in methanol for 2 hours at 4°C, then in acetone for 1 hour at room temperature, and then embedded them in Epon 812. We stained sections of the carotid bodies on grids with 0.8% lead citrate in 100 mM NaOH for 5 minutes in an atmosphere of oxygen before examining them with a Siemens Elmiskop 1 electron microscope.

II. Quantitative Methods

To help characterize different types of glomus cells and nerve endings, we measured the diameters of vesicles contained by these structures.[3] For these quantitative studies, we calibrated the electron

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[3]To avoid the effects on ultrastructure of subtle physiological differences (in blood pressure, PaO_2, $PaCO_2$, etc.) that may exist among animals at the time the carotid bodies are fixed for electron microscopy, all the measurements of vesicles in nerve endings that we give are from one carotid body.

microscope by using a ruled diffraction grating with 2160 lines per mm (Ernest F. Fullam, Inc., Schenectady, New York) and then photographed vesicles at known magnifications. We enlarged electron microscopic negatives to yield a total magnification of X100,000 on photographic prints and measured the diameters of vesicles in nerve endings and glomus cells on these prints by using a Zeiss Particle Size Analyzer (Model TGZ-3). Although we measured only those vesicles whose membranes were clearly defined, this technique overestimated the number of vesicles present (by 6% according to Heuser and Reese (40)) because some vesicles appear in two sections. We did not attempt to compensate for this inaccuracy. We measured areas of nerve endings and glomus cells in prints either directly with a compensating polar planimeter or by calculating them from the weight of the paper occupied by the structure being measured.

RESULTS

I. General Description of the Carotid Body of the Long-Evans Rat

In the Long-Evans rat, the carotid body lies in dense connective tissue between the internal and external carotid arteries adjacent to the superior cervical sympathetic ganglion (Fig. 1). It is innervated by the carotid sinus nerve, which is a branch of the glossopharyngeal nerve, and by sympathetic nerves from the cervical sympathetic trunk and ganglion.[4] The carotid body is roughly egg-shaped, measures 0.6 x 0.6 x 1.5 mm, and contains about 8700 glomus cells, 2300 sheath cells, and 25 ganglion cells.[5] Tightly packed collections of these cells

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[4]To study the gross anatomical features of the innervation of the carotid body, we examined whole-mounts of the carotid body and its neighboring nerves, ganglia, and blood vessels. To prepare these whole-mounts, we perfused rats with our aldehyde/peroxide fixative, removed the carotid body, nerves, ganglia, vessels, and accompanying tissue from each side as a single specimen, blackened the nerves with osmic acid, then dehydrated the tissue with methanol and cleared it in benzyl benzoate.

The vagus and glossopharyngeal nerves are contiguous as they pass through the jugular foramen of the skull. At that point a few axons usually leave the vagus and enter the glossopharyngeal nerve. However, we were unable to find a contribution from the extracranial portion of the vagus nerve to the carotid body. Occasionally we found a small nerve (about 10 microns in diameter) leaving the pharyngeal branch of the vagus nerve and joining the carotid sinus nerve midway between the carotid body and the glossopharyngeal nerve. This nerve travelled rostrally with the carotid sinus nerve, away from the carotid body. We found no contributions of the superior laryngeal nerve to the carotid body.

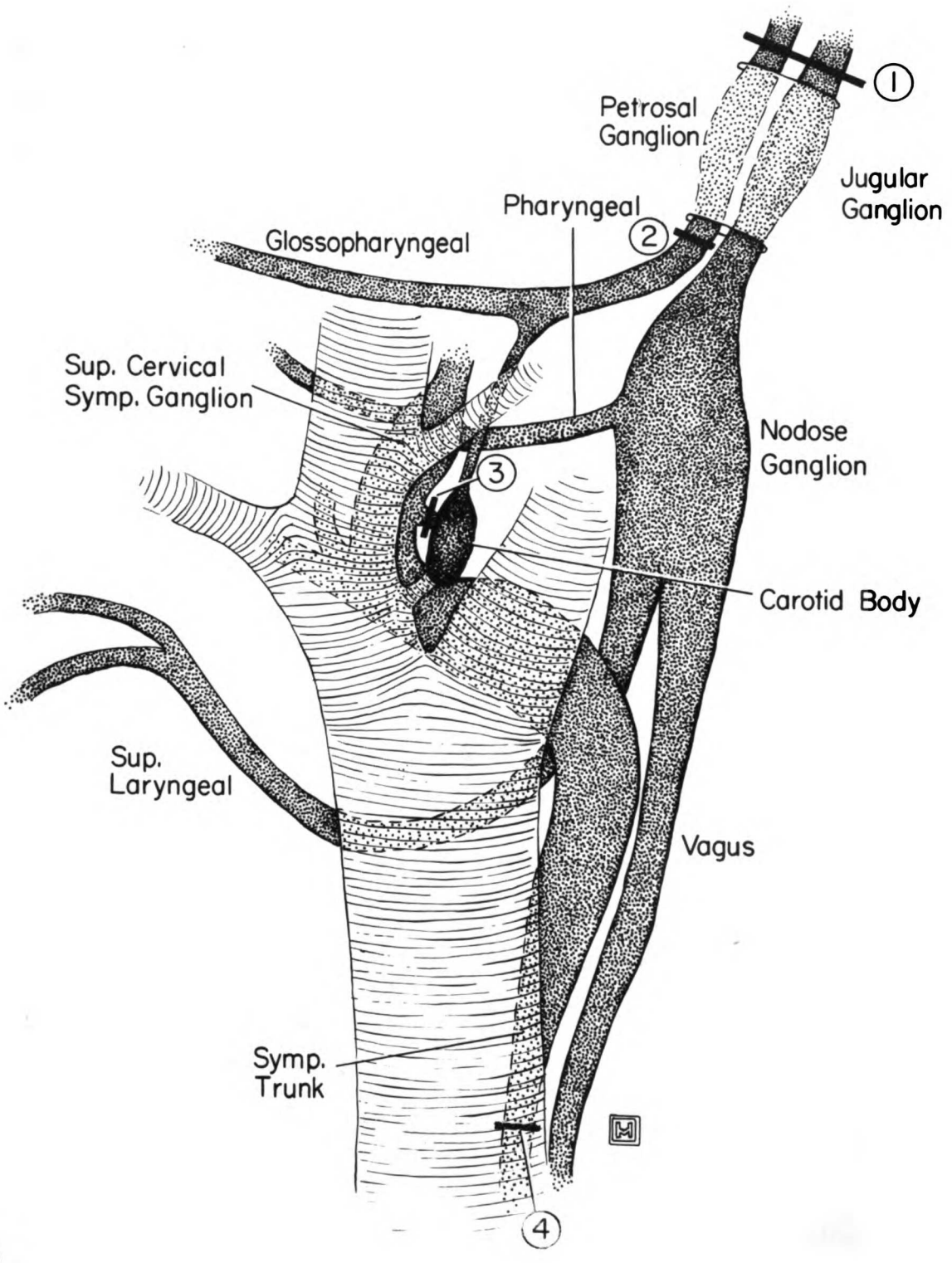
[5]We counted all glomus, sheath, and ganglion cells in serial 10 micron sections of a carotid body embedded in paraffin. Since we could not always distinguish glomus cells from sheath cells by light microscopy, we determined their proportions by electron microscopy (79% were glomus cells and 21% were sheath cells, Table II). By combining data from these light and electron microscopic studies, we calculated the total number of glomus cells and sheath cells.

Table II: Frequency* of Glomus and Sheath Cells.

Type of Cell	Glomus	Sheath
Frequency (Mean ± S.D.)	79 ± 3	21 ± 3
Range	73 - 85	15 - 27
Number of Cells Evaluated	5785	1501
Number of Carotid Bodies Evaluated	28	28

*Expressed as percent of total number of glomus and sheath cells.

Fig. 1. This drawing illustrates the relationship of the carotid body to adjacent nerves and ganglia (stippled) and arteries (contoured) in the Long-Evans rat. In our study of the innervation of glomus cells, we cut the glossopharyngeal nerve at (1) central to the petrosal ganglion or at (2) peripheral to the ganglion. To examine the sympathetic innervation of glomus cells and blood vessels, we cut postganglionic sympathetic nerves at (3) and preganglionic nerves at (4). The carotid sinus nerve (unlabelled) extends rostrally from the carotid body to join the glossopharyngeal nerve about 1 mm below the petrosal ganglion (for simplicity, branches of the carotid sinus nerve from the carotid sinus are omitted).


Petrosal Ganglion
Jugular Ganglion
Pharyngeal
Glossopharyngeal
Sup. Cervical Symp. Ganglion
Nodose Ganglion
Carotid Body
Sup. Laryngeal
Vagus
Symp. Trunk
1
2
3
4

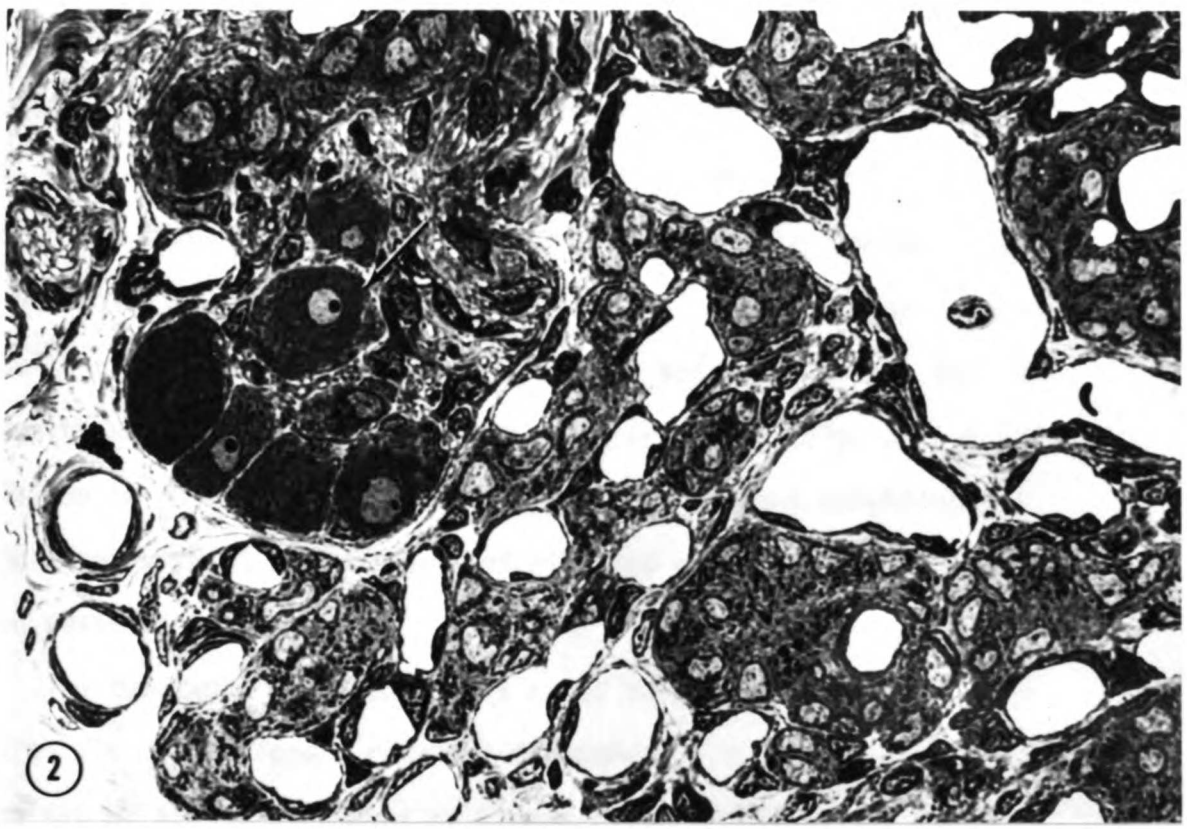
1

surround the abundant, tortuous, thin-walled blood vessels (Fig. 2). Glomus cells have a globular cell body, 8-15 microns in diameter, which has one or more cytoplasmic processes. Some processes are more than 40 microns long. The cytoplasm of glomus cells characteristically contains a large population of vesicles with electron-dense cores. Sheath cells are structurally similar to satellite cells in autonomic ganglia, have cytoplasmic processes that surround glomus cells, and do not contain dense-cored vesicles. Ganglion cells, 15-30 microns in diameter, occur separately or in small groups at the periphery of the carotid body where the carotid sinus and sympathetic nerves attach (Fig. 2).

II. Types of Glomus Cells

The carotid body of some species contains more than one type of glomus cell. In several electron microscopic studies two types of glomus cells (light and dark) have been distinguished on the basis of the elctron density of their cytoplasm (1,37,43,53,60). This criterion alone is not reliable because "dark cells" can be an artifact of fixation (21,22); therefore, additional criteria have been used. Kobayashi distinguished two types of glomus cells (chromaffin and nonchromaffin) in the dog carotid body on the basis of their capacity to reduce chromium salts (chromaffin reaction) and the size and electron density of their dense-cored vesicles (45). He found only nonchromaffin cells in the rat carotid body (46). Morita *et al.* distinquished four types of glomus cells in the cat carotid body (59), using size, shape, electron density, and number of dense-cored vesicles as

Fig. 2. This light micrograph illustrates a group of parasympathetic ganglion cells (upper left) where the carotid sinus nerve enters the carotid body. The arrow points to a ganglion cell with a round pale nucleus and a prominent dark nucleolus. Clusters of glomus cells and sheath cells surrounding thin-walled blood vessels fill most of the remainder of the micrograph. Blood vessels were emptied of blood by perfusing the fixative. Epon section, 1 micron thick, stained with toluidine blue. X700.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criteria for making this distinction.

Although glomus cells in the rat carotid body heretofore have been treated as a homogeneous population (18,42), our studies revealed two different types, which we call type A and type B. We distinguished the two types on the basis of the diameter and concentration of their dense-cored vesicles. The mean diameter of vesicles in type A cells is significantly larger than that of vesicles in type B cells (Figs. 3,4,5 and Table III), although the smallest vesicles of type A cells overlap in size the largest vesicles of type B cells (Fig. 6). Furthermore, dense-cored vesicles in type A cells are more than twice as abundant as those in type B cells (Table III). While the two types of glomus cells are similar in size, type A cells usually have a smooth, globular contour with few if any processes, and type B cells have an irregular contour with several long, thin processes (Fig. 3). A and B cells can be distinguished readily by these prominent morphological differences without measurements of size and concentration of dense-cored vesicles.

The two types of glomus cells exist in nearly equal proportions (Table IV). In different rats the frequency of type A varied from 33 to 69% of the total number of glomus cells. However, in each rat the frequency of type A cells was not significantly different in the right and left carotid bodies.[6] Most glomus cells can be classified as either type A or type B, but subtypes of A or B, not distinguishable by our criteria, may exist.

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[6]The mean difference in the frequency of type A glomus cells in the right and left carotid bodies of 6 rats was 2%. This difference was found not to be significant by using a paired t test.

Table III: Diameter and Concentration of Vesicles in Sections of Type A and B Glomus Cells.

	Vesicle Diameter (nm)		Vesicle Concentration*	
Type of Glomus Cell	A	B	A	B
Mean ± S.D.	116 ± 20	90 ± 11	3.4 ± 1.3	1.4 ± 0.6
p**	<0.001		<0.001	
Number of Glomus Cells Counted	6	10	50	26
Number of Vesicles Measured	530	334	5254	1159

*Vesicles per micron^2 of section(glomus cell cytoplasm, excluding the nucleus).

**Unpaired t test.

Table IV: Frequency* of Type A and B Glomus Cells.

Type of Glomus Cell	A	B
Frequency (Mean ± S.D.)	51 ± 10	49 ± 10
Range	33 - 69	31 - 67
Number of Glomus Cells Evaluated	1909	1799
Number of Carotid Bodies Evaluated	19	19

*Expressed as percent of total number of glomus cells.

Fig. 3. This electron micrograph illustrates two types of glomus cells in the rat carotid body. The type A glomus cell (below) is oval in shape and contains conspicuous large dense-cored vesicles. The type B glomus cell (above) has an irregular shape with a process extending down the right margin of the micrograph (arrow). It contains dense-cored vesicles that are smaller and less abundant than those in type A cells and tend to accumulate along the plasma membrane. X14,000.

Fig. 4. Large dense-cored vesicles (mean diameter 90 nm) from a type B glomus cell. X70,000.

Fig. 5. Large dense-cored vesicles (mean diameter 116 nm) and three synaptic vesicles without dense cores (mean diameter 53 nm) from a type A glomus cell. X70,000.

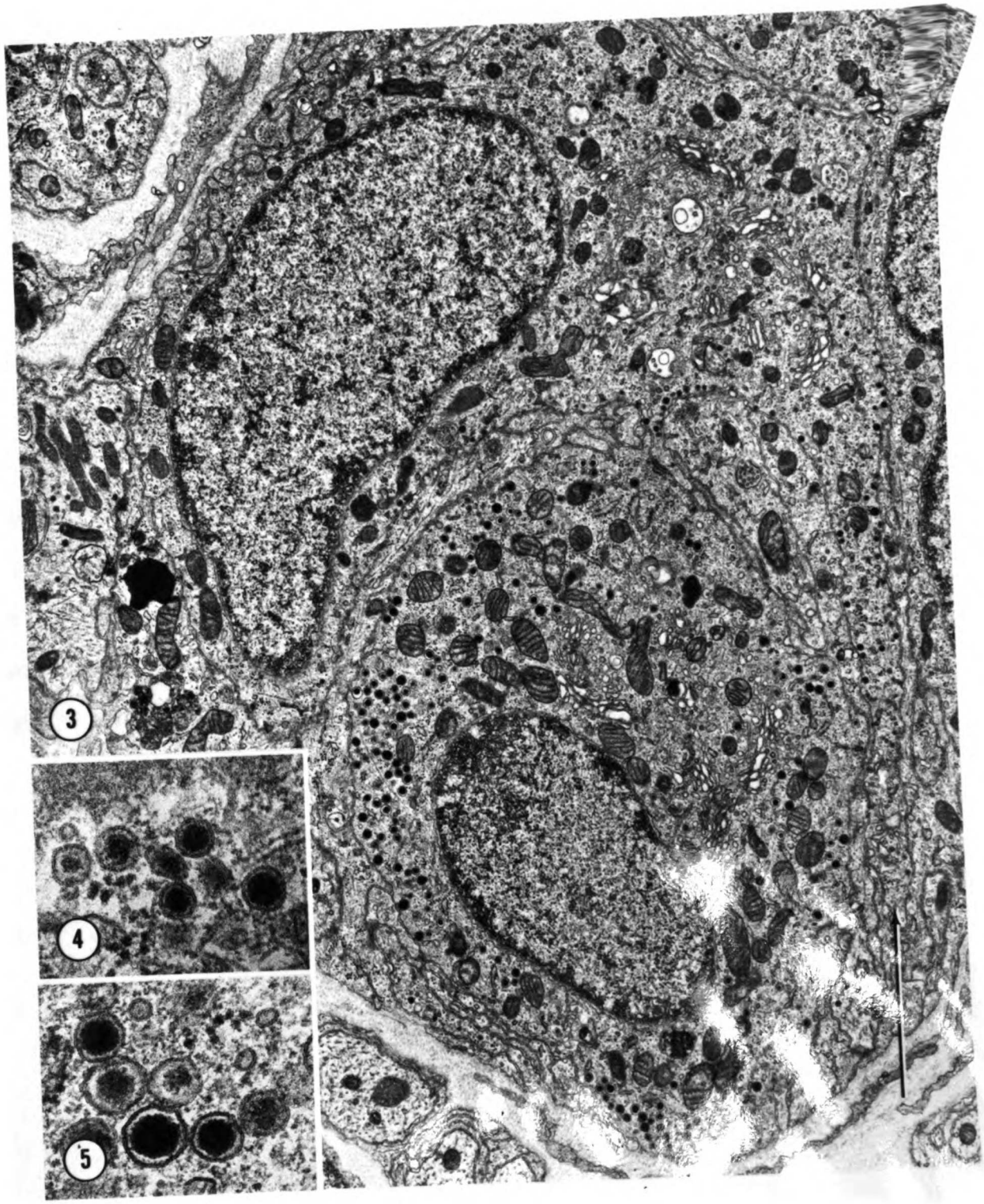
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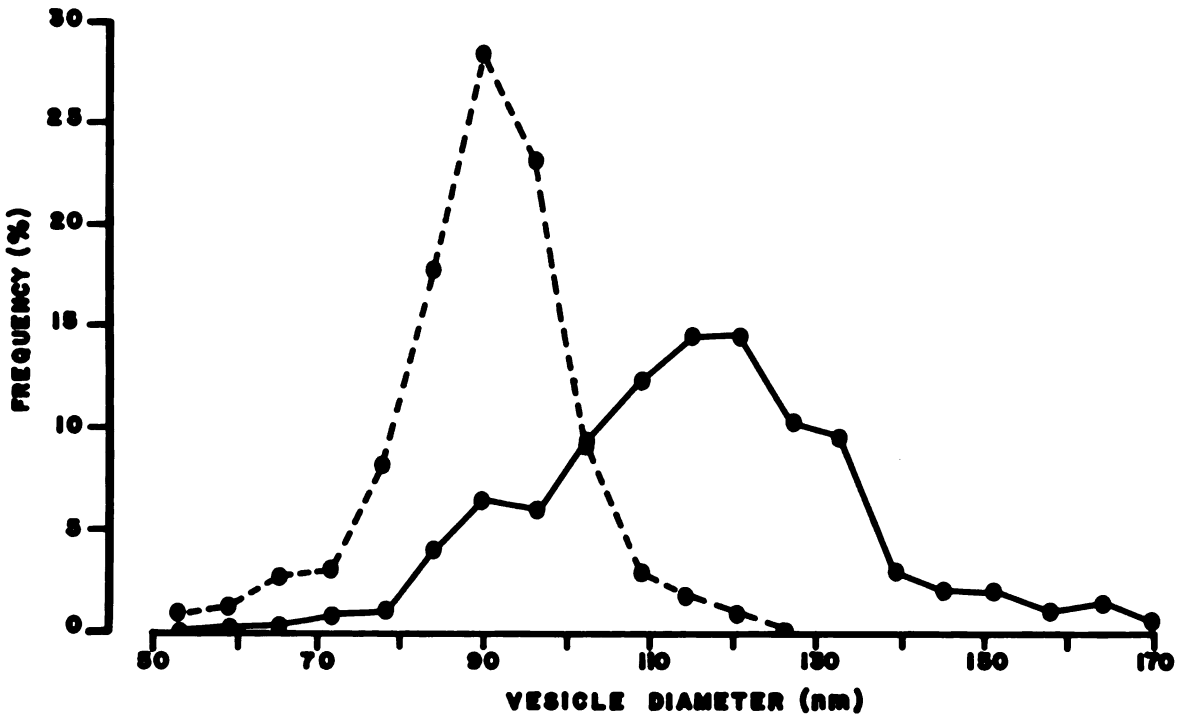
Fig. 6. This graph compares the size of large dense-cored vesicles in type A glomus cells (mean diameter 116 nm, solid line) and in type B cells (mean diameter 90 nm, broken line). The curves are based on measurements of 530 vesicles in 6 type A cells and 334 vesicles in 10 type B cells.


30
25
20
15
10
5
0
FREQUENCY (%)
50
70
90
110
130
150
170
VESICLE DIAMETER (nm)

6

III. Types of Nerves Ending on Glomus Cells

In describing the types of nerves which end on glomus cells, we use the terms afferent and efferent to define the direction of nerve conduction. Afferent nerves conduct toward the central nervous system (away from the carotid body) and efferent nerves conduct in the opposite direction. The terms presynaptic and postsynaptic designate the direction of synaptic transmission. In distinguishing afferent and efferent axons, we assume that, in the glossopharyngeal nerve, axons arising from cell bodies in its sensory (petrosal) ganglion[7] are afferent, and axons arising from cell bodies in the brain are efferent. These assumptions are based on results from neuroanatomical studies by Ramón y Cajal (71) and de Castro (25). We located the cell bodies of axons ending in the carotid body by determining which lesions caused these nerves to degenerate (assuming that axons and endings degenerate when severed from their cell body).

A. Glomus Cell Innervation by the Glossopharyngeal Nerve.

To determine the contribution by the glossopharyngeal nerve to the afferent and efferent innervation of glomus cells, we studied two groups of rats. In one group of 5 rats, we cut the right glossopharyngeal nerve peripheral to the petrosal ganglion to eliminate

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[7]Because of the proximity of the superior and inferior ganglia of the glossopharyngeal nerve in the rat, we consider them a single ganglion in this paper and use the term petrosal ganglion. In species in which the two ganglia are separate, the rostral ganglion is called the superior or jugular ganglion or the ganglion of Ehrenritter, and the caudal ganglion is called the inferior or petrous ganglion or the ganglion of Andersch.

travel in the glossopharyngeal nerve (lesion 2 in Fig. 1). In the other group of 5 rats, we cut the right glossopharyngeal nerve central to the petrosal ganglion to eliminate only endings of efferent axons that travel in the glossopharyngeal nerve (lesion 1 in Fig. 1).[8,9] One rat from both groups was killed at each of the following times after surgery: 1/4, 1, 4, 10, and 25 days. The left carotid bodies in all 10 rats served as controls. After perfusing the animals with fixative, we carefully exposed the glossopharyngeal nerve again to determine if the nerve had been cut completely and at the correct location. When the operation was found to be unsuccessful, the animal was discarded and the experiment repeated. When it was successful, we prepared the carotid bodies for electron microscopy and determined the effect of the operations on nerves endings on glomus cells.

1. Method for quantitating effects on glomus cell innervation caused by cutting the glossopharyngeal nerve. To compare the number

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[8] The sites peripheral and central to the petrosal ganglion at which we cut the glossopharyngeal nerve in the two groups of animals were separated by a distance of 3-4 mm. This value is based on the following measurements: 1) the carotid sinus and glossopharyngeal nerves (from the carotid body to the brain stem), 6 mm; 2) the carotid sinus nerve (from the carotid body to the glossopharyngeal nerve), 1.5-2.5 mm; 3) the glossopharyngeal nerve (from the entrance of the carotid sinus nerve to the caudal pole of the petrosal ganglion), 1 mm; 4) the petrosal ganglion, 2 mm; and 5) the glossopharyngeal rootlets (from the rostral pole of the ganglion to the brain stem), 1 mm.

[9] We anesthetized rats for surgery by injecting sodium methohexital (70 mg/kg) intraperitonally. In one group of rats, we exposed and then cut the glossopharyngeal nerve in the neck at its exit from the jugular foramen. In the other group, we exposed the dorso-lateral surface of the right cerebellar hemisphere, retracted it medially to expose the nerves, and cut the roots of the glossopharyngeal nerve near the surface of the brain stem. We also cut the spinal accessory nerve and roots of the vagus nerve. We used sutures to repair the dura, reattach muscles, and close the skin incision.

of nerves ending on glomus cells in the control and experimental carotid bodies, we counted glomus cells and nerve endings on glomus cells visible by electron microscopy in a single section (80 nm thick) from each carotid body. These counts were expressed as a ratio of nerve endings to glomus cells. We counted all glomus cells (both types A and B) whose nucleus appeared in the section. Only about half of the glomus cells in the section were visible, because the copper grid supporting the section in the electron microscope obscured nearly 50% of the section. To maximize the number of glomus cells in each section and to standardize the process of sampling, we embedded each carotid body whole in plastic and cut sections for electron microscopy from a plane through the center of its long axis. This procedure enabled us to examine an average of 200 glomus cells (range 110-355) in the section from each carotid body.

We counted all nerve endings on all glomus cells, not just on those cells whose nucleus appeared in the section. Structures counted as nerve endings satisfied three criteria: they 1) contained a uniform population of synaptic vesicles; 2) did not contain ribosomes; and 3) were enclosed by or next to a glomus cell or a sheath cell. Glomus cells and sheath cells did not meet these criteria. Glomus cells sometimes contained aggregates of synaptic vesicles, but invariably contained ribosomes too. Sheath cells never contained synaptic vesicles. Autonomic nerve endings on blood vessels were not counted because they seldom were surrounded by glomus or sheath cells.

2. Afferent innervation of glomus cells. The ratio of the number of nerve endings on glomus cells to the number of glomus cells averaged 1.0 in the control carotid bodies (Table V). The ratio of nerve endings

Table V: Ratio of Nerve Endings to Glomus Cells after Glossopharyngeal Nerve Is Cut Central or Peripheral to Petrosal Ganglion.

	Mean	S.D.	Range	Number Examined	
				Carotid Bodies	Glomus Cells
Controls	1.0	0.3	0.6 - 1.4	10	2092
Central	1.0*	0.1	0.9 - 1.1	5	1037
Peripheral	0.06**	0.04	0.01 - 0.1	4***	970

Significance of difference between control group and experimental group evaluated by Cochran's approximation test (85).

*not significant

**significant at the 5% level

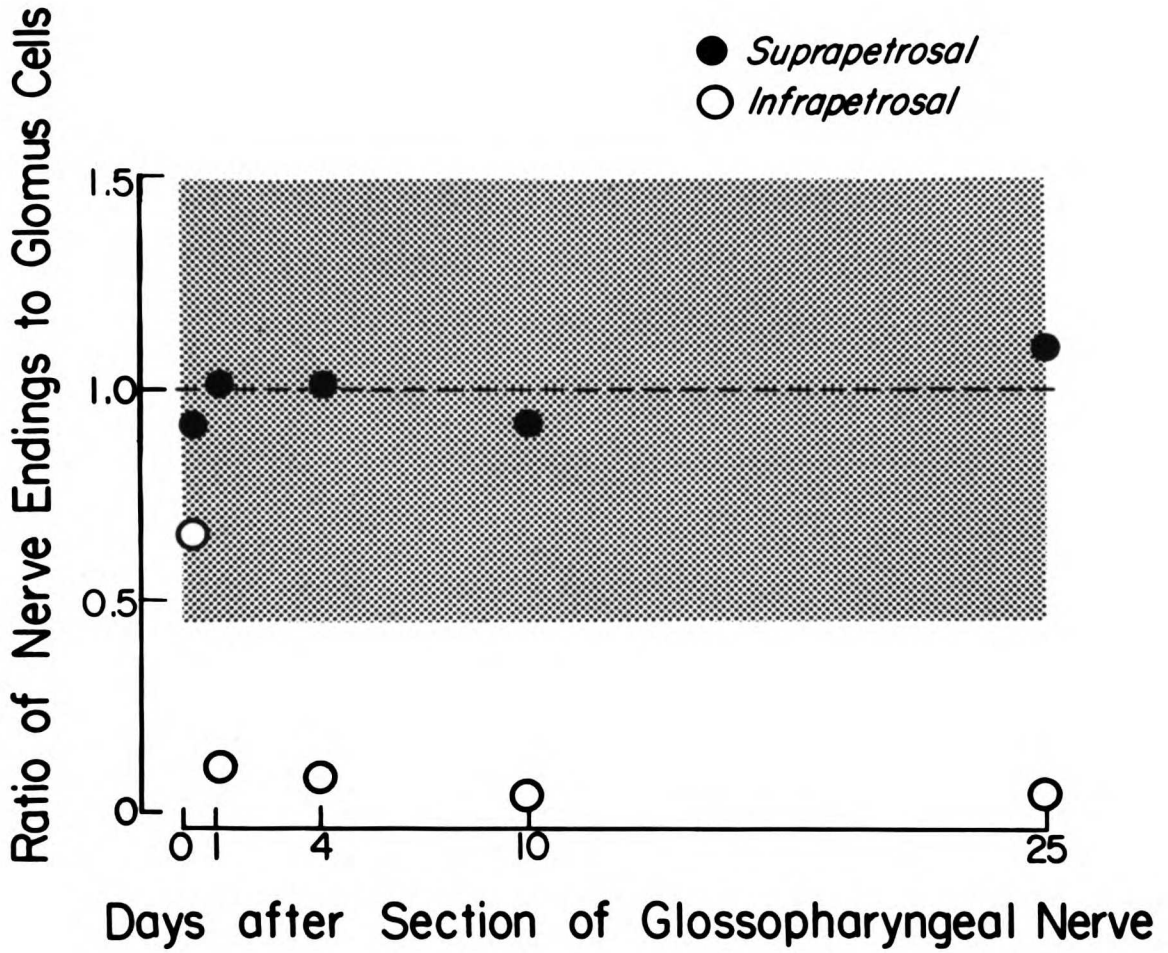
***carotid bodies 1, 4, 10 and 25 days after we cut the nerve

to glomus cells differed in the two experimental groups. In carotid bodies from the 5 rats in which we cut the glossopharyngeal nerve central to the petrosal ganglion, we found no morphological signs of degeneration of nerve endings on glomus cells, and the mean ratio of nerve endings to glomus cells did not deviate significantly from the control value (Fig. 7 and Table V). However, in carotid bodies from rats in which we cut the nerve peripheral to the ganglion, the ratio was significantly less than that of the controls (Table V). Six hours after we cut the glossopharyngeal nerve, the ratio had not decreased significantly, but many of the nerve endings on glomus cells were degenerating (Figs. 8-11). One day after we cut the nerve, the ratio was less than 10% of the control value, and at 10 and 25 days after surgery was less than 5% of the control value (Fig. 7). However, at all times normal endings were present on a few glomus cells.

Because there was more than a 95% reduction in the number of nerve endings on glomus cells after we cut the glossopharyngeal nerve peripheral to the petrosal ganglion but no measurable decrease after we cut the nerve central to the ganglion, at least 95% of endings have cell bodies in the petrosal ganglion. Therefore, we postulate that most nerves ending on glomus cells are afferent, and that the few normal endings remaining 25 days after surgery do not have their cell bodies in the petrosal ganglion and do not have axons in the glossopharyngeal nerve.

3. Degeneration of afferents. Several ultrastructural features characterized the degenerating afferent nerve endings. However, we have not determined the precise sequence in which afferent endings change structurally as they degenerate. Endings degenerate rapidly

Fig. 7. This figure illustrates that most nerves ending on glomus cells have their cell bodies in the petrosal ganglion. It compares the effect on glomus cell innervation of cutting the glossopharyngeal nerve central to the petrosal ganglion (suprapetrosal, solid circles) and cutting the nerve peripheral to the ganglion (infrapetrosal, open circles). The mean ratio of the number of nerve endings to the number of glomus cells in single sections from 10 control carotid bodies (2092 glomus cells) is 1.0 (broken line). The stippled region indicates the 95% confidence interval for the mean. After suprapetrosal section the ratio did not deviate significantly from the control value, but after infrapetrosal section the ratio decreased to less than 5% of the control value.


Suprapetrosal
Infrapetrosal
Ratio of Nerve Endings to Glomus Cells
1.5
1.0
0.5
0
0 1 4 10 25
Days after Section of Glossopharyngeal Nerve

(7)

and the process of degeneration apparently is not synchronized in all afferents; therefore, several stages of degeneration are present in the same carotid body.

Structural changes in afferent endings present 6 hours after the nerve was cut include: 1) endings separating from glomus cells and being enveloped by processes of sheath cells (Fig. 8); 2) mitochondria in endings becoming swollen or condensed (Figs. 8,9,10); 3) cytoplasmic matrix of endings aggregating to form flocculent electron- dense precipitates (Fig. 9); 4) synaptic vesicles in endings becoming clumped (Fig. 11); 5) entire endings condensing with their contents into electron-dense masses (Fig. 10); and 6) plasma membranes of endings disappearing and contents of endings being phagocytized by sheath cells (Fig. 11).

One day after the nerve was cut, a few endings on glomus cells appeared normal; the remainder of those still present exhibited the same degenerative changes that were prominent at 6 hours. Some sheath cells contained remnants of nerve endings, e.g., mitochondrial fragments, vesicles, whorls of membrane, and amorphous electron-dense precipitates, all presumably in lysosomes. However, we were impressed by the dearth of abnormal features, except for the reduction in the number of nerve endings on glomus cells.

Four days after the glossopharyngeal nerve was cut, most of the endings had disappeared from glomus cells. At this time there was little structural evidence of the events of four days earlier. Most of the debris had been cleared.

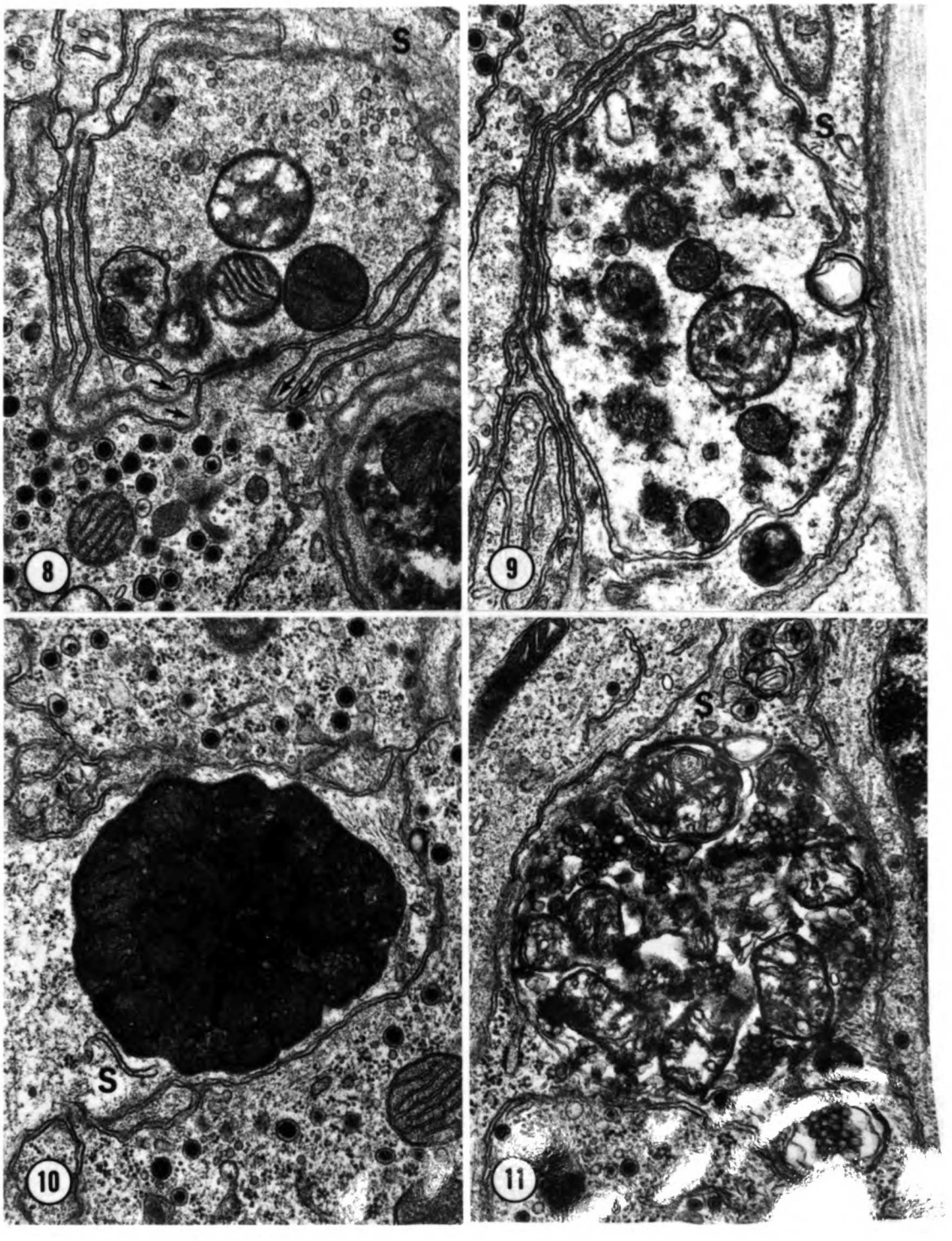
Figs. 8-11. Each of these four degenerating afferent nerve endings exhibits a different combination of structural changes 6 hours after axonal transection. S, sheath cell. X30,000.

Fig. 8. This ending has swollen mitochondria, is separating from a type A glomus cell (with large dense-cored vesicles), and apparently is being enveloped by processes of sheath cells (arrows). A portion of another degenerating ending is in the lower right corner.

Fig. 9. The cytoplasmic matrix of this ending has formed flocculent electron-dense precipitates. The ending is completely surrounded by a sheath cell.

Fig. 10. This ending has become condensed and appears as an electron-dense mass surrounded by a sheath cell.

Fig. 11. Synaptic vesicles in this ending are clumped and mitochondria are fragmenting. The ending has lost its plasma membrane and apparently is being phagocytized by a sheath cell.


S
8
S
9
S
10
S
11

B. Glomus Cell Innervation by Sympathetic Nerves.

To determine whether nerve endings on glomus cells which do not come from the glossopharyngeal nerve come from sympathetic nerves, we studied two groups of rats. In one group (3 rats) we removed the left superior cervical sympathetic ganglion (lesion 3 in Fig. 1) and cut the left carotid sinus nerve, then prepared the carotid bodies for electron microscopy 1/4, 1, and 20 days after the operation. We determined the ratio of nerve endings to glomus cells in the same manner as in the previous experiments. Six hours after the operation, many endings were degenerating; one day after the operation, the number of endings was only 1% of normal; and twenty days after the operation, we found no endings on glomus cells. Consequently, we postulate that the few nerves ending on glomus cells that do not travel in the glossopharyngeal nerve travel instead with the sympathetics.

In the other group (2 rats) we cut the left superior cervical sympathetic trunk (lesion 4 in Fig. 1) instead of removing the sympathetic ganglion. We killed both rats 10 days after surgery. These carotid bodies had a normal complement of afferent endings but no endings resembling those remaining on glomus cells after the glossopharyngeal nerve is cut (see description of endings below). Therefore, we postulate that the few sympathetic nerves innervating glomus cells are preganglionic sympathetics and that they are efferent.

C. Structural Differences Between Endings of Afferent and Efferent Nerves.

To characterize the structure of afferent and efferent nerve endings we determined their size, measured the diameter of their vesicles, and

calculated the concentrations of their vesicles and mitochondria.

1. Size and shape of nerve endings. Nerve endings on glomus cells vary in size and shape. Although investigators have distinguished two types of nerve endings by using size and shape of endings as criteria (6,29,60), Biscoe and Pallot (13) reconstructed endings from electron micrographs of serial sections, and found that a single axon can give rise both to small and to large endings.

In the rat carotid body some endings on glomus cells have the form of small boutons (Fig. 12), and others the form of large calyces (Fig. 13). Our experiments showed that all calyceal endings and most boutons are connected to afferent axons (Figs. 12,13, 14). However, a few boutons are connected to preganglionic sympathetic axons (Figs. 12,15, 28). Because some afferents and all efferents have bouton-shaped endings, and because calyceal endings appear bouton-like when cut in some planes of section, we could not distinguish afferent and efferent endings by their size and shape alone.

2. Size of vesicles in nerve endings. Endings of both afferent and efferent nerves contain two populations of vesicles: synaptic vesicles with electron-lucent cores and large vesicles with electron-dense cores. Afferent endings, having either a calyceal or bouton shape, contain synaptic vesicles with a mean diameter of 61 nm (Fig. 16). Efferent boutons contain synaptic vesicles with a mean diameter of 53 nm (Fig. 16). The size difference of synaptic vesicles in afferent and efferent endings (Table VI) is sufficient to be apparent in electron micrographs (compare Fig. 17 with Fig. 18). Afferent and efferent endings contain dense- cored vesicles with a mean diameter of 95-97 nm (Table VI).

Fig. 12. This electron micrograph illustrates afferent and efferent nerve endings near a type A glomus cell. S, sheath cell. X14,000. The inset shows (within the dotted line) the region appearing in the micrograph and outlines the glomus cell (G), efferent nerve ending (E), afferent nerve endings (cross-hatching), and preterminal afferent axons (cross-hatching plus asterisk).

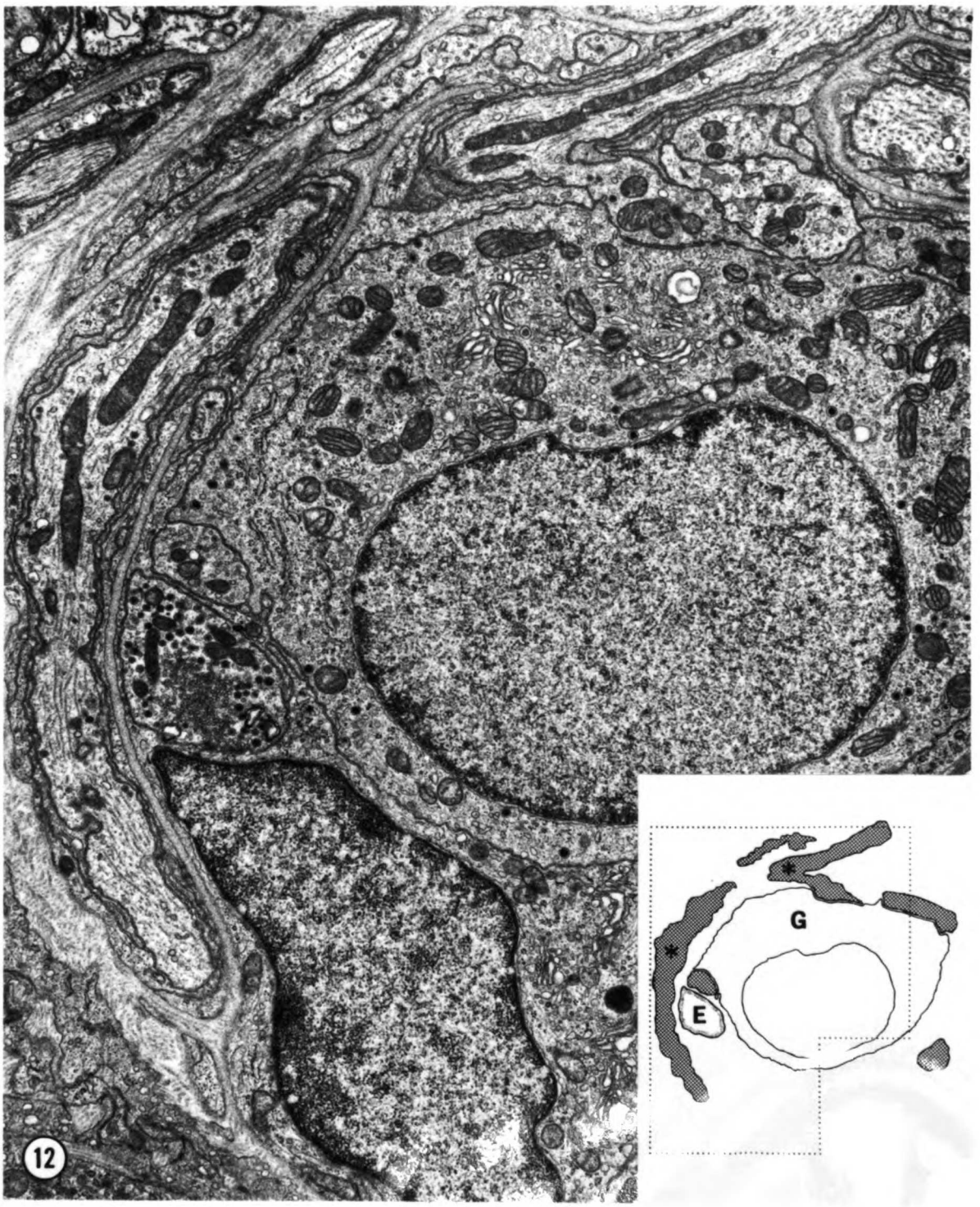
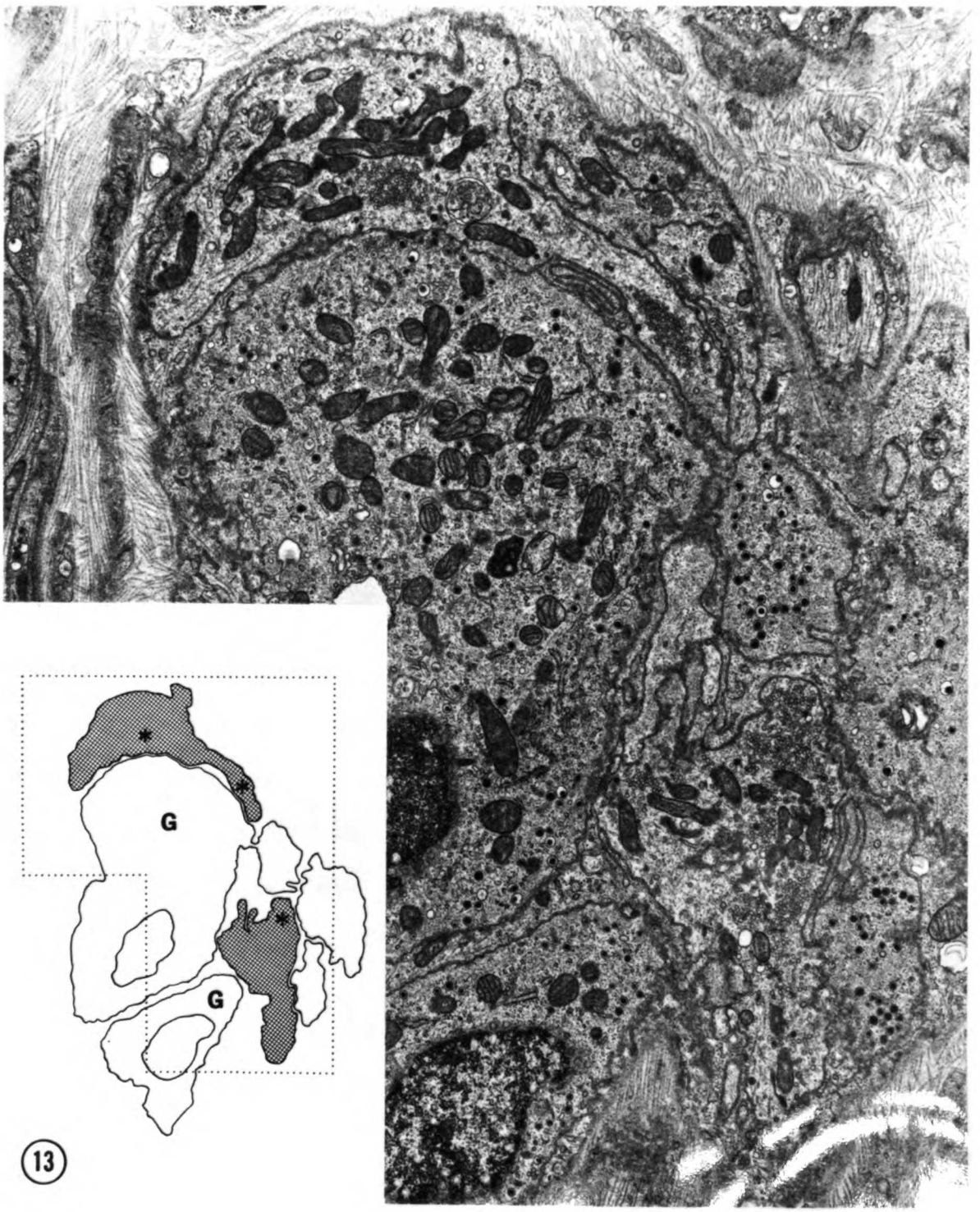

G
E
12

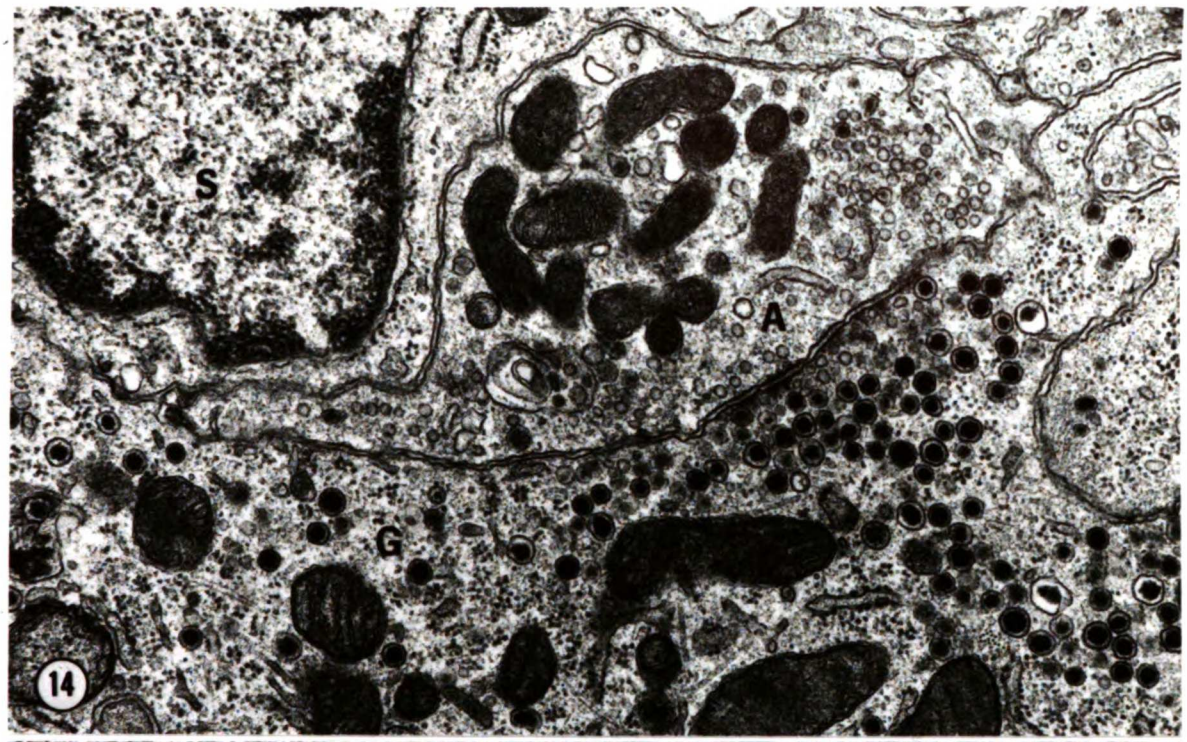
Fig. 13. This electron micrograph illustrates two portions of a large afferent nerve ending next to two type A glomus cells. X 14,000. The inset shows (within the dotted line) the region appearing in the micrograph and outlines the glomus cells (G) and afferent nerve endings (cross-hatching). Some aggregates of synaptic vesicles in afferent nerve endings are marked by asterisks.



13

Fig. 14. A (glossopharyngeal) afferent nerve ending (A) with scattered synaptic vesicles is shown next to a type A glomus cell (G). Most nerve endings on glomus cells resemble this one, although size and shape of endings vary tremendously. S, sheath cell. X30,000

Fig. 15. A (preganglionic sympathetic) efferent nerve ending (E) with densely-packed synaptic vesicles is presynaptic to a type A glomus cell (G). The glossopharyngeal nerve was cut peripheral to the petrosal ganglion one day before fixation and most afferent nerve endings in the carotid body had degenerated. Endings similar to this one comprise less than 5% of endings on glomus cells in the normal carotid body. S, sheath cell. X30,000.


S
A
G
14

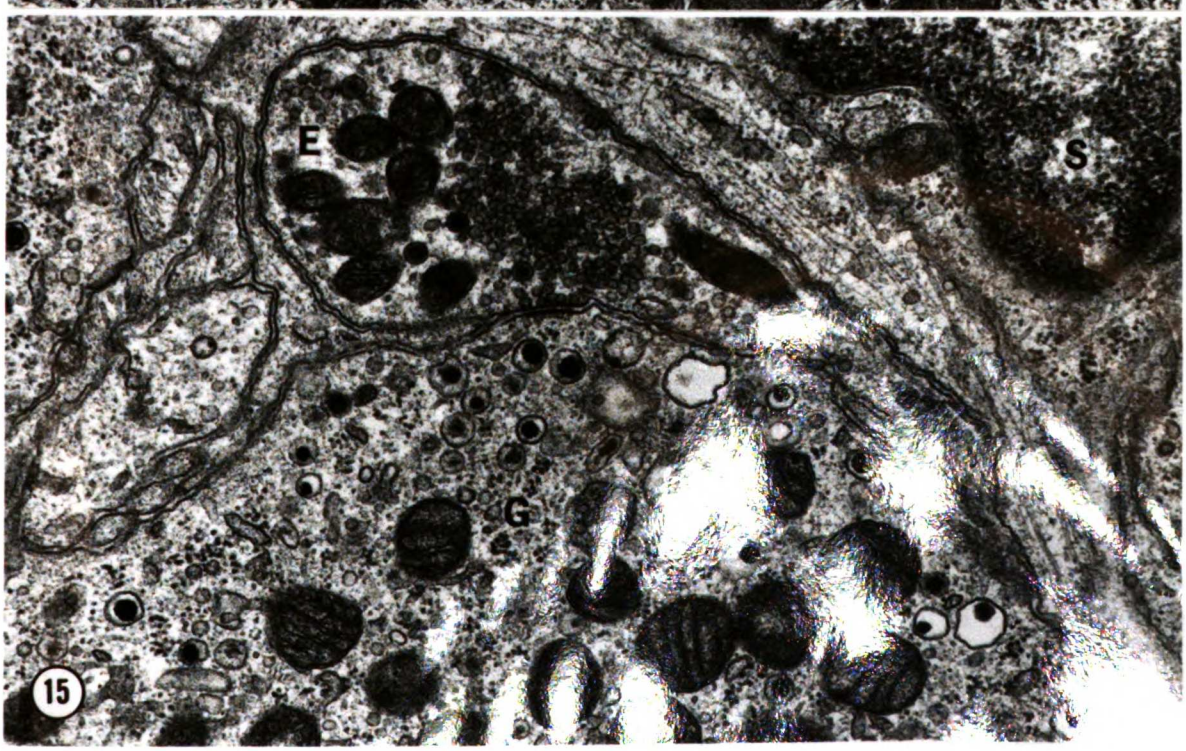
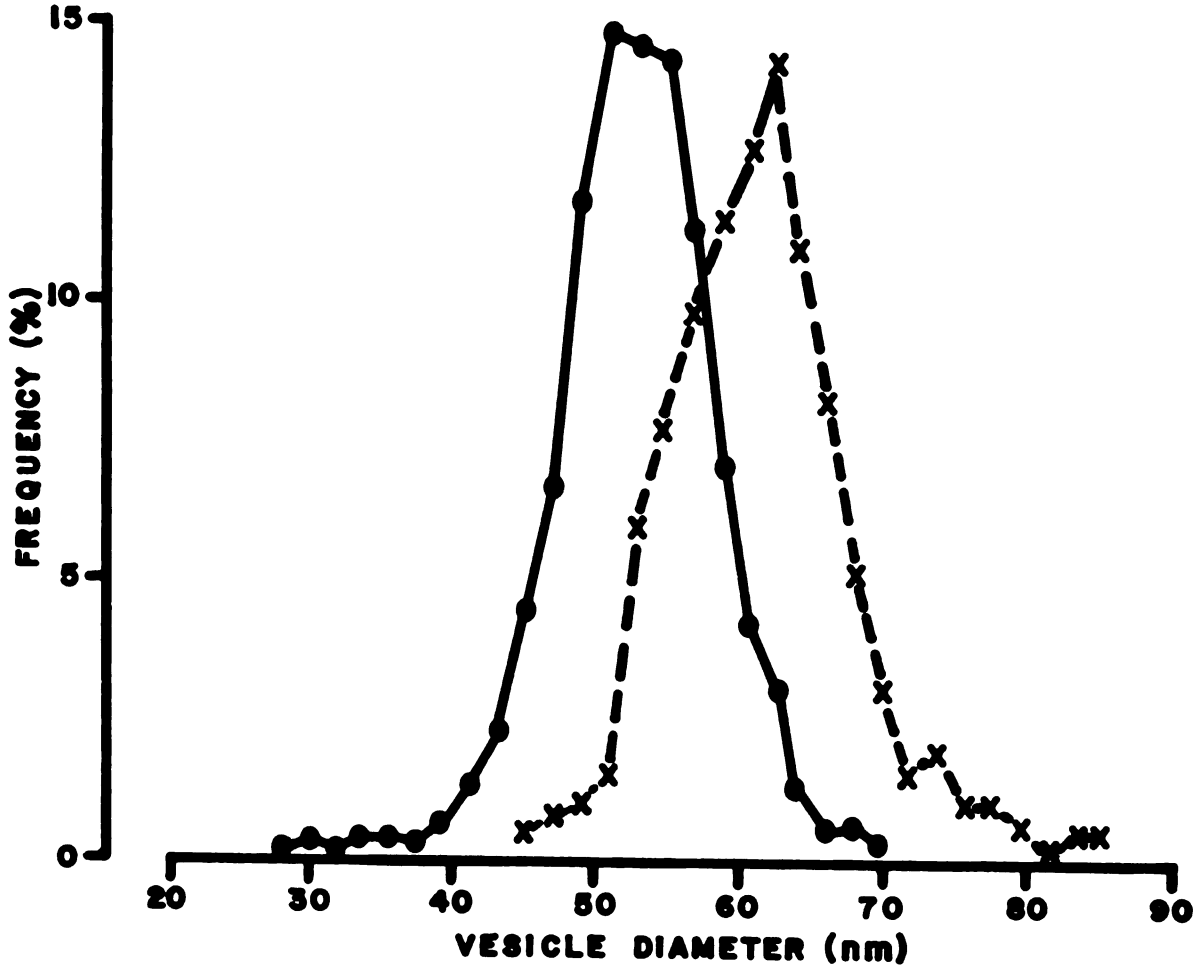

E
S
G
15

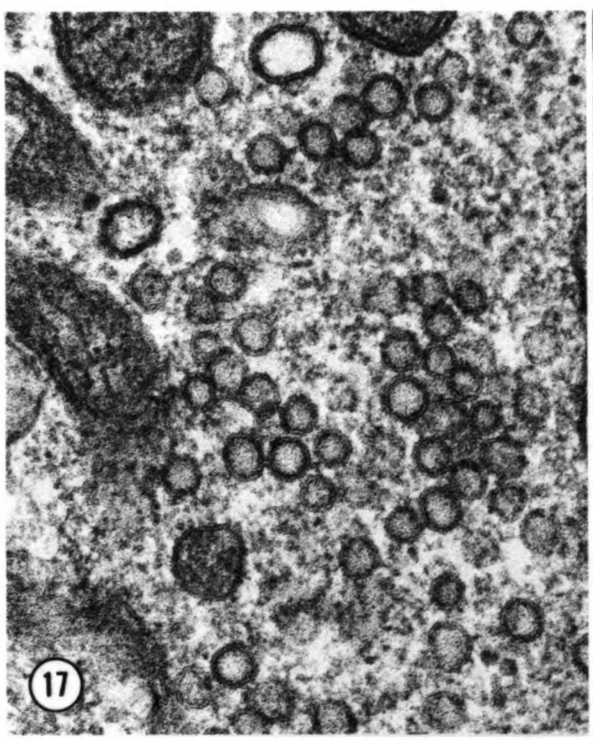
Fig. 16. This graph compares the size of synaptic vesicles in afferent nerve endings (mean diameter 61 nm, broken line) and efferent nerve endings (mean diameter 53 nm, solid line). The curves are based on measurements of 1647 vesicles in 28 afferent nerve endings and 2539 vesicles in 19 efferent endings.



(16)

Fig. 17. These relatively large, scattered synaptic vesicles in an afferent nerve ending have a mean diameter of 61 nm. X90,000.

Fig. 18. These relatively small, closely packed synaptic vesicles in an efferent nerve ending (on a type A glomus cell) have a mean diameter of 53 nm. Note the large dense-cored vesicles which are nearly five times as frequent as are similar vesicles in afferent nerve endings. X90,000.



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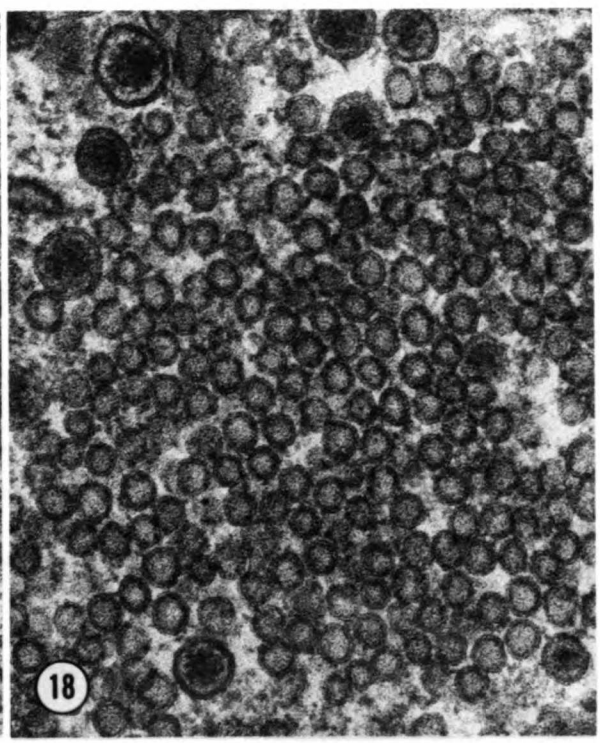


18

Table VI: Diameter* of Vesicles in Sections of Nerve Endings on Glomus Cells.

Type of Ending	Afferent	Efferent	p**
Synaptic Vesicles	61 ± 7	53 ± 6	<0.001
Large Dense-Cored Vesicles	95 ± 14	97 ± 11	N.S.
Number of Endings	28	19	
Number of Synaptic Vesicles	1647	2539	
Number of Large Dense-Cored Vesicles	88	309	

*Outside diameter in nm (mean ± S.D.)

**Unpaired t test for differences between afferent and efferent nerve endings; N.S. = not significantly different.

3. Concentration of vesicles and mitochondria in nerve endings

The mean concentration of synaptic vesicles in afferent nerve endings is 29 per micron^2 of cytoplasm, in efferent endings 76 per micron^2 (Table VII). A characteristic of efferent endings is their closely-packed, relatively small synaptic vesicles (compare Figs. 14 with 15, and 17 with 18), a characteristic they share with nerve endings on ganglion cells and blood vessels (see Sections V and VI), but not with afferent endings. While localized aggregations of synaptic vesicles exist in some afferent endings (Fig. 13), the mean concentration of synaptic vesicles is comparatively low.

The mean concentration of large dense-cored vesicles in afferent endings is 2 per micron^2, in efferent endings 9 per micron^2 (Table VII). A high concentration of large dense-cored vesicles is peculiar to efferent endings on glomus cells, being at least twice that of any other type of nerve ending in the carotid body. The mean concentration of mitochondria is the same in afferent and efferent nerve endings (Table VII).

IV. Synaptic Connections of Glomus Cells

Both afferent and efferent nerves are connected to glomus cells by synapses. Synapses are common between nerves and type A cells, but rare between nerves and type B cells. More than 99% of 1392 nerve-glomus cell synapses that we examined in sections from 19 carotid bodies involved type A cells; less than 1% involved type B cells. In fact, nerve endings usually were separated from type B cells by sheath cells. Consequently, our data apply to synapses involving nerves and type A

Table VII: Concentration* of Vesicles and Mitochondria in Sections of Nerve Endings on Glomus Cells.

Type of Ending	Afferent	Efferent	p**
Synaptic Vesicles	29 ± 14	76 ± 32	< .001
Large Dense-Cored Vesicles	2 ± 1	9 ± 3	< .001
Mitochondria	2 ± 1	2 ± 1	N.S.
Number of Endings	28	19	
Area per Ending (Micron^2)	2 ± 1	2 ± 1	N.S.

*Vesicles or mitochondria per micron^2 of section (mean ± S.D.).

**Unpaired t test for differences between afferent and efferent endings; N.S. = not significantly different.

cells. We assume that most type B cells are not innervated by afferent or efferent nerves.

A. Criteria for Identifying Synapses.

We used the same features to identify synapses in the carotid body that characterize chemical synapses elsewhere in the nervous system: synaptic vesicles at a specialized junction involving the pre- and postsynaptic membranes (5,68). The synaptic junction is distinctive because cytoplasmic densities are attached asymmetrically to the pre- and postsynaptic membranes (Fig. 23). The cytoplasmic density on the presynaptic membrane is divided into roughly cone-shaped, presynaptic dense projections. These projections form a lattice near which synaptic vesicles aggregate. The cytoplasmic density on the postsynaptic membrane forms the postsynaptic web. We did not consider puncta adherentia as part of the structure of synapses, although they have been so considered by some investigators who have studied the carotid body (1,19,39). These junctions, in which cytoplasmic densities are attached symmetrically to apposing membranes (68), are common between nerve endings and glomus cells and between adjacent glomus cells (7,50).

B. Synapses Between Afferent Nerves and Glomus Cells.

Afferent nerve endings and glomus cells are linked by four types of synaptic connections: 1) endings presynaptic to glomus cells; 2) endings postsynaptic to glomus cell bodies; 3) endings postsynaptic to processes of glomus cells; and 4) endings connected by reciprocal synapses to glomus cells. The connections presumably do not define four different types of afferent endings, but merely different regions of afferent endings.

We determined the proportion of afferent endings displaying each type of connection in single sections, not from serial reconstructions. These proportions indicate the relative frequency of each connection, not the total number of synaptic connections of an average afferent nerve ending or that some endings have no synaptic connections with glomus cells.

1. Afferent nerves presynaptic to glomus cells. Afferent nerve endings are presynaptic to glomus cells. About 20% of endings in each section contain vesicles that aggregate near synaptic junctions (Fig. 19). The junctions have one to three presynaptic dense projections, which are spaced about 150 nm peak to peak and extend 50-70 nm into the cytoplasm of the ending. The postsynaptic web extends about 30 nm into the glomus cell cytoplasm and about 220 nm laterally along the postsynaptic membrane. The synaptic cleft is about 13 nm wide and contains granular, electron-dense material appearing as an indistinct band equidistant from the pre- and postsynaptic membranes (Fig. 19). This band occupies about half the width of the cleft.

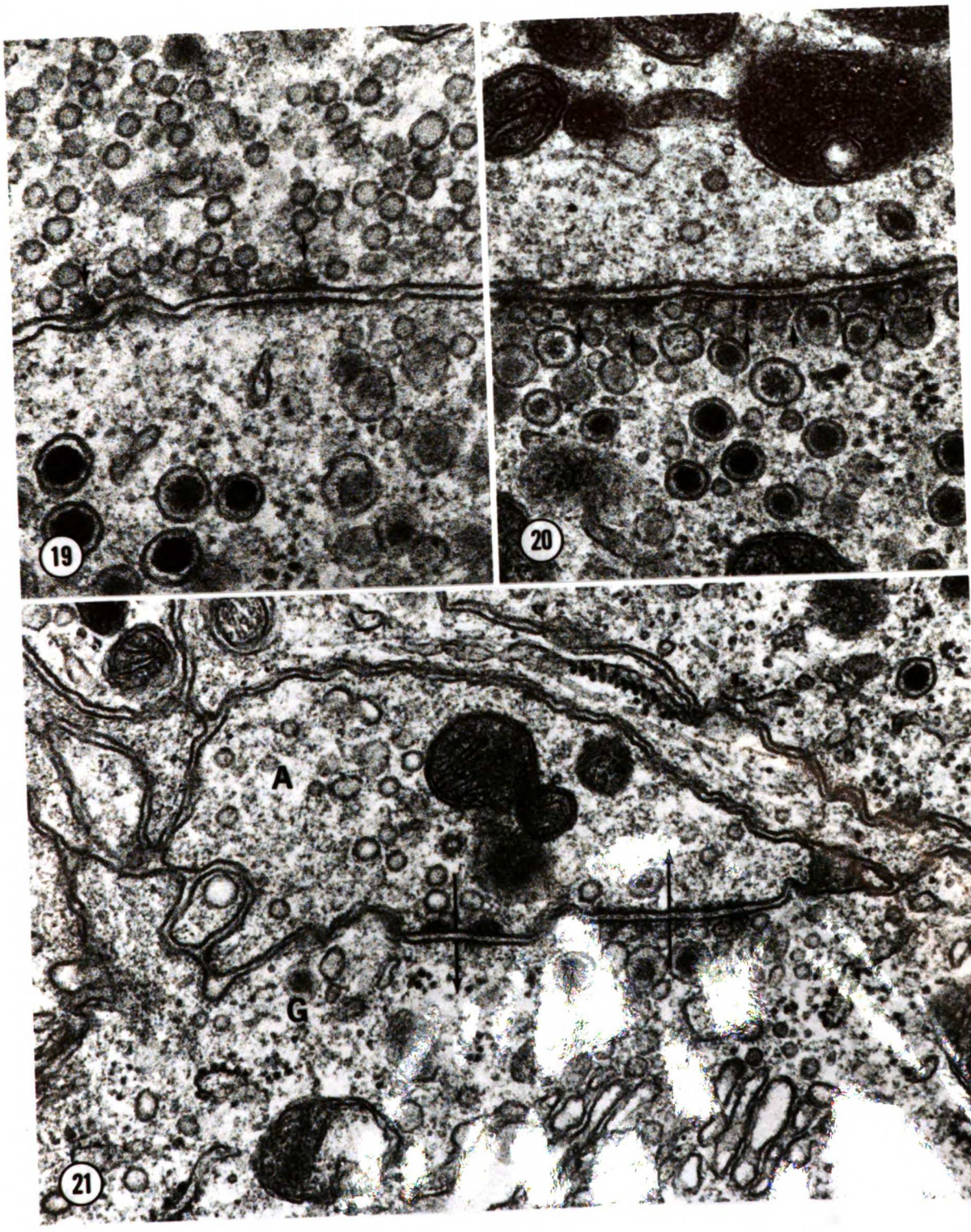
Both calyceal and bouton-shaped afferent endings are presynaptic to glomus cells. While many endings in a section have no synapses, others have two or three. Because all afferent endings on glomus cells contain synaptic vesicles, we presume that all afferents are presynaptic to glomus cells and that the small size and limited distribution of synaptic junctions over the extensive plasma membrane of endings restrict the number of synapses appearing in single sections.

2. Afferent nerves postsynaptic to glomus cell bodies. In addition to being presynaptic, afferent endings are postsynaptic to glomus cells. Synapses involving glomus cell bodies differ in structure from those involving glomus cell processes; consequently, we discuss them separately.

Fig. 19. This afferent nerve ending (above) is presynaptic to a type A glomus cell. Synaptic vesicles in the ending accumulate near presynaptic dense projections (arrows) which are part of the synaptic junction. X70,000.

Fig. 20. This afferent nerve ending (above) is postsynaptic to a type A glomus cell. Synaptic vesicles and large dense-cored vesicles in the glomus cell accumulate near presynaptic dense projections (arrows) which are part of a broad synaptic junction. Relatively few vesicles are present in this region of the nerve ending. X70,000.

Fig. 21. This afferent nerve ending (A) is connected to a type A glomus cell (G) by a reciprocal synapse. Arrows mark the two components of the reciprocal synapse. One component resembles the synapse shown in Fig. 19; the other resembles the synapse in Fig. 20. X55,000.


19
20
A
G
21

About 30% of afferent endings are postsynaptic to glomus cell bodies in single sections. Two types of vesicles accumulate at the synaptic junctions: large dense-cored vesicles and synaptic vesicles (Figs. 14, 20,23). Large dense-cored vesicles at synapses (mean diameter 116 nm) are typical of those present throughout the glomus cell cytoplasm. Synaptic vesicles (mean diameter 53 nm) rarely have an electron-dense core, accumulate mainly at synapses, and form a population distinct in size from large dense-cored vesicles (Fig. 22). The ratio of synaptic vesicles to large dense-cored vesicles averaged 1:1 (range 1:4 to 8:1) at ten synapses in one carotid body.[10] The synaptic junction at these synapses is made conspicuous by up to ten presynaptic dense projections, which are spaced about 110 nm peak to peak and extend 60-90 nm into the glomus cell cytoplasm (Figs. 20,23). Each junction occupies as much as 1.5 microns of the synaptic membrane. The postsynaptic web extends about 30 nm into the cytoplasm of the ending. The synaptic cleft, about 15 nm wide, contains electron-dense material that forms a prominent granular band midway between the pre- and postsynaptic membranes and fills half the width of the cleft.

Both calyceal and bouton-shaped afferent endings are involved in this type of synapse. Furthermore, one glomus cell may form several synapses with an ending, and an ending may be connected synaptically to several glomus cells.

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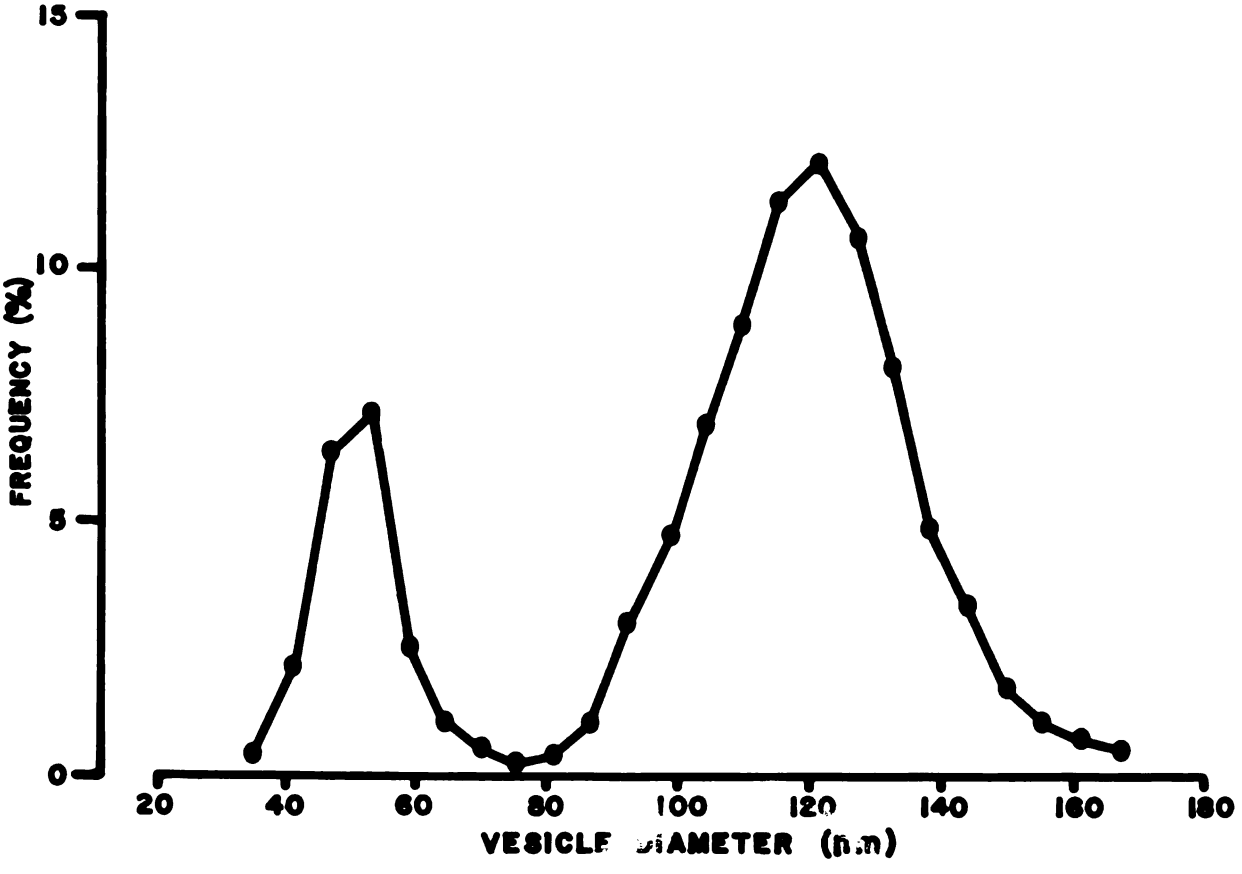
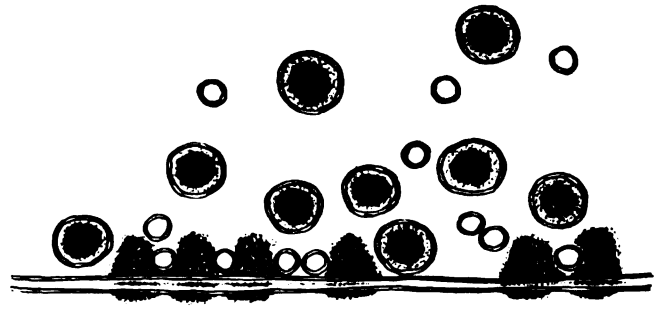
[10] To calculate the ratios of these two types of vesicles at synapses, we counted the vesicles in a rectangular region of the glomus cell cytoplasm adjacent to synaptic junctions. The length of this rectangle equalled the lateral extent of the synaptic junction and the width was 1 micron.

Fig. 22. This graph illustrates the bimodal distribution of vesicles based on size in type A glomus cells. The minor peak (left) is formed by synaptic vesicles (mean diameter 53 nm), most of which lack dense cores (see Figs. 20,26). Synaptic vesicles are abundant at synapses but account for only 20% of vesicles in glomus cell bodies. The major peak is formed by large dense-cored vesicles (mean diameter 116 nm).


15
10
5
0
FREQUENCY (%)
20
40
60
80
100
120
140
160
180
VESICLE DIAMETER (nm)

(22)

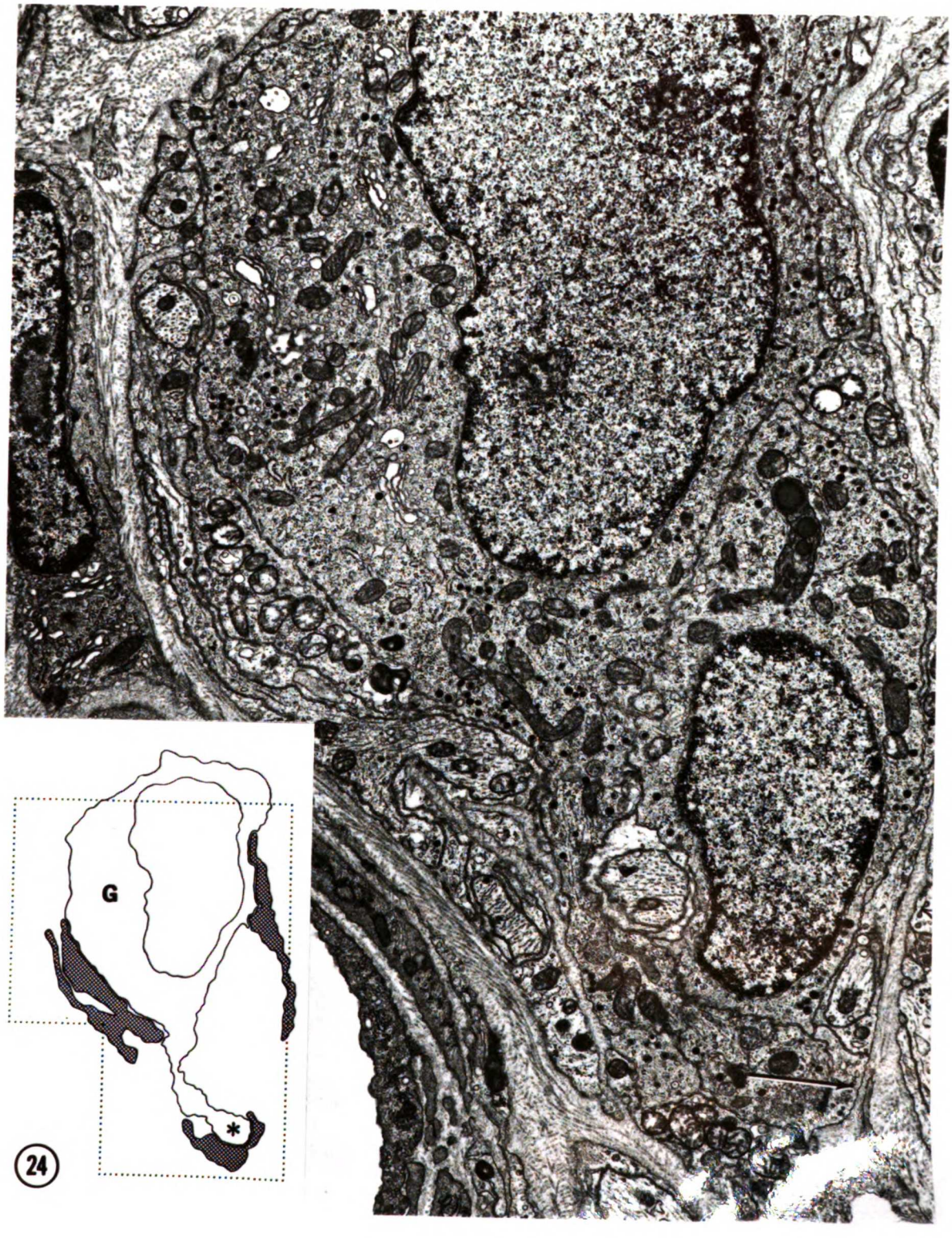
Fig. 23. This drawing summarizes the structure of synapses at which type A glomus cells (above) are presynaptic either to afferent nerves or to other glomus cells. Synaptic vesicles and large dense-cored vesicles accumulate at a synaptic junction which is characterized by prominent presynaptic dense projections, a thin dense band in the synaptic cleft, and a small postsynaptic web. X70,000.



3. Afferent nerves postsynaptic to glomus cell processes. Glomus cell processes, like dendrites of neurons, contain most types of organelles found in the cell body and extend tens of microns from the cell body. These processes, like their cell bodies, are presynaptic to afferent nerve endings. This point may explain the observation that some "axons" are presynaptic to afferent nerve endings (88).

In single sections, about 5% of afferent endings are postsynaptic to cell processes which resemble nerve endings: they contain many synaptic vesicles - a few with dense cores - and few large dense-cored vesicles (Figs. 26,27). Because many processes have no attachment to their cell body in the plane of section appearing in single electron micrographs, the identity of the processes puzzled us initially. We now have evidence that they are dendritic processes of type A glomus cells, not endings of axons. 1) Type A cell processes which are presynaptic to afferent endings have the same structure as the processes in question (Fig. 24). 2) Operations in which we cut the glossopharyngeal nerve and removed the superior cervical sympathetic ganglion did not eliminate the processes. 3) The processes contain synaptic vesicles and large dense-cored vesicles with mean diameters identical to those of vesicles in type A cell bodies. 4) The processes contain large dense-cored vesicles with a mean diameter more than 30% larger than that of large dense-cored vesicles in efferent nerve endings on glomus cells (Fig. 25), although their synaptic vesicles have a mean diameter the same as that of synaptic vesicles in efferent endings. 5) The processes contain ribosomes, some free in the cytoplasm and others attached to membranous cisternae, but nerve endings do not (compare Fig. 27 with 28). Consequently, we assume that the processes

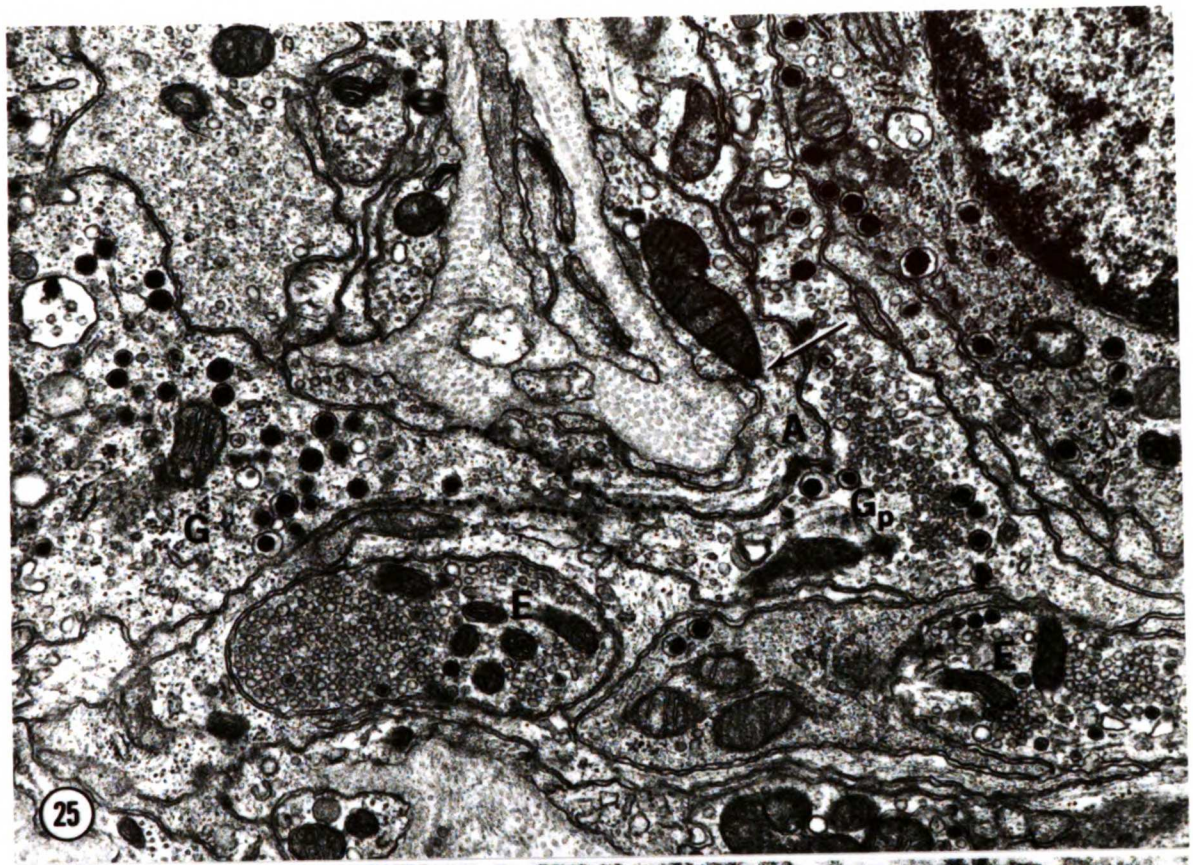
Fig. 24. This electron micrograph illustrates a type A glomus cell with a process that is presynaptic to an afferent nerve ending (arrow). X14,000. The inset shows (within the dotted line) the region appearing in the micrograph and outlines the glomus cell (G) and afferent nerve endings (cross-hatching). The asterisk marks the region of the glomus cell process which contains an accumulation of synaptic vesicles and is presynaptic to an afferent nerve ending.



24

Fig. 25. This micrograph shows a type A glomus cell (G) giving rise to a process (G_p) which is presynaptic (arrow) to an afferent nerve ending (A). Synaptic vesicles are abundant in the process near the synapse. Compare this process with two efferent nerve endings (E) which contain synaptic vesicles similar in size but contain large dense-cored vesicles smaller than those in glomus cells. X24,000.

Fig. 26. A type A glomus cell process (G_p) presynaptic to an afferent nerve ending (A) is shown at high magnification in this micrograph. Only one presynaptic dense projection (arrow) appears in this section of the synapse. Synaptic vesicles in the glomus cell process greatly outnumber large dense-cored vesicles (compare with Fig. 20, which shows a glomus cell body presynaptic to an afferent nerve ending). Large dense-cored vesicles in the process are identical in size to those in glomus cell bodies. X90,000.


A
G
Gp
E
E
25

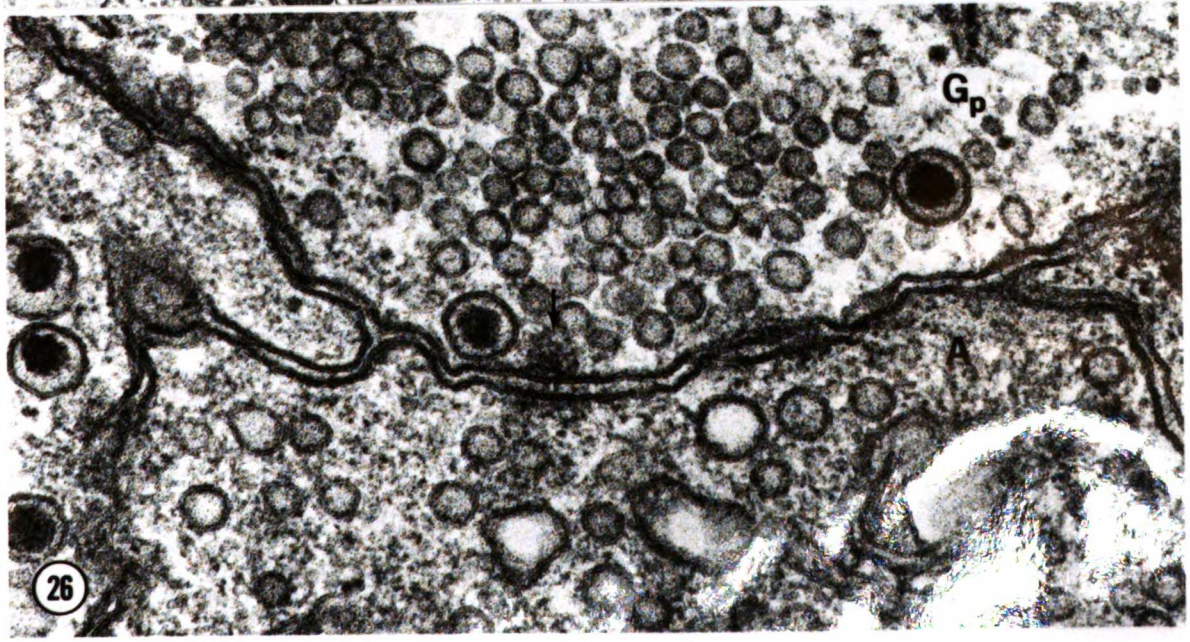
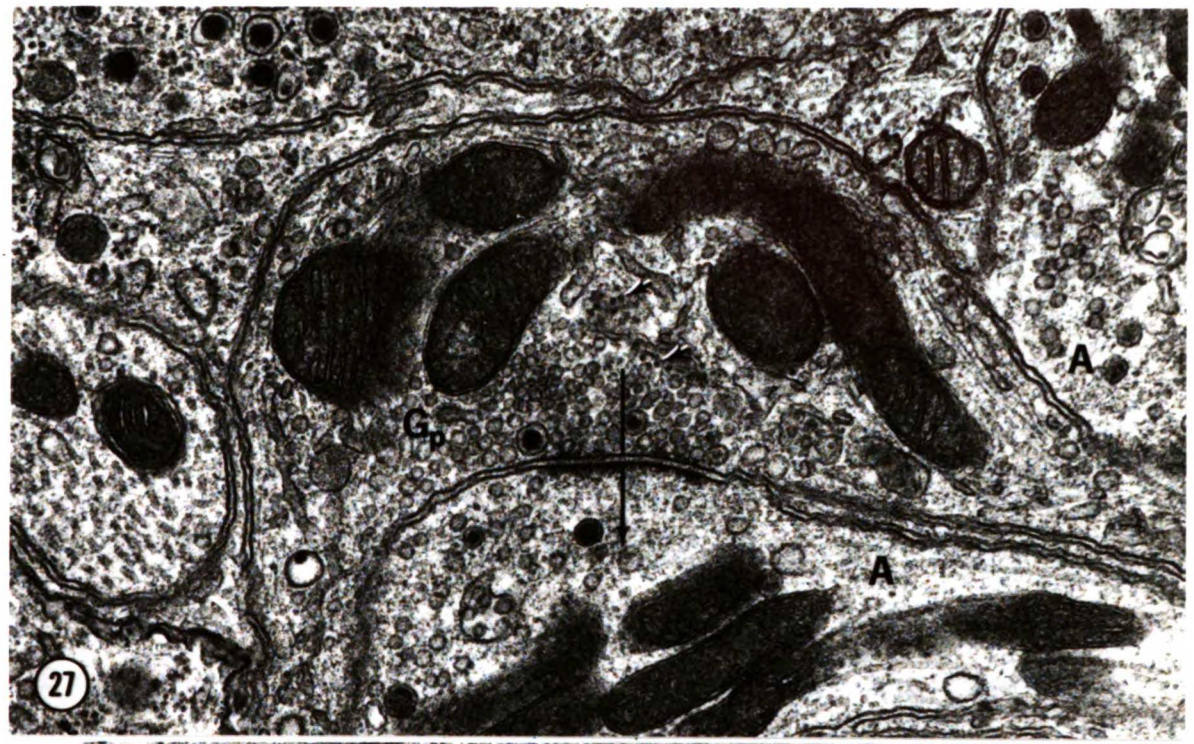

Gp
A
26

Fig. 27. This type A glomus cell process (G_p), which is presynaptic (long arrow) to an afferent nerve ending (A), contains ribosomes (short arrows). Compare this process with the efferent nerve ending in Fig. 28. X38,000.

Fig. 28. This (preganglionic sympathetic) efferent nerve ending (E) which is presynaptic (arrow) to a type A glomus cell (G) contains synaptic vesicles and large dense-cored vesicles, but no ribosomes (compare with the glomus cell process in Fig. 27). X38,000.



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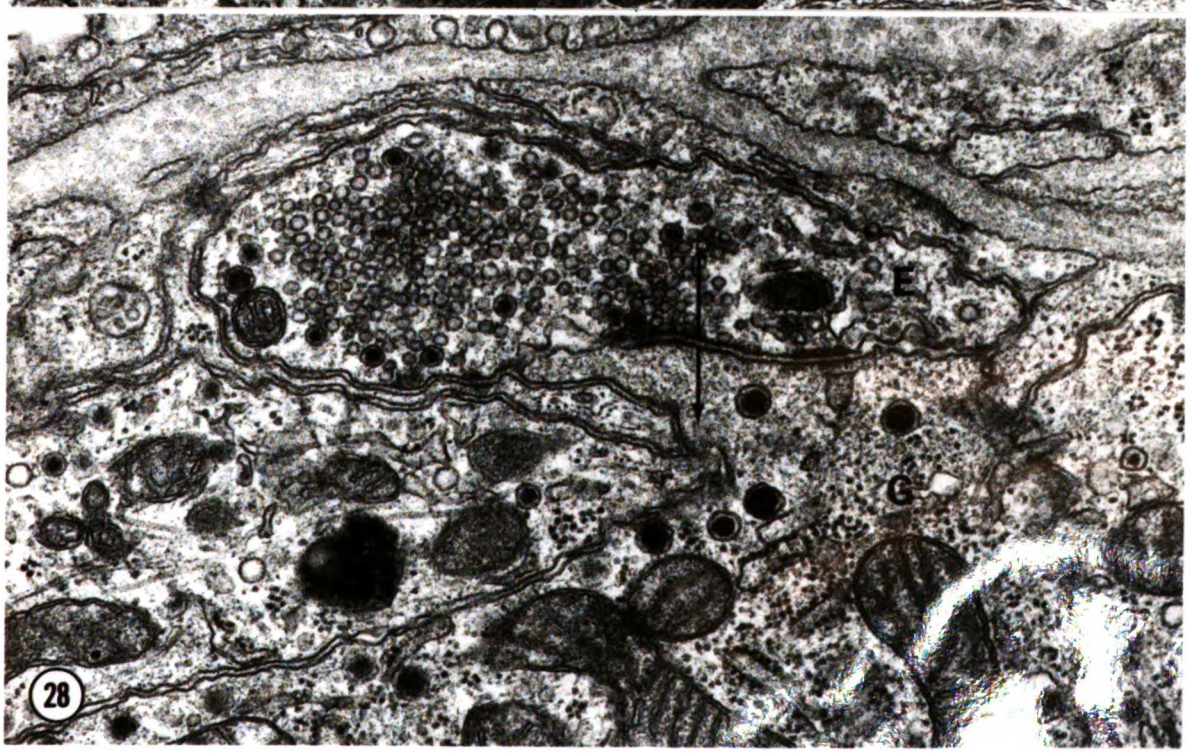


28

arise from type A cells.

An interesting feature of synapses between glomus cell processes and afferent nerve endings is that they have a structure different from that of synapses between glomus cell bodies and afferent endings. This difference is based on the proportion of synaptic vesicles and large dense-cored vesicles near the synaptic junction (see footnote 10, page 27). While synapses involving glomus cell bodies have approximately equal proportions of the two types of vesicles, synapses involving glomus cell processes have many more synaptic vesicles (compare Fig. 20 with 26). The ratio of synaptic vesicles to large dense-cored vesicles averaged 25:1 (range 9:1 to 75:1) in ten synapses involving processes in one carotid body. Such regional differences in the proportions of the two types of vesicles at synapses apparently result from absolute - not merely relative - differences in their concentrations.

4. Reciprocal synapses between afferent nerves and glomus cells. Reciprocal synapses are paired synaptic contacts between two neural structures, usually dendrites (72). One structure is presynaptic to the other at one synapse and postsynaptic to the other at an adjacent synapse. Reciprocal synapses have been found in several parts of the central nervous system (27,30,72,89), but not heretofore in the peripheral nervous system.

In the carotid body, about 5% of afferent endings in single sections have paired synapses joining them with glomus cells. These paired synapses have the configuration typical of reciprocal synapses (Fig. 21). We do not know the total number of reciprocal synapses between afferent endings and glomus cells because we have not examined serial sections, but many single synapses may be part of reciprocal synapses. The two

components of reciprocal synapses are identical structurally to the two types of synapses that join afferent endings with glomus cell bodies (compare Figs. 19 and 20 with 21). We have not yet found a clear example of a reciprocal synapse between an afferent ending and a glomus cell process.

C. Synapses Between Efferent Nerves and Glomus Cells.

Efferent nerve endings are presynaptic to glomus cells(Figs. 15,28). We have found no efferent nerves presynaptic to afferent endings, postsynaptic to afferent endings or glomus cells, or connected by reciprocal synapses to glomus cells. In efferent endings presynaptic to glomus cells, synaptic vesicles are densely packed near synaptic junctions, achieving concentrations several times that found in afferent endings presynaptic to glomus cells (compare Fig. 21 with 28). While both afferent and efferent nerves innervate some glomus cells (Fig. 12), the infrequency of efferent endings leads us to believe that many glomus cells are innervated only by afferent nerves.

D. Synapses Between Adjacent Glomus Cells.

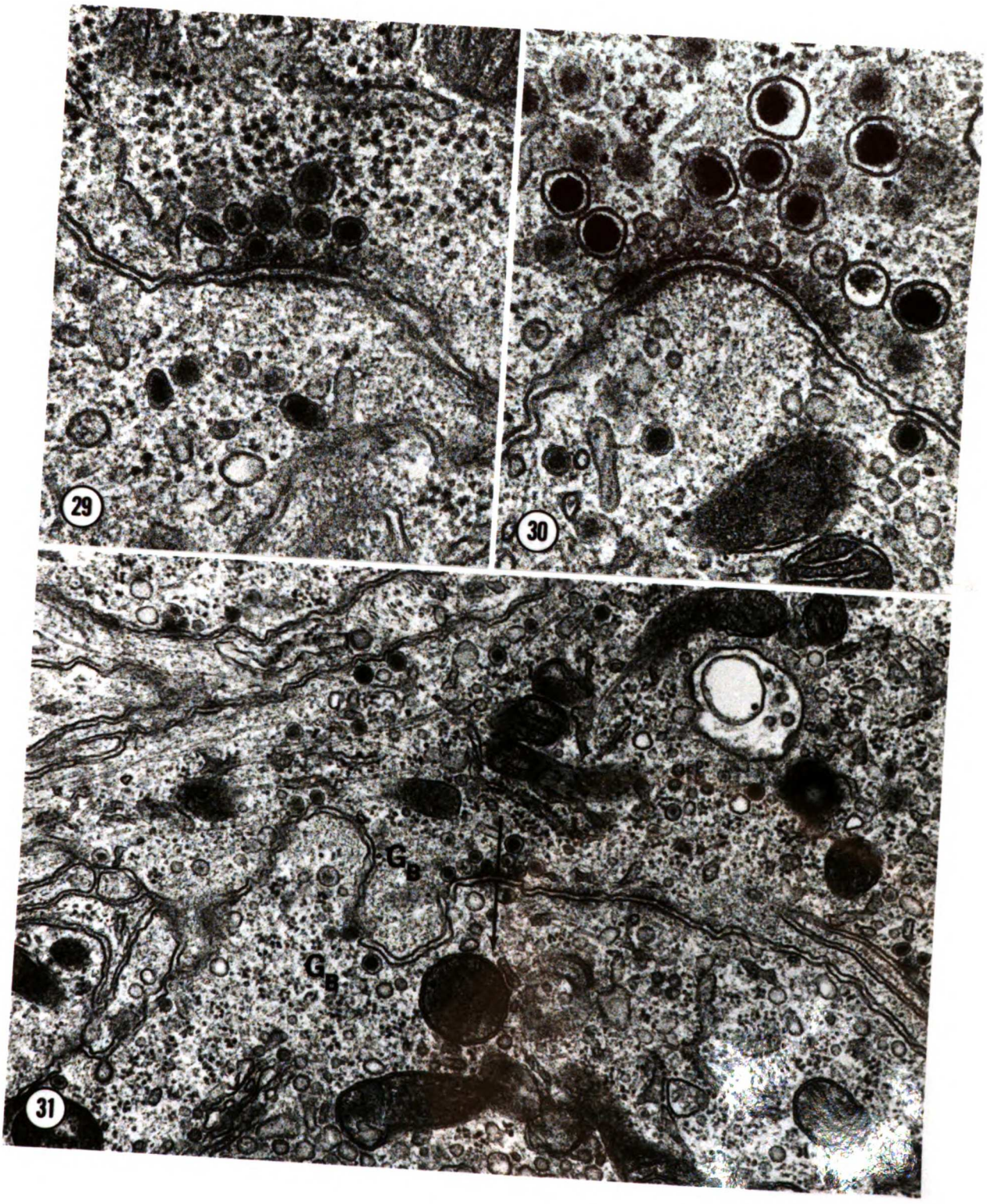
Glomus cells are presynaptic not only to afferent nerve endings, but also to other glomus cells. But unlike synapses between glomus cells and nerve endings, synapses between two glomus cells involve both type A and type B cells. We have found synapses between adjacent type A cells and between adjacent type B cells (Figs. 29, 31). In addition, some type A cells are presynaptic to B cells (Fig. 30), and some B cells are presynaptic to A cells.

Synapses at which type A cells are presynaptic to other glomus cells

Fig. 29. A synapse between two type B glomus cells is shown at high magnification in this micrograph. Large dense-cored vesicles (mean diameter 90 nm) accumulate at the synaptic junction. 70,000X.

Fig. 30. This micrograph shows a synapse between a type A glomus cell (above) and a type B glomus cell. Large dense-cored vesicles (mean diameter 116 nm), typical of those throughout the cytoplasm of type A glomus cells, accumulate together with synaptic vesicles at the synaptic junction. Note the similarity of this synapse and the one shown in Fig. 20, in which an afferent nerve ending is the postsynaptic structure. X70,000.

Fig. 31. Two type B glomus cells (G_B) are shown in this micrograph. A synapse between the two cells is marked by the arrow. Synapses between type B glomus cells and other glomus cells are common, but synapses between type B cells and afferent or efferent nerve endings are rare. X38,000.


29
30
G_B
G_B
31

are similar in structure to synapses at which A cells are presynaptic to afferent nerves (compare Fig. 20 with 30). Both small vesicles and large dense-cored vesicles accumulate at synaptic junctions. Synapses at which type B cells are presynaptic are distinctive because dense-cored vesicles with a mean diameter of 90 nm accumulate at synaptic junctions (Fig. 29). Some adjacent glomus cells are connected by several synapses and some of these synapses have the configuration of reciprocal synapses. Furthermore, glomus cell processes, which may be either presynaptic or postsynaptic, interconnect glomus cell bodies separated sometimes by tens of microns. Although we have not determined the precise frequency of each type of synapse between glomus cells, more type B cells than type A are presynaptic to other glomus cells.

V. Types of Ganglion Cells

About 25 autonomic ganglion cells are present in the rat carotid body (83). Embryological studies indicate that they are of two types: parasympathetic, migrating into the carotid body from the glossopharyngeal nerve, and sympathetic, migrating from the sympathetic ganglion (74,83). De Castro found that parasympathetic ganglion cells are innervated by axons from the glossopharyngeal nerve and that sympathetic ganglion cells are innervated by sympathetic nerves (24).

We studied ganglion cells in the carotid bodies of the same ten rats used in our study of the afferent innervation of glomus cells (Section III A). Of 14 ganglion cells appearing in sections from the control carotid bodies, 85% had nerve endings on their cell bodies

or dendrites in the plane of section that we examined (Fig. 32). Many endings were presynaptic to dendrites, fewer were presynaptic to cell bodies (Fig. 33). The preganglionic endings were bouton-shaped and contained synaptic vesicles slightly larger in size but similar in concentration to those in endings of efferent nerves on glomus cells. (Compare data in Table VIII with those in Tables VI and VII.)

Cutting the glossopharyngeal nerve either peripheral or central to the petrosal ganglion caused degeneration of nerve endings on 16 of 18 ganglion cells that we examined. One day after surgery, about 80% of ganglion cells had degenerating nerves next to them (Fig. 34). At 4, 10, and 25 days after surgery, the process of degeneration was complete, but 10% of ganglion cells still had normal endings on them. These data suggest that many ganglion cells are parasympathetic and are innervated by nerves whose cell bodies are in the brain and whose axons travel in the glossopharyngeal and carotid sinus nerves.

To study the innervation of sympathetic ganglion cells, we cut the cervical sympathetic trunk in two rats and examined ganglion cells in their carotid bodies ten days later. We found two denervated ganglion cells which contained in their cytoplasm clusters of synaptic vesicles, about 10% of which had dense cores. These probably are sympathetic ganglion cells, once innervated by preganglionic nerves from the sympathetic trunk. Neurons in sympathetic ganglia (36) and noradrenergic nerve endings (32) contain similar clusters of synaptic vesicles with dense cores.

Although our experiments suggest that parasympathetic ganglion cells outnumber sympathetic, ganglion cells are not scattered throughout the carotid body. Instead, they are concentrated at the periphery

TABLE VIII: Diameter* and Concentration** of Vesicles in Sections of Preganglionic Endings on Ganglion Cells.

	Synaptic Vesicles	Large Dense-Cored Vesicles
Diameter	57 ± 6	94 ± 8
Concentration	68 ± 34	5 ± 5
Number of Endings Evaluated	11	11
Number of Vesicles Evaluated	1236	67

*Outside diameter in nm (mean ± S.D.).

**Vesicles per micron^2 of section (mean ± S.D.).

Fig. 32. This electron micrograph shows an autonomic ganglion cell (center) with several preganglionic nerve endings (arrows) nearby. Ganglion cells are at the periphery of the carotid body and rarely contact glomus cells (G). The nucleus of a satellite cell, whose processes form a thin covering of the ganglion cell, is in the lower right corner of the micrograph. V, blood vessel. X7000.

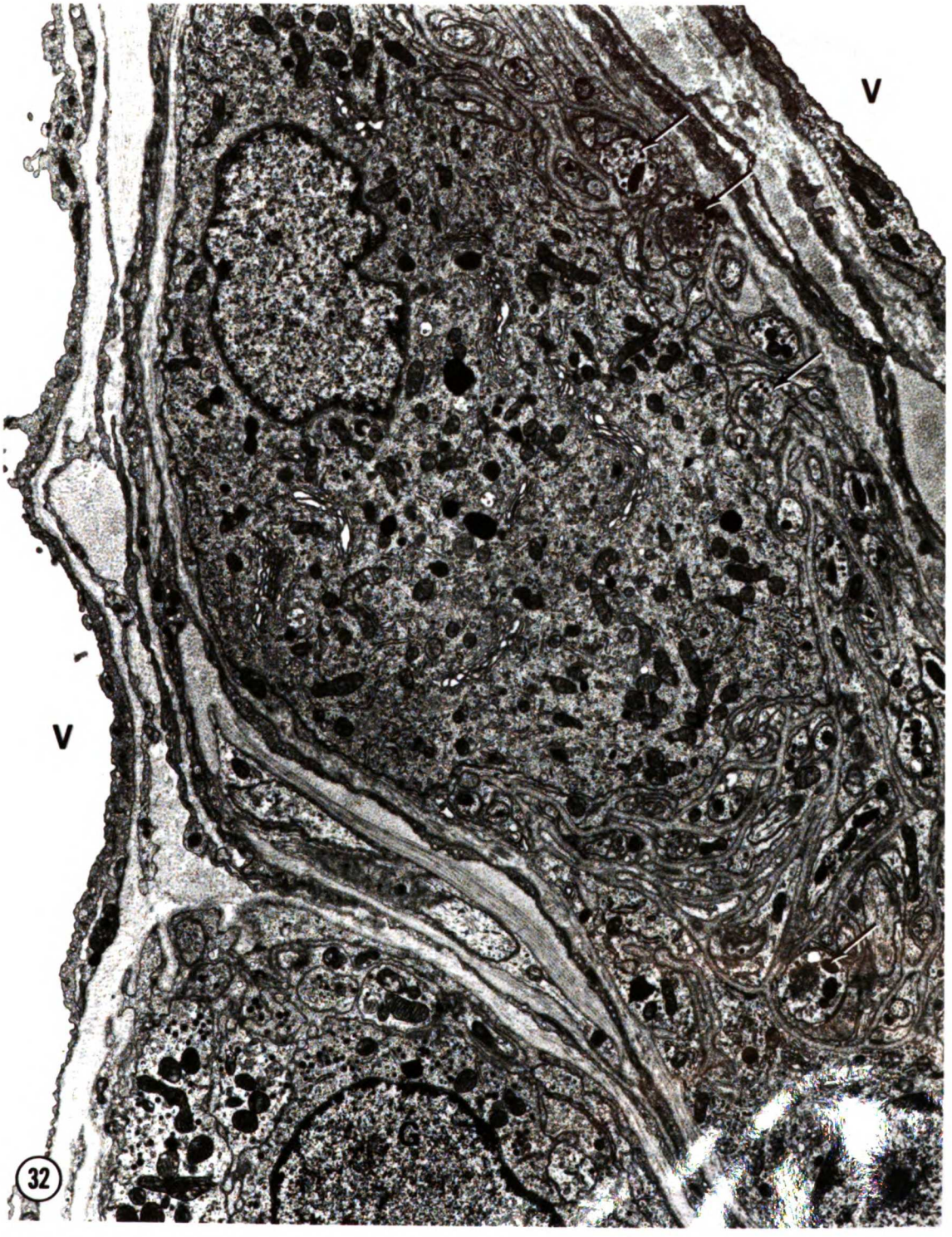
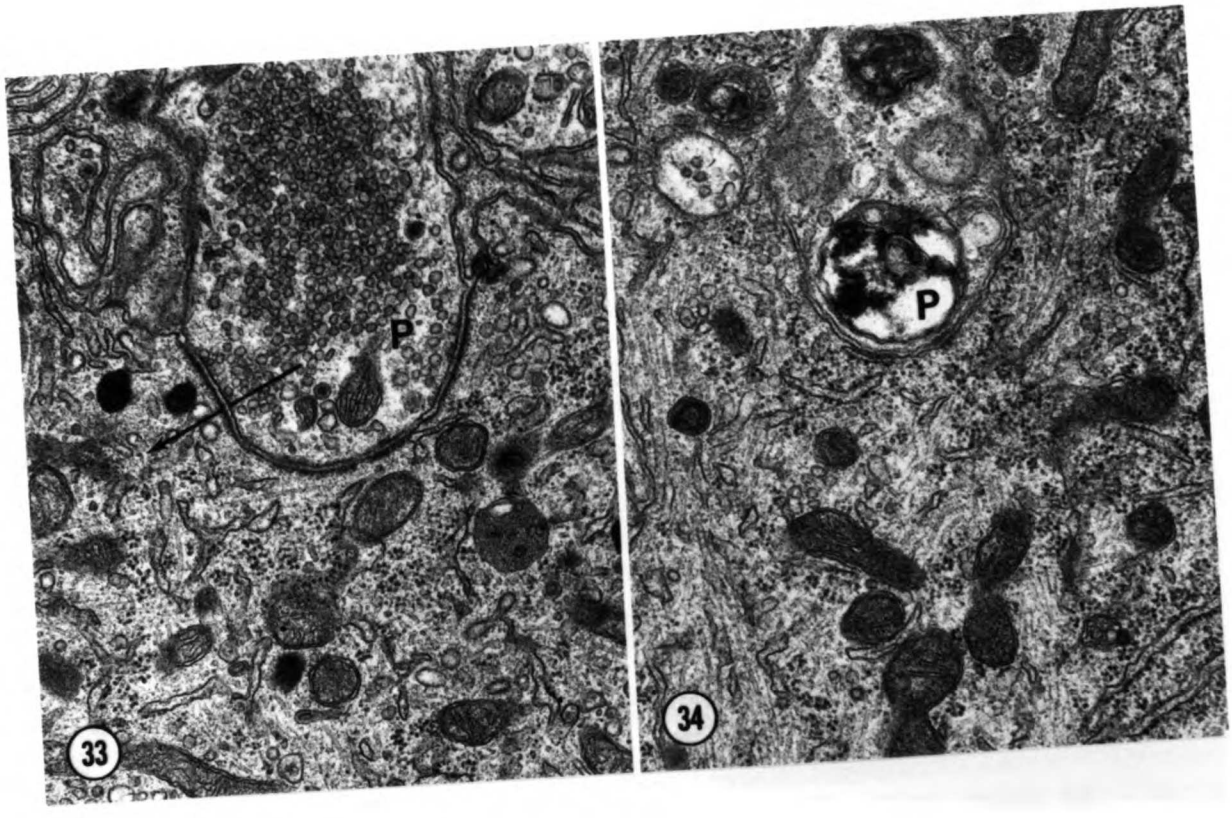

V
V
32

Fig. 33. This preganglionic nerve ending (P) is presynaptic (arrow) to an autonomic ganglion cell. Such nerve endings contain larger synaptic vesicles and fewer large dense-cored vesicles than do efferent nerve endings on glomus cells. X30,000.

Fig. 34. This micrograph shows a degenerating nerve ending (P) on a ganglion cell (prepared for electron microscopy one day after the glossopharyngeal nerve was cut central to the petrosal ganglion). Such preganglionic parasympathetic nerves do not end on glomus cells. X30,000.


P
33
P
34

near connections with the glossopharyngeal nerve rostrally and near connections with the sympathetic ganglion medially. The relatively small proportion of sympathetic ganglion cells that we found may have resulted from nonuniform sampling.

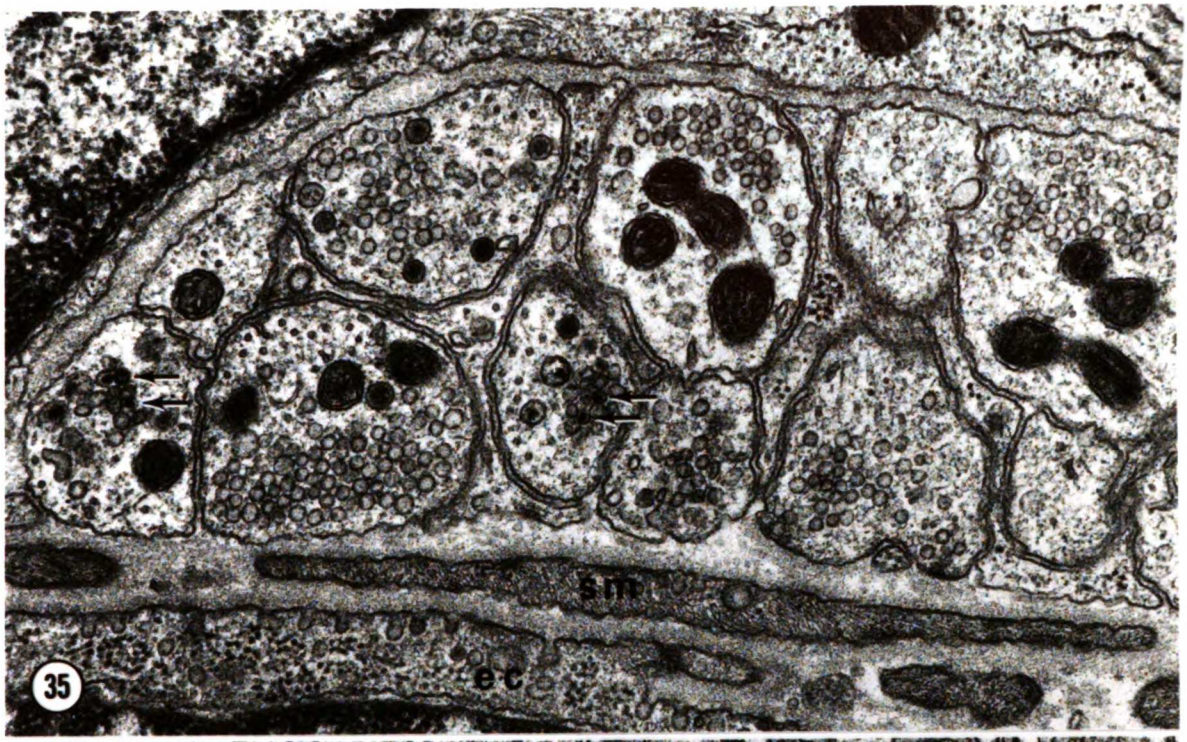
VI. Types of Nerves Ending on Blood Vessels

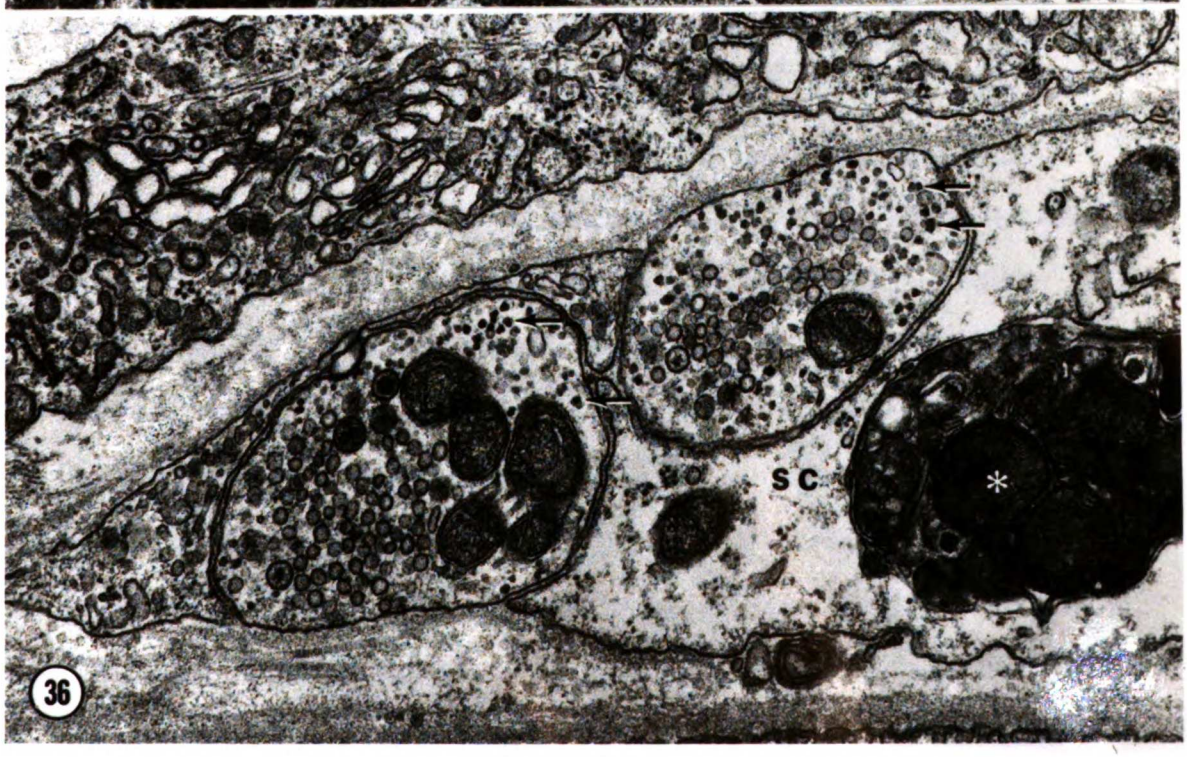
Many blood vessels in the carotid body are innervated. Some vascular nerves in the cat carotid body have their cell bodies in the superior cervical sympathetic ganglion (17), and Biscoe and Silver found that some of these nerves have acetylcholinesterase activity; they suggested that these were cholinergic (15). Kobayashi found that some vascular nerves in the rat carotid body contain monoamines (46); these may be noradrenergic. We found that two types of nerve endings are abundant on blood vessels (Fig. 35). One type contains synaptic vesicles with dense cores, characteristic of noradrenergic nerves (32); the other contains vesicles without dense cores. The neurotransmitter of the latter vascular nerves is unknown, although cholinergic nerves contain synaptic vesicles similar in appearance (40). Both types of vascular nerve endings contain a few large dense-cored vesicles.

To determine the sources of these vascular nerves, we studied two groups of rats. In one group (3 rats) we removed the left superior cervical sympathetic ganglion, then prepared the carotid bodies for electron microscopy 1/4, 1, and 20 days after surgery. At 1/4 and 1 day after surgery, many nerve endings on blood vessels were degenerating; but at 20 days the process of degeneration was complete

Fig. 35. The two types of vascular nerve endings shown in this micrograph are abundant on blood vessels. Sympathetic (noradrenergic) nerve endings contain synaptic vesicles with dense cores (arrows). Parasympathetic vascular nerve endings (without arrows) contain synaptic vesicles without dense cores (these endings differ from efferent nerve endings on glomus cells which contain smaller synaptic vesicles). Both types of vascular nerve endings often contain large dense-cored vesicles. sm, smooth muscle cell. ec, endothelial cell. X38,000.

Fig. 36. These vascular nerve endings are shown one day after we cut the carotid sinus nerve and removed the superior cervical sympathetic ganglion, nodose ganglion, and cervical vagus nerve. Parasympathetic nerve endings (containing synaptic vesicles without dense cores) contain glycogen (arrows) but are not degenerating. They probably arise from ganglion cells within the carotid body. Sympathetic nerve endings (containing synaptic vesicles with dense cores) are degenerating (asterisk); consequently, most arise from ganglion cells outside the carotid body. sc, Schwann cell. X38,000.


sm
ec
35


sc
*
36

and most of the nerve endings with dense-cored synaptic vesicles were gone. Endings that remained on blood vessels contained synaptic vesicles without dense cores. We conclude that most vascular nerves containing synaptic vesicles with dense cores arise from noradrenergic neurons in the superior cervical sympathetic ganglion and that most nerves containing synaptic vesicles without dense cores arise elsewhere from neurons of another type.

In the other group (2 rats) we severed all extrinsic neural connections of the carotid body by cutting the left carotid sinus nerve and removing the left superior cervical sympathetic ganglion, nodose ganglion, and cervical vagus nerve. The carotid bodies were prepared for electron microscopy 1 and 20 days after surgery. Many vascular nerve endings were degenerating at 1 day (Fig. 36), and at 20 days after surgery few endings with dense-cored synaptic vesicles remained. At both times vascular endings with synaptic vesicles lacking dense cores appeared normal except for an accumulation of glycogen in their cytoplasm (Fig. 36). These endings presumably arise from parasympathetic ganglion cells in the carotid body. The few surviving endings with dense-cored synaptic vesicles presumably arise from sympathetic ganglion cells in the carotid body. Hereafter we use the term sympathetic for vascular nerves with dense-cored synaptic vesicles and parasympathetic for nerves with synaptic vesicles without dense cores.

Sympathetic vascular endings contain dense-cored vesicles of two sizes: synaptic vesicles and large dense-cored vesicles (Table IX). About 25% of synaptic vesicles in sympathetic endings contained dense cores. (This proportion varies somewhat in different rats.) In some synaptic vesicles the dense core appears as a discrete spot about half

Table IX: Diameter* and Concentration** of Vesicles in Sections of Vascular Nerve Endings.

	Synaptic Vesicles		Large Dense-Cored Vesicles	
Type of Ending	Sympathetic	Parasympathetic	Sympathetic	Parasympathetic
Diameter	58 ± 7	59 ± 6	112 ± 13	99 ± 12
p***	N.S.		< 0.001	
Concentration	63 ± 32	76 ± 49	3 ± 2	1 ± 2
p***	N.S.		N.S.	
Number of Endings	5	13	5	13
Number of Vesicles	464	1848	21	33

*Outside diameter in nm (mean ± S.D.).

**Vesicles per micron^2 of section (mean ± S.D.).

***Unpaired t test; N.S. = not significantly different.

the diameter of the vesicle (Fig. 35), while in others it is a diffuse, granular precipitate. Processes of glomus cells contain synaptic vesicles, a few having dense cores, and large vesicles, most having dense cores, but their synaptic vesicles are significantly smaller and large dense-cored vesicles significantly larger than those in sympathetic nerve endings.

Parasympathetic vascular nerve endings also contain two types of vesicles: synaptic vesicles and large dense-cored vesicles (Table IX). These are similar to vesicles in afferent and efferent nerve endings on glomus cells; but differences exist (compare data in Tables VI, VII, and IX). In afferent nerve endings synaptic vesicles are significantly larger and less numerous than those in parasympathetic vascular endings. In efferent nerve endings on glomus cells, synaptic vesicles are significantly smaller and large dense-cored vesicles more numerous than those in parasympathetic vascular endings. These data support our concept that efferent nerves ending on glomus cells are a type different than parasympathetic nerves ending on blood vessels and that parasympathetic ganglion cells in the carotid body do not innervate glomus cells.[11]

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[11] We found a few examples of sympathetic nerve endings which are presynaptic to glomus cells and apparently do not innervate blood vessels. These endings contain synaptic vesicles with dense cores. The ratio (number of sympathetic nerves ending on glomus cells to number of glomus cells in single sections) is about 2:1000. We have not determined whether these endings arise from ganglion cells in the carotid body or in the sympathetic ganglion. Kondo found nerve endings similar to these near glomus cells in the guinea pig carotid body (50).

DISCUSSION

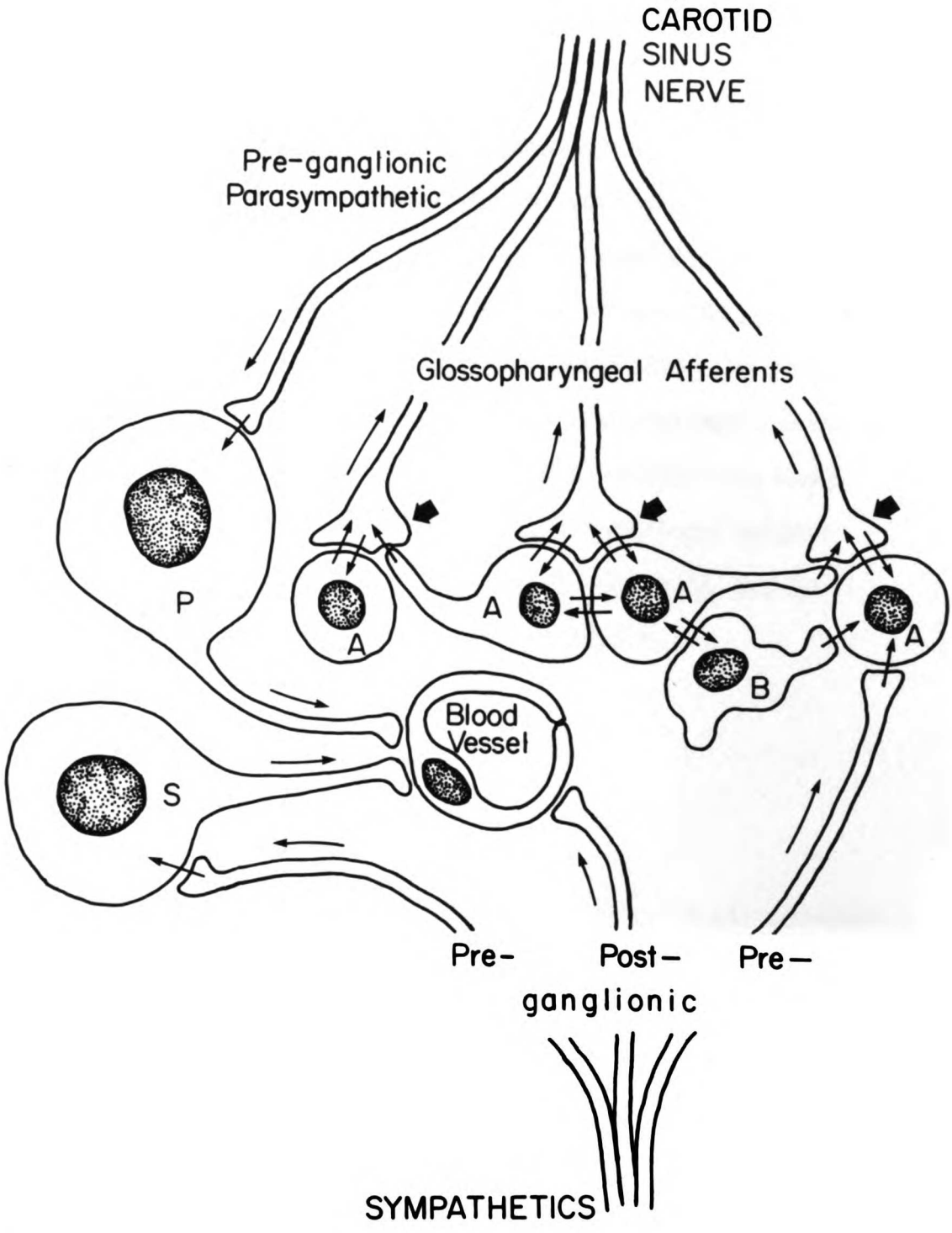
I. Neural Circuits in the Carotid Body

Our data indicate that the synaptic organization of the rat carotid body is more complicated than previous studies suggest (reviewed in 10 and 47). At least six types of nerves, two types of glomus cells, and two types of ganglion cells comprise neural circuits that give the carotid body its characteristic chemoreceptive properties (Fig. 37).

Most of the nerves ending on glomus cells are afferent; a few are efferent. Afferent nerves have sensory endings which form reciprocal synapses with glomus cells; efferent nerves are preganglionic sympathetic axons whose endings are presynaptic to glomus cells. Afferent nerves carry chemoreceptive information from the carotid body via the carotid sinus and glossopharyngeal nerves to the brain; efferent nerves are part of a feedback circuit that enters the carotid body through the cervical sympathetic ganglion. Efferent axons form the glossopharyngeal nerve which enter the carotid body end on ganglion cells, not on glomus cells.

The existence of reciprocal synapses between glomus cells and afferent nerves supports the recent but well-documented concept that axonal endings are not the only sites at which neurons transmit information. Presynaptic dendrites and presynaptic perikarya are known to exist in many parts of the nervous system (72). In the carotid body glomus cells possess no axons, but are presynaptic to afferent nerves; and afferent nerve endings are receptive regions of sensory neurons, but are presynaptic to glomus cells.

Fig. 37. This diagram summarizes neural circuits which we postulate exist in the rat carotid body. Directions of nerve conduction and synaptic transmission are shown by thin arrows. Reciprocal synapses are indicated by two adjacent arrows pointing in opposite directions. We hypothesize that hypoxia depolarizes afferent nerve endings (bold arrows) and that glomus cells (A, type A; B, type B) are interneurons which adjust the sensitivity of chemoreceptive nerve endings through their reciprocal interactions. Glomus cells are innervated by afferent- but not efferent - axons from the carotid sinus nerve and by (preganglionic) efferent axons from the sympathetic trunk. Blood flow is regulated by vascular nerves from parasympathetic ganglion cells (P), which are innervated by preganglionic axons from the carotid sinus nerve, and from sympathetic ganglion cells (S), which are innervated by preganglionic axons from the sympathetic trunk.


CAROTID
SINUS
NERVE
Pre-ganglionic
Parasympathetic
Glossopharyngeal Afferents
P
A
A
A
A
B
Blood
Vessel
S
Pre-
Post-
ganglionic
Pre-
SYMPATHETICS

While the chemoreceptive message sent to the brain from the carotid body presumably is derived from an interaction of afferent nerves, efferent nerves, and the glomus cells they innervate, the two vasomotor pathways in the carotid body also are important in chemoreception because they regulate blood flow. One pathway is sympathetic and the other parasympathetic (Fig. 37). The sympathetic pathway, which may cause vasoconstriction (69), consists of preganglionic axons which end on ganglion cells in the superior cervical sympathetic ganglion (and a few in the carotid body), and postganglionic axons which enter the carotid body and innervate vascular smooth muscle. The parasympathetic pathway, which may cause vasodilation (62,63), consists of preganglionic axons which arise in the brain, travel in the glossopharyngeal and carotid sinus nerves, and end on ganglion cells in the carotid body, and postganglionic axons which innervate vascular smooth muscle.

II. What are Glomus Cells?

Kohn, in 1900, postulated that the carotid body was a paraganglion homologous to the adrenal medulla (49). Consequently many investigators thereafter thought that glomus cells were secretory, similar to chromaffin cells of the adrenal medulla (reviewed by Adams (4)), although glomus cells of most species reduce chromium salts less completely than do adrenal medullary cells. De Castro, in 1926, determined that glomus cells are innervated principally by axons from the glossopharyngeal nerve rather than from sympathetic nerves (24). Thus emerged the concept that the carotid body is a parasympathetic analogue of the adrenal

medulla, and glomus cells secrete substances that act in opposition to those secreted by adrenal medullary cells. De Castro himself invalidated this concept when in 1928 he established that sensory axons from the glossopharyngeal nerve innervate glomus cells (25). He suggested that glomus cells taste the blood in adjacent vessels and transmit information gathered therefrom to the sensory nerves. On the basis of de Castro's suggestion, glomus cells have been assumed to be receptor cells.

Today the sensory function of the carotid body is no longer at issue. However, some investigators have proposed that glomus cells have a nonsensory function. The predominant proposal is that glomus cells are secretory. Pearse includes glomus cells in the class of endocrine cells which secrete polypeptide hormones (66). He notes the similarity of glomus cells to other cells in this group: all contain biogenic amines; all take up precursors of biogenic amines; and all have secretory vesicles 100-200 nm in diameter with electron-dense contents. Pearse includes adrenal chromaffin cells in this class also. Recently Tramezzani et al. argued that glomus cells secrete a hormone which stimulates erythropoiesis (86). This controversial suggestion elicited similar studies by other investigators, who failed to verify the conclusions of Tramezzani's group (34,38,65). Nonetheless, the structural resemblance of glomus cells to some endocrine cells is undeniable.

In 1970 Biscoe and his colleagues hypothesized that glomus cells are secretory cells which, by releasing catecholamines, regulate the sensitivity of neighboring chemoreceptive nerve endings (12). They assumed that glomus cells are innervated by efferent, not afferent,

nerves. Our data oppose this assumption.

Thus, concepts of glomus cell function have changed as information has accumulated. An enduring theme, repeatedly reinforced by inferences from studies done during the past 75 years, is that glomus cells are similar to adrenal chromaffin cells. Both are 1) small, globular, catecholamine-containing cells possessing large, dense-cored vesicles, 2) innervated, and 3) situated around large, thin-walled blood vessels lined by fenestrated endothelial cells. But major differences abound. Unlike glomus cells, chromaffin cells 1) are not surrounded by sheath cells, 2) do not have dendrite-like processes, 3) do not have accumulations of small synaptic vesicles, 4) are not presynaptic to adjacent nerves, 5) are not innervated by sensory nerves, 6) contain more norepinephrine or epinephrine than dopamine, and 7) characteristically display a strong chromaffin reaction.

During our studies of the rat carotid body, we were impressed that glomus cells have structural characteristics of neurons. Like most neurons, glomus cells 1) are enveloped by glial-like sheath cells, 2) have dendritic processes arising from their cell bodies, 3) have a synaptic input and a synaptic output, and 4) form synapses characterized by vesicles at an asymmetrical junction between the pre- and postsynaptic membranes.

Glomus cells share these features with most neurons; however, glomus cells have other features which they share only with certain types of neurons (Table X). Most neuron-like features we have observed in glomus cells of the rat carotid body have been identified in glomus cells of other species. Glomus cells probably are innervated and surrounded by sheath cells in all species, but only in the mouse (48),

Table X: Features of Glomus Cells in Rat Carotid Body Shared with Certain Types of Neurons.

Features of Glomus Cells	Neurons Sharing Feature
1) No axon	amacrine cells of retina (27) granule cells of olfactory bulb (72) small intensely fluorescent (SIF) cells of sympathetic ganglia (probably) (56,81)
2) Presynaptic cell bodies	amacrine cells of retina (33) some neurons of lateral geniculate body (55) some neurons of optic tectum (80) SIF cells of sympathetic ganglia (56,81)
3) Presynaptic dendrites	amacrine cells of retina (27) mitral, granule, tufted, and periglomerular cells of olfactory bulb (72,89) some neurons of thalamus (70) some neurons of motor cortex (82) SIF cells of sympathetic ganglia (56,81)
4) Reciprocal synapses with other neurons	amacrine and bipolar cells of retina (27) mitral, granule, and periglomular cells of olfactory bulb (72,89) some neurons of lateral geniculate body (30)
5) Large dense-cored vesicles in addition to synaptic vesicles	hypothalamic neurosecretory cells (64) some amacrine cells of retina (27) SIF cells of sympathetic ganglia (81) sympathetic ganglion cells (36)

guinea pig (47,50), rabbit (87,88), and some birds and amphibia (47) is there morphologic evidence of glomus cells presynaptic to nerves. In each case, both large dense-cored vesicles and small clear vesicles accumulate at the synaptic region. We infer from our data that the postsynaptic structures at these synapses are afferent nerves. We have found these synapses in the cat carotid body, although they are much less frequent there than in the rat carotid body. If one function of glomus cells is to regulate the sensitivity of afferent nerve endings, this function may be accomplished in some species by synapses which do not have typical structural specializations of the pre- and postsynaptic membranes. Synapses without these structural specializations occur in other parts of the peripheral nervous system (73); they are typical of contacts between autonomic nerves and vascular smooth muscle in the rat carotid body.

Glomus cells probably receive information via synapses in most species (see below), but reciprocal synapses interconnecting glomus cells and nerve endings have not been described before. We found them in rats, and we presume that they exist in other species. A published electron micrograph of a nerve endings and glomus cell in the rabbit carotid body (Verna (87), Plate III, Fig. b), which probably contains a reciprocal synapse, supports our presumption.

Generalizations about glomus cells must be made cautiously, because more than one type exist. One of the two types we have identified in the rat is densely innervated; the other is either sparsely innervated or not innervated at all. We postulate that glomus cells without direct innervation transmit or receive information via their synaptic connections with other glomus cells. The carotid bodies of some species

contain a few cells which display a strong chromaffin reaction. Kobayashi suggested that efferent nerves end on chromaffin cells present in the dog carotid body (45). Similarly, we found efferent nerve endings on chromaffin cells present in the cat carotid body. These nerve endings do not degenerate with the afferent nerve endings after the carotid sinus nerve is cut (unpublished observations), and may be derived from preganglionic sympathetic neurons. Perhaps the few chromaffin cells in the dog and cat carotid body are homologous to chromaffin cells in the adrenal medulla.

From the input-output synaptic relationship of glomus cells with efferent and afferent nerves, we infer that glomus cells are interneurons (internuncial neurons). Interneurons do not simply function as relays between the neurons which they link together; they are "repositories of the output pattern for which the (incoming)...signal is but the trigger." (Schiebels (79)).

One interneuron which glomus cells resemble morphologically is the small intensely fluorescent (SIF) cell of sympathetic ganglia. These small, probably axonless neurons are postsynaptic to incoming preganglionic nerves and presynaptic to sympathetic ganglion cells (56,81). In the rat these interneurons contain large dense-cored vesicles, in size indistinguishable from the large dense-cored vesicles of type A glomus cells. Although both glomus cells and SIF cells contain catecholamines, usually they do not reduce chromium salts (28, 46). Synapses connecting SIF cells to ganglion cells resemble synapses connecting glomus cells to afferent nerve endings: both contain large dense-cored vesicles and small clear vesicles near the presynaptic membrane. The function of SIF cells is unknown. One hypothesis is

that they regulate the activity of adjacent ganglion cells: in response to an excitatory input from preganglion nerves, SIF cells release dopamine, which evokes slow inhibitory potentials in ganglion cells (54).

A second interneuron which glomus cells resemble is the granule cell, the most abundant neuron of the olfactory bulb. These small, axonless cells link several types of neurons to mitral cells, the principal output neurons of the olfactory bulb (72). Synapses between dendrites transmit information from granule cells to mitral cells. Most of these dendro-dendritic synapses are reciprocal: mitral cells excite granule cells, and granule cells inhibit mitral cells (72). These synapses probably produce their effect by initiating graded postsynaptic potentials, not action potentials (72). Consequently, these synapses complete feedback loops which process information in an analog rather than a digital fashion. By their local inhibitory effects, granule cells participate in the integrative function of the olfactory bulb.

A third interneuron which glomus cells resemble is the amacrine cell of the retina. The dendrites of these small, axonless neurons are a pathway for lateral communication between bipolar cells (27). Like glomus cells, amacrine cells not only are connected by reciprocal synapses to neurons carrying sensory information, but also are connected by synapses to each other. Some amacrine cells contain dopamine - a further resemblance to glomus cells (51,52).

Although we cannot exclude the possibility that glomus cells have a neuroendocrine function, we conclude that they are interneurons which regulate the activity of chemoreceptive nerves.

III. Are Afferent Nerve Endings Presynaptic Dendrites?

Our data support the experimental evidence of de Castro (25) and Hess and Zapata (39) that most nerves ending on glomus cells are afferent. We believe that Biscoe and his associates (10,11,12) concluded incorrectly that glomus cells are innervated only by efferent nerves. They compared neither the rate nor the magnitude of degeneration caused by cutting the glossopharyngeal nerve peripheral to the petrosal ganglion with that of cutting the nerve centrally. Furthermore, they assumed that nerves in the carotid body degenerate slowly after their axons are cut. Our studies and those of others (1) have shown that endings begin to degenerate a few hours after axonal transection. The reduction in the number of nerve endings on glomus cells that they found months after cutting the glossopharyngeal nerve intracranially may have resulted from a process analogous to atrophy of disuse, because the operation disconnected afferent axons from their central projections. Because Biscoe's group studied cats, we repeated our experiments on cats and obtained results quantitatively similar to those derived from rats (57).

Several investigators have proposed that glomus cells have both an afferent and efferent innervation (3,6,43,47,88), although none has attempted to determine the source or proportion of efferent nerve endings. We have found that some glomus cells have a dual innervation, but the identity of the two types of endings can be confused because both afferent and efferent nerves contain synaptic vesicles and are presynaptic to glomus cells. Some investigators have used qualitative structural features to classify nerve endings, e.g., endings with few synaptic vesicles, or endings with many vesicles (3,6,29,43), but we have found

such criteria unreliable for distinguishing efferent and afferent endings. Although we have quantitative morphological criteria which permit us to distinguish the two types, our selective denervation studies produced the strongest evidence for a dual innervation.

Attempts to classify the receptive endings of sensory nerves using standard nomenclature for neuronal components have caused considerable debate. To reconcile the controversy, Bodian suggested that afferent endings be regarded as dendrites, because dendrites function as the principal receptive site of most neurons and need not arise from the cell body (20). We find this view helpful in understanding the function of afferent nerve endings in the carotid body. When regarded as dendrites, afferent nerve endings on glomus cells are analogous to presynaptic dendrites in other parts of the nervous system. Some presynaptic dendrites in the brain, like those in the carotid body, are connected to interneurons by reciprocal synapses (30,72,89).

We conclude that most of the nerves innervating glomus cells are afferent and that endings of these afferent nerves are dendrites, both pre- and postsynaptic to glomus cells.

IV. Function of Afferent Nerve Endings and Glomus Cells

We hypothesize that dendritic endings of afferent nerves are chemoreceptors sensitive to hypoxia, that glomus cells are interneurons which adjust the sensitivity of the afferent nerve endings, and that glomus cells and afferent nerves interact through reciprocal synapses. This concept opposes the traditional hypothesis introduced by de Castro (25),

that glomus cells are receptors which release an excitatory transmitter in response to hypoxia. Eyzaquirre has defended the notion that this transmitter is acetylcholine, which stimulates chemoreceptive nerves and is present, along with cholinesterases, in the carotid body (29). However, while glomus cells contain catecholamines (23,46), there is no evidence that they contain acetylcholine. Furthermore, hypoxia continues to excite chemoreceptive nerves after the action of acetylcholine is blocked pharmacologically (26,75,77). Consequently Biscoe and others have proposed alternate hypotheses.

Biscoe suggested recently that long, uniformly thin afferent nerve endings, not in contact with glomus cells, are chemoreceptors and that glomus cells regulate the sensitivity of receptive nerves by releasing catecholamines (10). This concept is appealing, because it contains a feedback mechanism for modulating the receptor and is not subject to the same criticisms as the previous hypothesis; but we have no evidence for such "free" nerve endings in the rat, and others have doubted their existence in the cat (29,39). We postulate that large afferent endings on glomus cells - considered to be efferent in Biscoe's hypothesis - have a chemoreceptive function. Nonetheless, we cannot exclude the possibility that several types of afferent nerves exist and that one type does not end on glomus cells.

Our hypothesis that endings of afferent nerves are chemoreceptors is supported by experiments in which we found that regenerated axons from the carotid sinus nerve are stimulated by hypoxia in the absence of glomus cells (58). These experiments suggest that glomus cells are not necessary for sensory transduction, and other studies suggest that they are part of an inhibitory feedback mechanism which regulates chemoreceptor sensitivity. Several investigators have shown that antidromic

electrical stimulation of most of the carotid sinus nerve depresses chemoreceptor activity recorded from a small strand split from the nerve (31,35,62,63,78). This depression, known as "efferent inhibition" of chemoreceptors, is considered to result from activation of an inhibitory feedback loop: stimulation of an excitatory neural input to glomus cells causes them to release a transmitter which decreases the sensitivity of chemoreceptive nerves.

The experimental phenomenon of "efferent inhibition" probably is mediated not by orthodromic stimulation of efferent nerves ending on glomus cells, but by antidromic stimulation of afferent nerves connected to glomus cells by reciprocal synapses. We reach this conclusion because our data indicate that glomus cells receive only an afferent innervation from the carotid sinus nerve, that afferent nerves are connected to glomus cells by reciprocal synapses, and that the inhibitory feedback loop remains intact after efferent - but not afferent - axons have been eliminated from the carotid sinus nerve by cutting the glossopharyngeal nerve central to its sensory ganglion (57).

The inhibitory transmitter released by glomus cells at their synapses with chemoreceptive nerves is thought to be dopamine, which exists in high concentrations in glomus cells (23). Sampson has shown that "efferent inhibition" is mimicked by injecting dopamine intra-arterially, and is blocked by drugs which abolish the inhibitory effect of dopamine (76,77). Although the chemical nature of the excitatory transmitter released by afferent nerve endings is unknown, it has some muscarinic properties (76,77). Probably afferent nerve endings are presynaptic dendrites and glomus cells are axonless interneurons which not only depolarize but also release transmitter

in a graded fashion.

The curve relating PaO_2 to firing rate of single chemoreceptive axons is hyperbolic, with the point of inflection at 60-70 torr (14). Drugs which block the inhibitory effect of dopamine tend to make the PaO_2/chemoreceptive response curve linear (57). Perhaps the glomus cell - afferent nerve inhibitory feedback loop is responsible in part for the non-linear responsiveness of chemoreceptors in the carotid body.

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