EFFECTS OF SINUS NERVE STIMULATION ON CAROTID BODY GLOMUS CELLS

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ABSTRACT

The sinus nerve or sympathetic trunk was stimulated unilaterally in one group of adult cats or Syrian hamsters while in another group the sinus nerve or sympathetic trunk was cut unilaterally and the animals were given reserpine. In a third group, atropine was administered prior to sinus nerve stimulation. All tissues were processed for the detection of primary monoamines. The carotid bodies on the operated sides were compared with those on the unoperated sides of the same animal in order to determine if amine depletion occurred following the experimental procedures. After sinus nerve stimulation alone, the density of the granules in the glomus cells was decreased, but changes were not noted in the granules following sympathetic nerve stimulation. Sinus nerve stimulation after atropine administration resulted in no change in granule density. Sinus nerve transection followed by reserpine treatment resulted in a greater decrease in granule density on the unoperated than on the operated side. Transection of the sympathetic components to the carotid body followed by reserpine injections resulted in a decrease in granule density in the glomus cells on both the operated and unoperated sides. These results suggest that the sinus nerve must be intact for reserpine to exert an effect and that the sinus nerve may contain efferent fibers which modulate amine secretion.

INTRODUCTION

The carotid body was first described by H. W. Taube in 1743 (cited by Adams, 1958) and was regarded as a ganglion. In 1900, Kohn reported that some cells of the carotid body exhibited a conspicuous chromaffin reaction (indicating the presence of catecholamines), and he postulated that the body was a paraganglion which received efferent fibers from the sympathetic nervous system. However, de Castro (1928) failed to localize catecholamines in the cells. He demonstrated that nerve terminals in the body degenerated when the ninth cranial nerve was cut below the level of the petrosal ganglion, but only slight degeneration was seen when the glossopharyngeal nerve was severed intracranially. From these experiments, he concluded that the nerve fibers were mostly afferent rather than efferent.

Following de Castro's morphological findings Heymans and his collaborators (1930) presented physiological evidence suggesting that the carotid body was a chemoreceptor which monitored changes in the circulating blood—a concept widely accepted by most physiologists. Electron microscope studies have shown that the terminals which appose the carotid body cells are morphologically efferent rather than afferent (Lever et al., 1959; Bisco and Stehbens, 1965). Biscoe and Stehbens (1967) and Ross (1967) noted that some of the nerve terminals associated with the parenchymal cells of the carotid body are still intact following section of the glossopharyngeal or ninth cranial nerve. These data suggested that there are efferent fibers, possibly postganglionic, which terminate in the carotid body. Ganglion cells in the vicinity

of or within the carotid body have been demonstrated in many species, but the presence of these cells has been neglected by most morphologists except for de Castro (1928, 1951) who suggested that their axons might possibly innervate blood vessels. However, the innervation of the blood vessels in the carotid body is considered by most workers to originate in the superior cervical (sympathetic) ganglion (Adams, 1958; Biscoe and Stehbens, 1967).

Using electron microscopic, radioautographic, and cytochemical methods, Chen and Yates (1969) demonstrated biogenic amines in the carotid body cells. These amines did not appear to be involved in the chemoreceptor function of the carotid body, as evidenced by the failure of the glomus cells to release cytochemically detectable amounts of amines in response to hypoxia (Chen, Yates, and Duncan, 1969). These observations suggested that the release of the amines from the cells might be controlled by an efferent innervation. On the basis of physiological data, Eyzaguirre and Uchizono (1961) and Biscoe and Sampson (1967) stated that there are some efferent fibers in the sinus nerve (a branch of the glossopharyngeal). The present research was undertaken to obtain additional evidence for an efferent innervation to the cells of the carotid body.

MATERIALS AND METHODS

Adult Syrian hamsters and cats were used for these studies. In one series of experiments, the sinus nerve or sympathetic components to the carotid body from the superior cervical ganglion were stimulated unilaterally (8 v; 1 msec duration; 10 msec intervals) for 30 min–1 hr. The opposite unstimulated side of each animal served as the control. In another series of experiments, the animals were given atropine (200 mg/kg) 30 min prior to stimulation of the sinus nerve (8 v; 1 msec duration; 10 msec intervals) for 1 hr. The opposite, unstimulated side of these animals served as the controls. In other experiments, the sinus nerve or sympathetic components to the carotid body from the superior cervical ganglion were cut unilaterally and the animals were given reserpine (1.25 mg/kg daily) for 2 days. All animals were sacrificed by perfusion through the left ventricle with a cold 3% solution of glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). In the stimulation experiments, the animals were lightly anesthetized with sodium nembutal.

Following fixation for 2–4 hr in 3% glutaraldehyde, all tissues were washed in 0.1 M phosphate buffer with 10% sucrose for 2 hr, and subsequently incubated in a solution of 2.5% potassium dichromate and 1% sodium sulfate in 0.2 M acetate buffer, pH 4.1 (Wood and Barrnett, 1964). This method has been used to study the effects of the catecholamine-depleting drug reserpine on the glomus cell granules. In this technique, monoamines react with glutaraldehyde to form a Schiff monobase which, in turn, reduces potassium dichromate, resulting in opaque deposits in which the amines are localized. Although quantitative data are lacking, Coupland and Hopwood (1966) state that the monoamine-glutaraldehyde complex is responsible for the opacity produced with dichromate. It has been shown (Chen, Yates, and Duncan, 1969) that the granules are reduced in density with the technique of Wood and Barrnett (1964) following treatment with reserpine. After incubation, the tissues were dehydrated in a graded series of ethanols and embedded in Epon 812 (Luft, 1961). Electron micrographs of unstained gold sections were made with an RCA EMU-3G microscope.

RESULTS

Two types of parenchymal cells occur within the carotid body of the cat and hamster; glomus or type I, and supporting or type II. The glomus cells are characterized by the presence of numerous membrane-bounded, electron-opaque granules within the cytoplasm. In tissues subjected to the dichromate incubation technique, the granules in the glomus cells were more electron opaque than in the cells of unstained tissues fixed with glutaraldehyde and osmium tetroxide. The other fine structural features of the glomus and supporting cells have been described elsewhere (Chen, Yates, and Duncan, 1969).

Large ganglion cells were observed within and immediately beneath the capsule of the carotid body. The ganglion cells in the hamster were dispersed and always present in each preparation.

Sinus Nerve Stimulation

The glomus cells on the unstimulated sides of the animals (Fig. 1) possessed granules which were equal in density to those of untreated controls. On the stimulated sides, the granules were consistently different from those of the unstimulated sides owing to a substantial decrease in their density (Fig. 2). In most of the glomus cells on the stimulated sides, the granules were distributed throughout the cytoplasm as in normal tissue, with no obvious concentration of granules along the peripheral margins of the cells. In animals in which atropine was administered prior to nerve

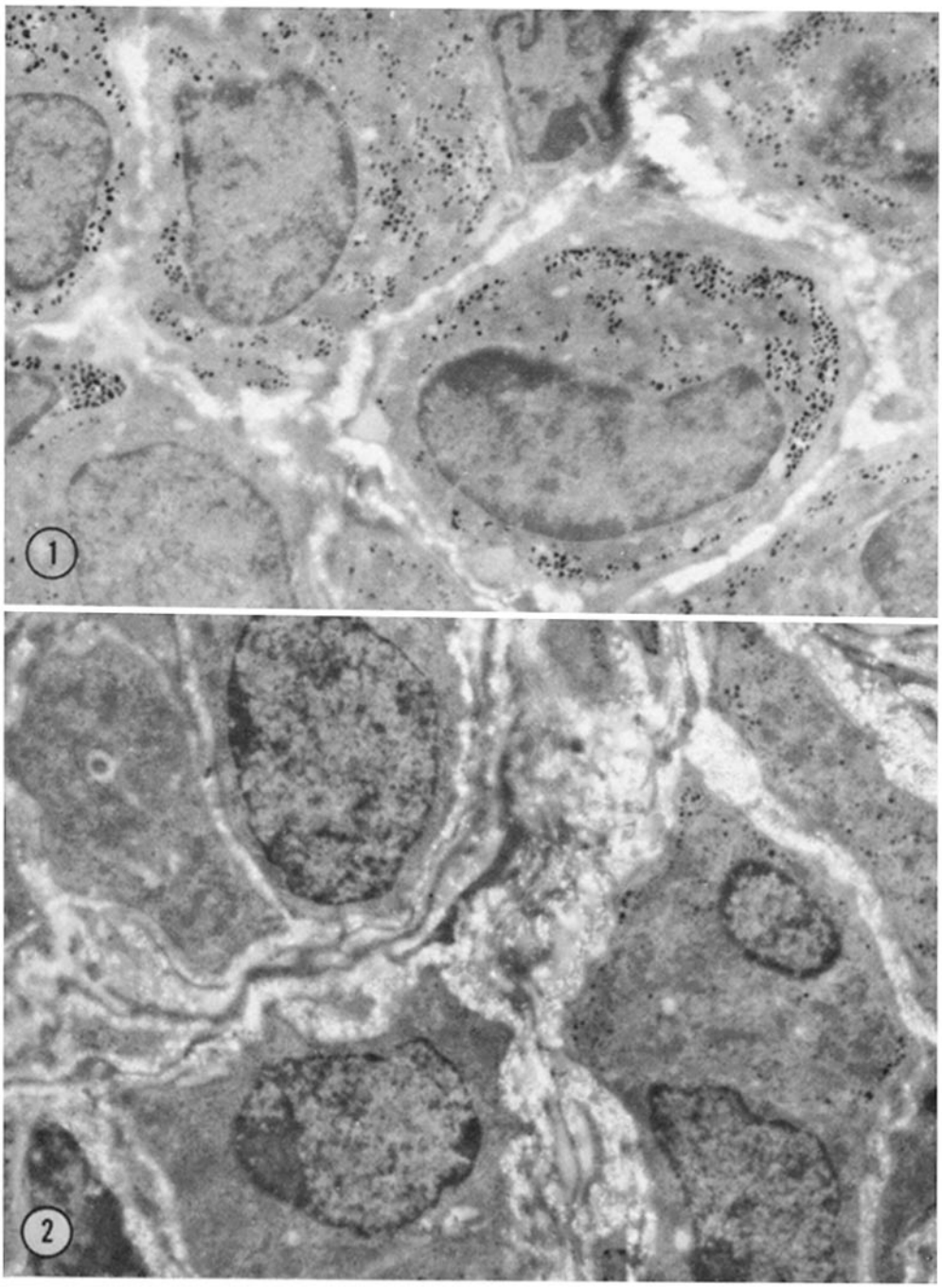

FIGURE 1 Electron micrograph illustrating the carotid body glomus cells from the unstimulated control side of the same animal shown in Fig. 2. Note the density and distribution of the granules. Unstained. × 6,500.

FIGURE 2 Electron micrograph illustrating the glomus cells from the stimulated (sinus nerve) side of the same animal shown in Fig. 1. Note that there is a decrease in the number of granules and that those visible are markedly reduced in density. Unstained. × 6,500.

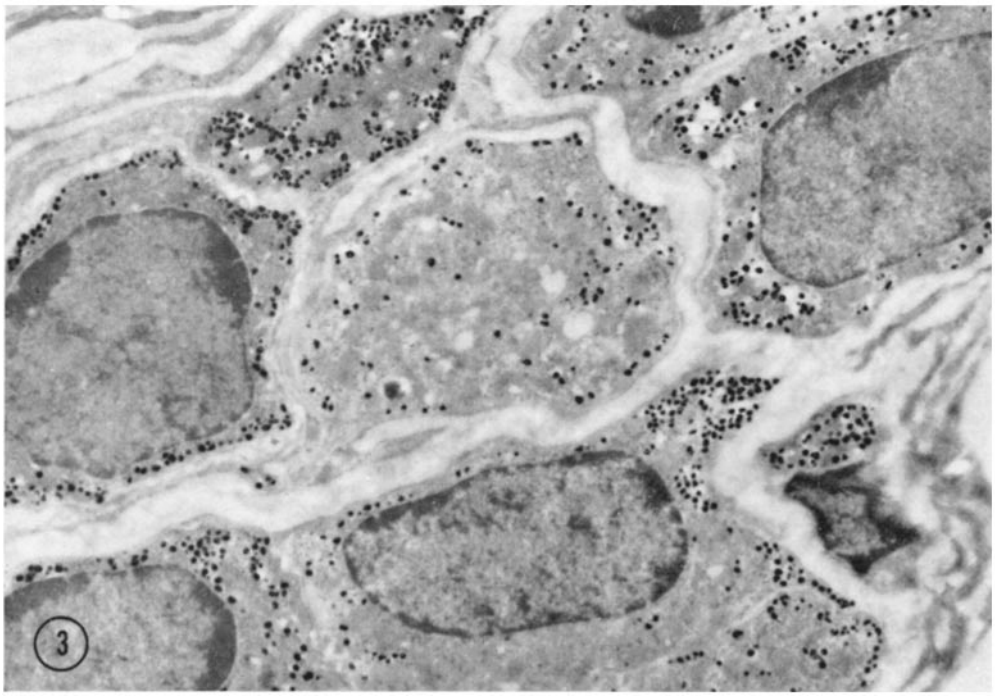

FIGURE 3 Micrograph of glomus cells from an animal in which atropine had been administered prior to sinus nerve stimulation. Note that the granules are approximately equal in density to those seen in Fig. 1. Unstained. × 6,500.

stimulation, no noticeable decrease in granule density was noted on the stimulated side (Fig. 3).

Sympathetic Nerve Stimulation

When the sympathetic components to the carotid body from the superior cervical ganglion were stimulated unilaterally, the granules in the glomus cells on the stimulated side were the same, in density and number, as those on the unstimulated side of the same animal (Figs. 4 and 5).

Nerve Transection and Reserpine Treatment

When the sinus nerve was transected unilaterally and the animal was subsequently given reserpine, the density of the granules in the glomus cells on the control (intact nerve) side was greatly reduced (Fig. 6). Such density reduction after reserpine treatment was reported in an earlier paper by Chen, Yates, and Duncan (1969). However, the granules in the glomus cells of the opposite side in which the nerve was transected were nearly equal in density to those of untreated control animals (Fig. 7), indicating that the sinus nerve must be intact for reserpine to exert its complete effect. Some reduction in granule density was noted in the transected side, indicating a direct though minor effect of reserpine on the glomus cell granules.

When the sympathetic components from the superior cervical ganglion were transected unilaterally and the animal was given reserpine, the granules in the glomus cells of the carotid body on both sides (transected and intact) displayed an equal decrease in density, indicating that these nerves need not be intact for reserpine to exert its effects.

DISCUSSION

The glomus cells of the carotid body when viewed with the electron microscope exhibit granules similar in appearance to those in the adrenal medulla which are known to contain catecholamines (Hagen and Barrnett, 1960). The effects of the amine-depleting drug reserpine on the glomus cell granules have been interpreted differently. Lever et al. (1959) noted a decrease in

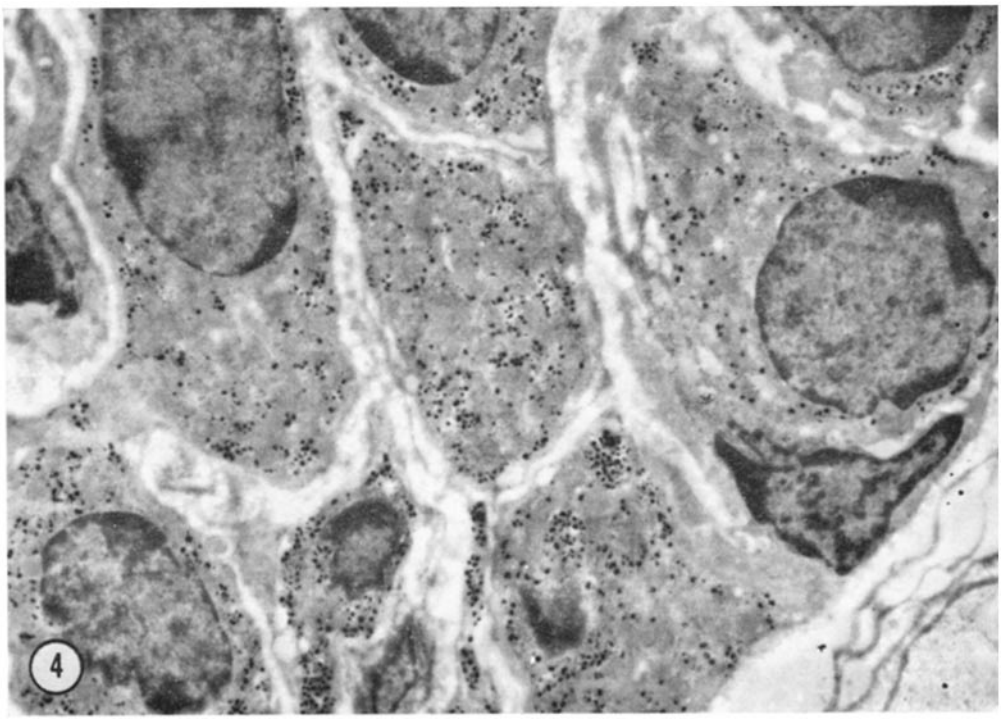

FIGURE 4 Electron micrograph illustrating glomus cells from the unstimulated control side of the same animal shown in Fig. 5. Note the density and distribution of the granules. Unstained. × 6,500.

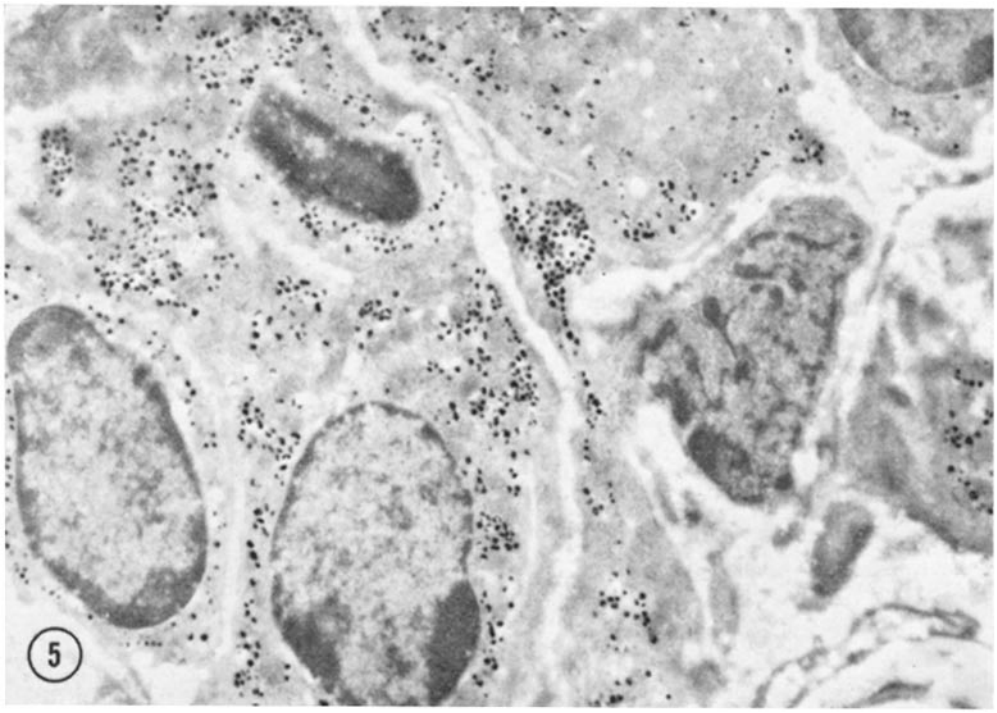

FIGURE 5 Micrograph illustrating the glomus cells from the stimulated (sympathetic nerve) side of the same animal shown in Fig. 4. Note that the granules have not decreased in density. Unstained. × 6,600.

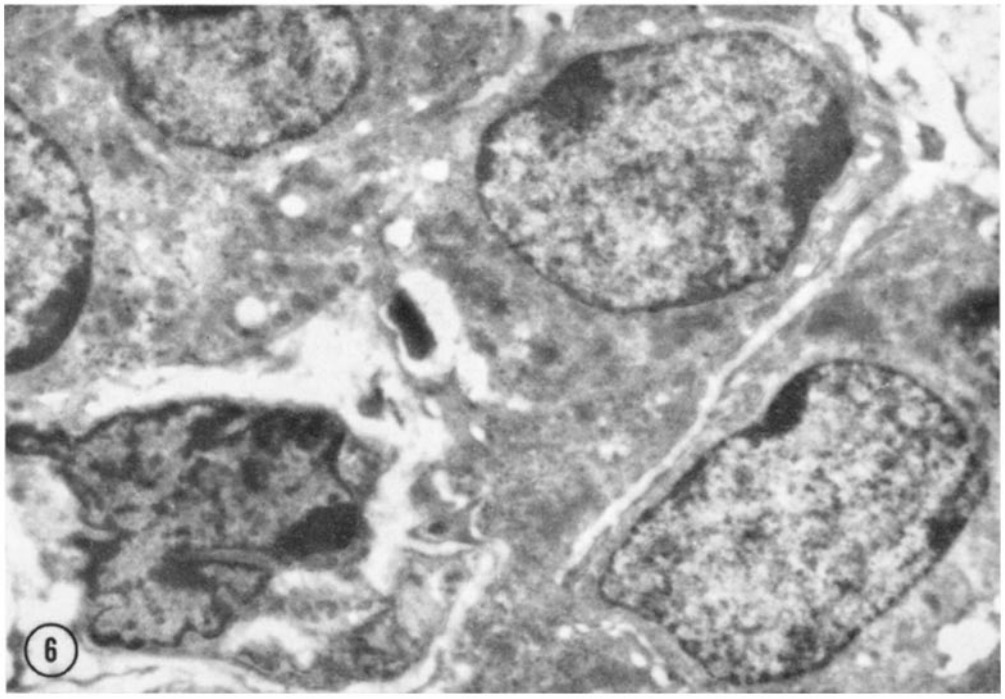

FIGURE 6 Micrograph illustrating the appearance of the glomus cells in an animal in which the sinus nerve was intact and which was given reserpine (compare with Fig. 7). Note that the glomus cell granules are not visible. Unstained. × 6,900.

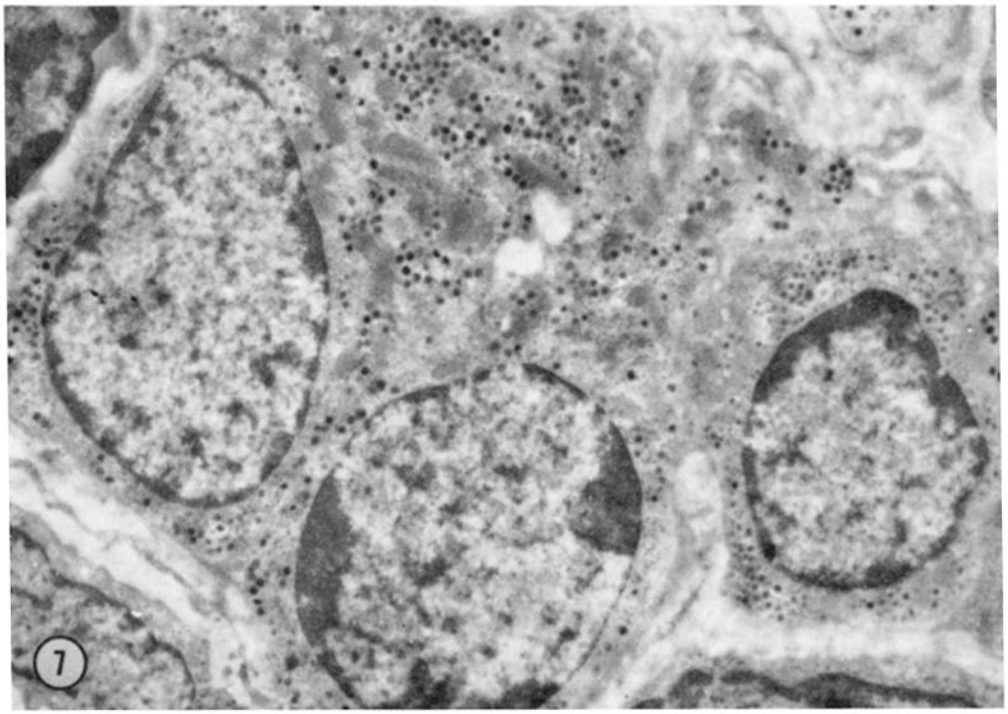

FIGURE 7 Micrograph illustrating the glomus cells of the carotid body of an animal given reserpine following section of the sinus nerve (compare with Fig. 6). Note that the granules are dense, indicating that the sinus nerve must be intact for reserpine to exert its full effect. Unstained. × 6,900.

granule numbers after osmium tetroxide fixation, but Duncan and Yates (1967) reported no such changes, following treatment with the drug, in tissues fixed in glutaraldehyde. Recently, using the catecholamine precursor, tritium-labeled 3,4 dihydroxyphenylalanine, Chen and Yates (1969) demonstrated positive grains of silver at the fine structural level which were localized predominantly over the cytoplasmic granules, strongly suggesting that the granules contain catecholamines. Other evidence (Chen, Yates, and Duncan, 1969) for the presence of catecholamines within the cells has been obtained by the special technique for the identification of monoamines as described by Wood and Barrnett (1964). The cytoplasmic granules appear very electron opaque after glutaraldehyde-dichromate incubation. Wood and Barrnett (1964) reported that this technique is specific for monoamines and that it depends upon the selective oxidation of amines by potassium dichromate. A similar technique was described by Coupland and Hopwood (1966), who showed that the monoamine-glutaraldehyde complex could be made more opaque by treatment of tissues with osmium tetroxide, potassium iodide, or potassium dichromate. We have shown that the opacity of the granules decreases following reserpine treatment. Fillenz (1968) reported fluorescence in the carotid body cells due to catecholamines. Following reserpine treatment, the fluorescence was greatly diminished, though not completely eliminated, and some granules were still present in the cells. These experiments strongly suggest that catecholamines are present within the cytoplasmic granules of the glomus cells, that the release of these amines occurs without complete granule disappearance, and that the decrease in density of the granules with glutaraldehyde-dichromate indicates amine depletion.

Electron microscopy has revealed three types of nerve terminals in the carotid body. One type (Duncan and Yates, 1967) contains granulated vesicles and is found adjacent to the blood vessels. The nerve fibers from which these endings arise degenerate following section of the sympathetic components to the carotid body from the superior cervical ganglion (Biscoe and Stehbens, 1967). The other two types of terminals are closely associated with the parenchymal cells (glomus and supporting) and contain synaptic vesicles. One type exhibits desmosome-like densities at the apposition of the axolemma and glomus cell, while the second is a basket-type ending (Al-Lami and Murray, 1968). The endings showing the densities degenerate following section of the sinus nerve (Biscoe and Stehbens, 1967). It is generally agreed that these fibers represent components of the sinus nerve which, according to Biscoe and Stehbens (1965), could be afferent or efferent although afferent fibers undoubtably terminate in the carotid body. We have noted nerve cell bodies in the vicinity of the carotid body in the hamster and cat along the sinus nerve. These cells may be the source of the effector nerve fibers as suggested by Watzka (1934).

Intracranial transection of the glossopharyngeal nerve resulted in no degeneration of the nerve terminals in the carotid body (de Castro and Rubio, 1968). However, transection of the nerve below the level of the petrosal ganglion resulted in partial degeneration of the terminals 30 days after the operation. An explanation for these results may be that some of the nerve terminals are efferent, perhaps postganglionic components. Our results support this idea since (*a*) sinus nerve stimulation resulted in a decrease in granule density indicating catecholamine depletion, (*b*) animals given the parasympathetic blocking agent atropine exhibited no decrease in granule density following stimulation of the sinus nerve, and (*c*) there are ganglion cells in the vicinity of the carotid body.

There is an increase in the rate of secretion of catecholamines from the adrenal medulla following reserpine administration. However, this increase is not noted in splanchnectomized animals (Kroneberg and Schumann, 1958). Using the quantitative technique for the detection of catecholamines as described by Anton and Sayre (1964), Wood and Benjamin (1970) noted that reserpine has less effect on denervated adrenals than on adrenals in which the splanchnic nerve was intact. They also reported no direct effect of reserpine on tissue catecholamines when slices of the adrenal medulla were incubated with the drug. The experiments of Kroneberg and Schumann (1958) and Wood and Benjamin (1970) suggest that the mechanism of action of reserpine is, at least in part, mediated through the efferent nerve supply to the adrenal medulla. The experiments with nerve transection followed by reserpine treatment indicate that the sinus nerve, but not the sympathetic component to the carotid body must be intact for reserpine to

exert its full effect, and they lend evidence for an efferent innervation of the glomus cells.

The presence of efferent fibers in the sinus nerve has been confirmed electrophysiologically (Eyzaguirre and Uchizono, 1961; Bisco and Sampson, 1967), but their significance has remained obscure. Our results suggest that the carotid body may be, in part, an accessory organ of internal secretion which liberates secretory substances into the circulatory stystem, and that the secretion of these substances is controlled by efferent nerve fibers, possibly parasympathetic. Experiments coupling functional studies of the carotid body with direct chemical analyses of amine content in the glomus cells are currently in progress.

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