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INNERVATION OF INTRAPULMONARY AIRWAY SMOOTH MUSCLE OF THE DOG, MONKEY AND BABOON

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ABSTRACT

Intrapulmonary airways of the dog, monkey and baboon were examined with the electron microscope. Differentiation of adrenergic and nonadrenergic nerves was facilitated by the use of 5-hydroxydopamine which was infused into the pulmonary arteries (500 μg/min for 30—45 min). In all species, nerves composed mainly of unmyelinated axons lay external to the muscularis and in the muscularis between bundles of smooth muscle cells. Nerves composed of varicose unmyelinated axons that ran parallel with the smooth muscle bundles contributed fibers that surrounded and entered these bundles. Most of the varicosities associated with airway smooth muscle were cholinergic, and longitudinal sections of the muscle bundles revealed elongate profiles of these varicosities. Most cholinergic varicosities in dog, monkey and baboon airway smooth muscle had no special morphologic relationships to the surrounding smooth muscle cells. Other cholinergic varicosities in the primates lay in depressions of the sarcolemmae. Some of these varicosities were apposed to the sarcolemmae and formed neuromuscular clefts devoid of electron-dense material. There were some adrenergic varicosities near bronchial smooth muscle cells of all species studied. Another type of nerve varicosity, which was present in all species, contained many large dense-core vesicles 90—120 nm in diameter and some small, agranular vesicles 40—60 nm in diameter.

INTRODUCTION

It has been shown that airway tone is regulated in part by neural mechanisms [21]. Also, nerves may be involved in airway pathology. There is

extensive evidence that in asthma, antigen—antibody reactions and chemical mediators released in these reactions can induce vagal reflex bronchoconstriction [38]. These mediators can constrict airway smooth muscle directly, but some of the effects of histamine and other mediators are blocked by atropine or cooling the vagus, suggesting that part of the bronchoconstriction in asthma is dependent on an intact vagal reflex [13,20]. Knowledge of the types of nerves that innervate the airways and their patterns of distribution would help us to understand the neural mechanisms that regulate airway tone and mediate pathologic increases in airway resistance. One would expect extensive innervation and many close nerve—muscle relationships in any smooth muscle system in which tone is predominantly neurogenic as opposed to those systems in which tone is myogenic or in which contraction is regulated by humoral substances other than neurotransmitters. Studies of several species have revealed neither abundant terminals nor close nerve—muscle associations in the airways [4,9,33]. Innervation of canine bronchial and bronchiolar smooth muscle by acetylcholinesterase-containing nerves and adrenergic nerves has been demonstrated [17], but little detailed information is available as to the distribution and fine structure of the nerve terminals that innervate intrapulmonary airway smooth muscle of dogs. Silva and Ross demonstrated nerve terminals around and within small bundles of bronchial smooth muscle cells in the cat [32], but Blumcke reported that nerves do not enter the bronchial smooth muscle bundles of the dog [4]. In studies on the nerves in the rhesus monkey lung, El-Bermani [14] and El-Bermani and Grant [15] found both acetylcholinesterase-containing and adrenergic peribronchial nerves. Richardson and Ferguson found cholinergic varicosities in guinea pig and human trachealis muscle, but failed to find any adrenergic varicosities [30]. No electron microscopic study of the innervation of intrapulmonary airways of the monkey or baboon has been reported. The present study of the fine structure of nerve terminals in the intrapulmonary airways of the dog, monkey and baboon was undertaken in order to compare the morphology of the neuroeffector systems in the airways of these species.

MATERIALS AND METHODS

Eight mongrel dogs, two rhesus monkeys and one baboon were used in this study. The animals were anesthetized with pentobarbital sodium (30 mg/kg i.v.).

Lung tissue from four dogs was prepared by the Falck—Hillarp method [16] for visualization of catecholamines. Blocks of fresh tissue were frozen by immersion in liquid propane cooled by liquid nitrogen. These blocks were freeze dried, treated with formaldehyde gas for 1 h at 80°C and embedded in paraffin. Sections were then obtained, mounted in paraffin oil and examined with a Reichert fluorescence microscope equipped with a mercury vapor lamp, KG-1/BG-12 primary filters and an OG-1 secondary filter.

Four dogs, two monkeys and one baboon were treated with 5-hydroxy-

dopamine and prepared for electron microscopy [35]. In three dogs, two monkeys and the baboon, 5-hydroxydopamine was infused into the artery of the left lower lobe at a rate of 500 μg/min for 30—45 min. In one dog, 3 mg of 5-hydroxydopamine was injected as a bolus into the bronchial artery supplying the left lower lobe [23].

After administration of 5-hydroxydopamine, the left lower lobe of each dog and monkey was perfused with 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 at a rate of 100—150 ml/min for 8—10 min. The baboon was perfused with the same fixative introduced into the left ventricle. The perfused lung tissue was removed and tissue blocks containing bronchi of various sizes were excised, immersed in cold fixative for 2 h, then placed in cold buffer overnight. The tissue was post-fixed in 1% osmium tetroxide for 2 h, dehydrated in ethanol, passed through propylene oxide and embedded in Maraglas. Sections were stained with uranyl acetate and lead citrate and examined with an RCA EMU 3G or 4A electron microscope.

RESULTS

Intrapulmonary bronchial smooth muscle cells of the dog, monkey and baboon are organized as long, slender bundles that encircle the bronchi, forming an incomplete muscularis. The smooth muscle cells of each bundle are separated from one another by glycocalyx and variable amounts of collagen except at those regions where the cells are joined by gap junctions. The general pattern of innervation was the same in all species studied. Nerves composed of unmyelinated axons ensheathed in Schwann cell processes formed a plexus external to the muscularis. These nerves surrounded individual smooth muscle bundles and entered these bundles to establish several types of relationships with smooth muscle cells.

Fluorescence microscopy

There were adrenergic nerves throughout the muscularis of the dog bronchi. By comparing transverse and longitudinal sections of the smooth muscle bundles, the parallel orientation of the terminals is revealed (Figs. 1 and 2).

Electron microscopy

Both methods of administration of 5-hydroxydopamine used in this study resulted in uptake of this osmiophilic substance into the synaptic vesicles of adrenergic terminals. Electron micrographs show profiles of two distinct types of nerve terminals in all species. Both types were partially covered by Schwann cell processes. The adrenergic varicosities contained small (40—60 nm) synaptic vesicles, some of which had intensely electron-dense cores (Figs. 3—6). Most of these varicosities also contained one or more large (75—120 nm) dense-core vesicles which appeared to have taken up 5-hydroxydopamine. The adrenergic nerves formed plexuses around the bundles of

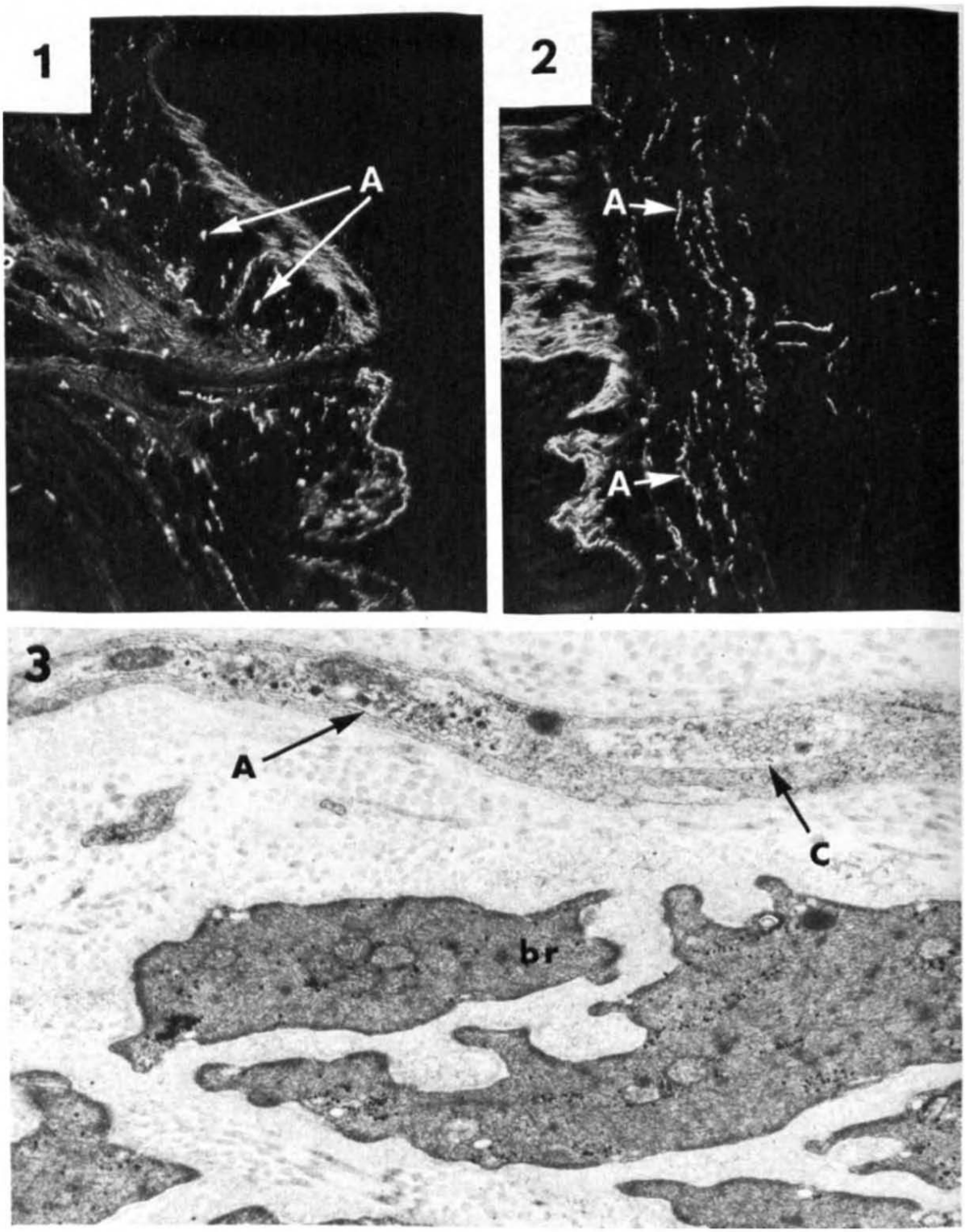

Fig. 1. Dog bronchial smooth muscle innervated by fluorescent adrenergic nerves (A) some of which are cut in cross-section so that they appear as bright dots. ×313.

Fig. 2. Dog bronchial smooth muscle innervated by fluorescent adrenergic nerves (A) sectioned longitudinally. ×313.

Fig. 3. Adrenergic terminal (A) and a terminal (C) having the fine structure characteristic of cholinergic terminals entwined in the same small nerve bundle. br, dog bronchial smooth muscle. ×25,012.

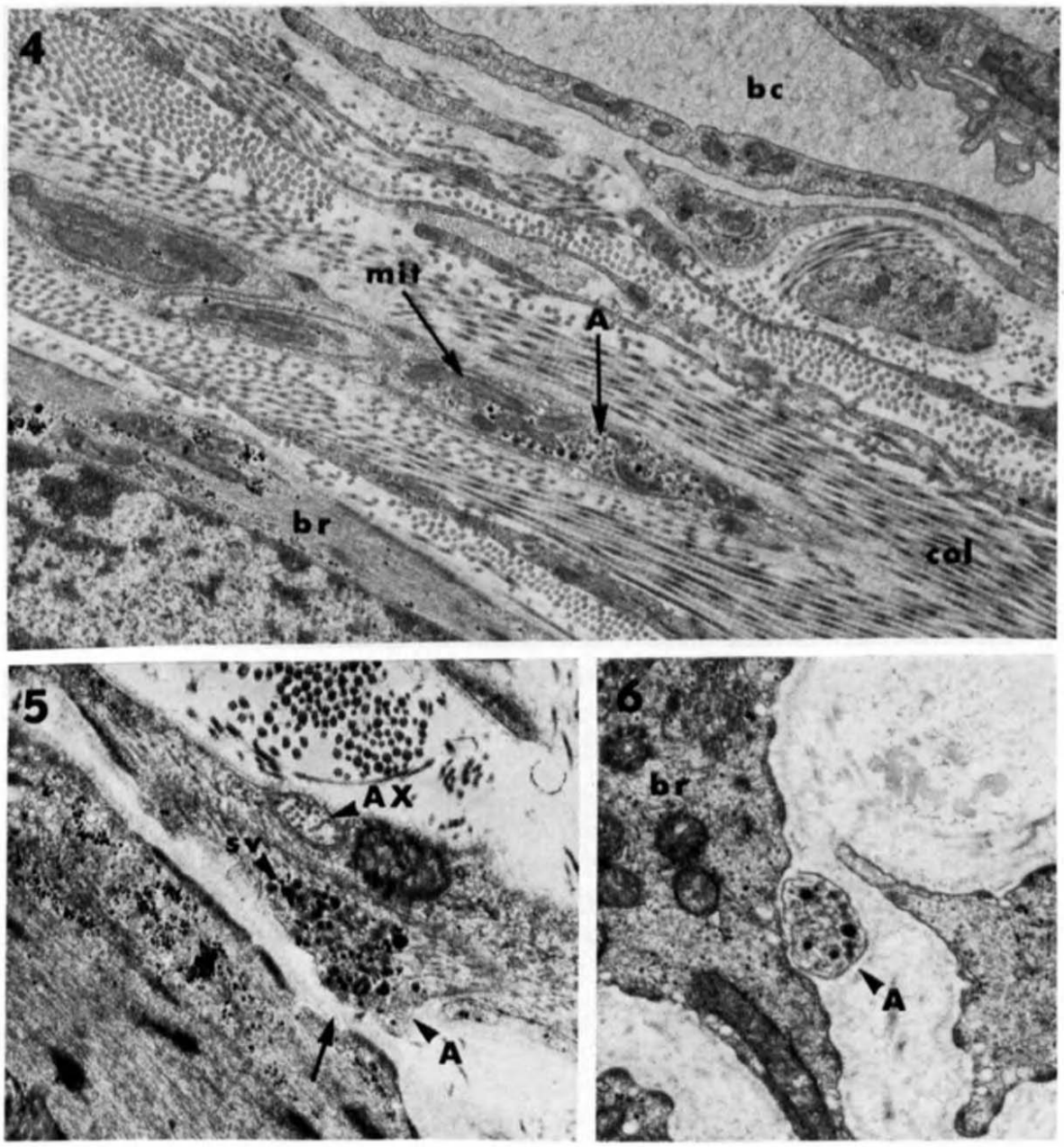

Fig. 4. Adrenergic terminal (A) lying between a bronchial smooth muscle cell (br) and a bronchial capillary (bc) of a monkey. mit, mitochondrion; col, collagen. ×15,750.

Fig. 5. Nerve with axon (AX), and an adrenergic terminal (A) in contact with the glycocalyx of a baboon bronchial smooth muscle cell (arrow). Synaptic vesicles (sv) in the adrenergic terminal have taken up the osmiophilic 5-hydroxydopamine. ×20,700.

Fig. 6. Close contact between an adrenergic terminal (A) and a baboon bronchial smooth muscle cell (br). ×27,900.

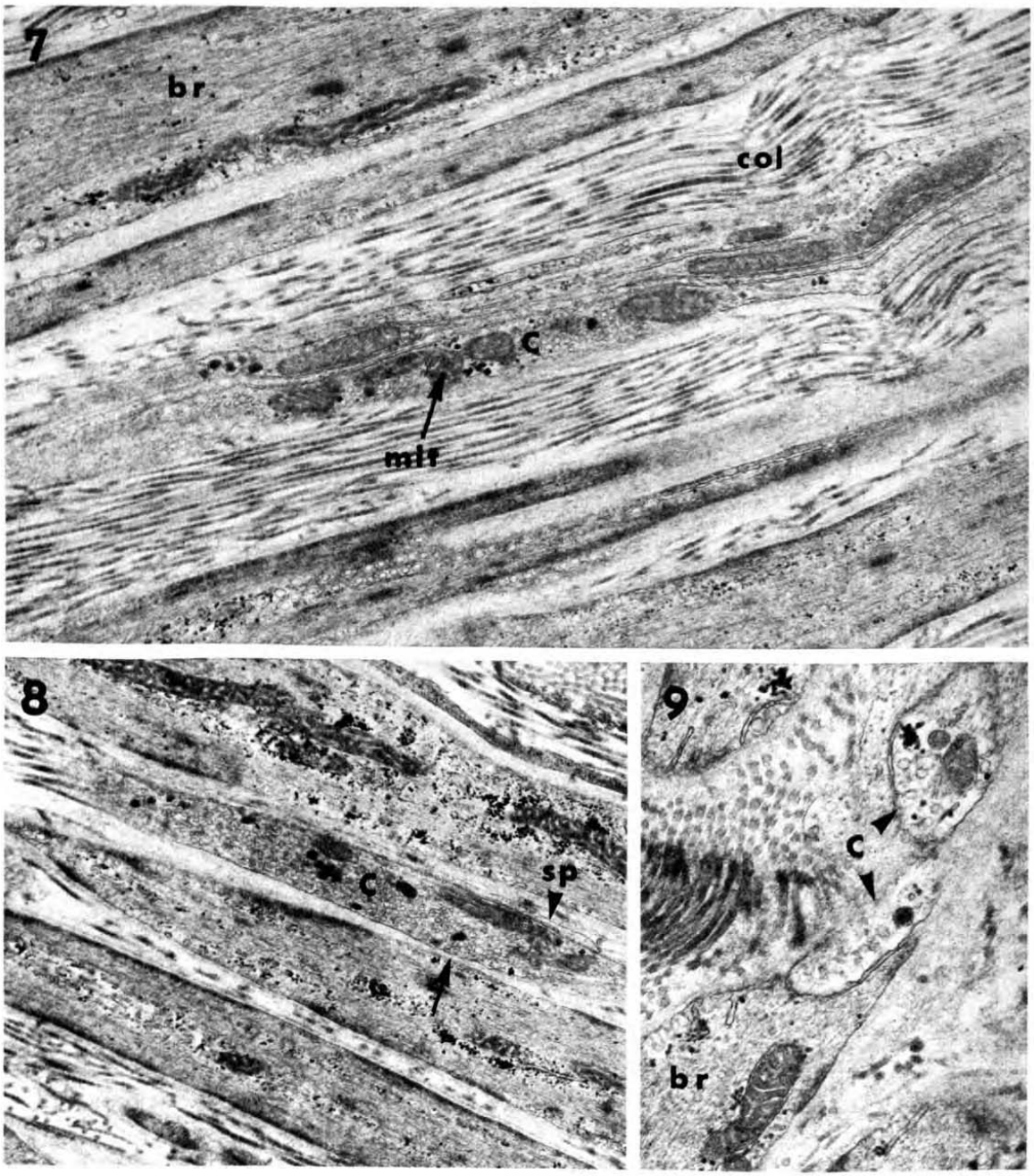

Fig. 7. Small nerve with an elongate cholinergic terminal (C) among several bronchial smooth muscle cells (br) of a monkey. mit, mitochondria; col, collagen. ×18,000.

Fig. 8. An elongate cholinergic terminal (C) in the bronchial wall of a monkey. The terminal is partially covered by the process of a Schwann cell (sp) but touches the glycocalyx of one smooth muscle cell (arrow). ×18,000.

Fig. 9. Close contact between a cholinergic terminal (C) and a bronchial smooth muscle cell (br) of a monkey. ×27,000.

smooth muscle (Figs. 1 and 2). From these plexuses, nerves entered the muscularis, and some varicosities in the dog and baboon were separated from the sarcolemmae of smooth muscle cells only by the glycocalyx (Fig. 5). Only one close contact between an adrenergic varicosity and a smooth muscle cell was found in this study (Fig. 6).

Cholinergic varicosities contained clusters of small agranular vesicles and most also contained some large dense-core vesicles (Figs. 7—13). In all the species examined, adrenergic and cholinergic terminals were intertwined in the same nerve bundles (Fig. 3). There was close proximity between some adrenergic and cholinergic varicosities. Some adrenergic and cholinergic varicosities were elongate and oriented parallel with the surrounding smooth muscle cells (Figs. 4, 7 and 8). Small nerves entered the smooth muscle bundles, but most of the cholinergic varicosities were separated from the smooth muscle cells by collagen fibrils and the glycocalyces of both nerve and muscle (Figs. 3, 7 and 10). Some cholinergic varicosities in the dog and monkey were separated from the surfaces of nearby smooth muscle cells only by the glycocalyx (Fig. 8, arrow). The most closely apposed neuromuscular junctions were found in the primates. In both the monkey and baboon, cholinergic varicosities lay adjacent to the sarcolemmae of smooth muscle cells (Figs. 9 and 11) or in depressions of the cell surfaces (Figs. 12 and 13). These close appositions of varicosity and muscle formed neuromuscular clefts of about 18 nm that were devoid of electron-dense material (Figs. 9, 11—13). The glycocalyx did not extend between the pre- and postsynaptic membranes but was reflected at the border and enclosed both nerve varicosity and smooth muscle cell (Fig. 12). There were several mitochondria in most adrenergic and cholinergic varicosities (Figs. 4, 7 and 10), and in some profiles, the mitochondrion was the predominant organelle (Fig. 11). However, most of these varicosities also contained some synaptic vesicles. One cholinergic varicosity (Fig. 13) appeared (because of the plane of section) to be enclosed by a smooth muscle cell. This varicosity also contained glycogen granules and a lamellar body, inclusions which were prominent in cholinergic terminals, especially in the baboon.

Another type of nerve varicosity, found in all species studied, contained some small agranular vesicles and clusters of large dense-core vesicles (Figs. 14 and 15). Both the large and small vesicles were in the same size range as those in cholinergic varicosities. Only the greater numbers of large vesicles distinguished these varicosities. Some such varicosities were in small nerve bundles and were enclosed by Schwann cell processes (Fig. 14). Others, however, were closely associated with smooth muscle cells and were naked (Fig. 15).

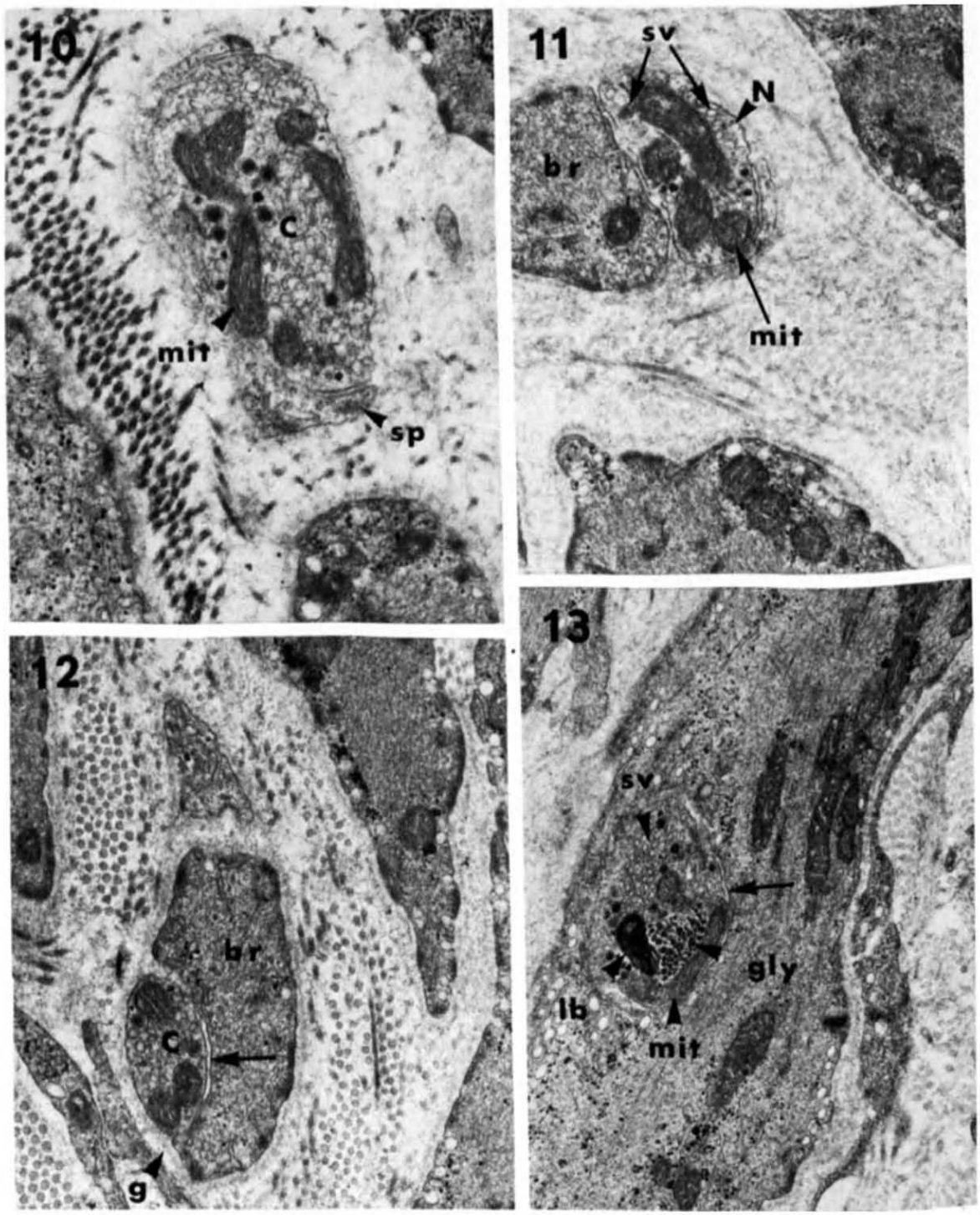

Fig. 10. Cholinergic terminal (C) in a bronchial wall of the baboon. mit, mitochondrion; sp, Schwann cell process. ×29,062.

Fig. 11. Close contact between a nerve varicosity (N) and a bronchial smooth muscle cell (br) of the baboon. The nerve contains several mitochondria (mit) but few synaptic vesicles (sv). ×29,062.

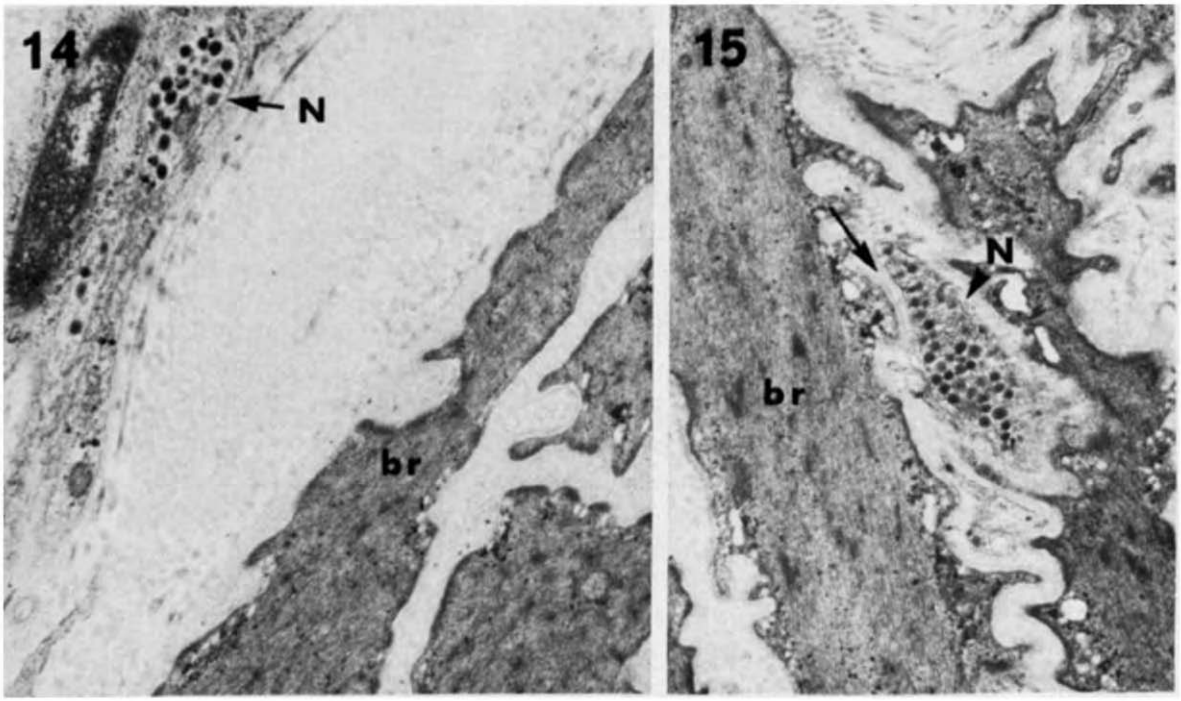

Fig. 14. Nerve varicosity (N) covered by Schwann cell processes and containing large dense-core vesicles in a bronchial wall of a dog. Bronchial smooth muscle (br). ×21,275.

Fig. 15. Nerve varicosity (N) containing large dense-core vesicles in a bronchial wall of the baboon. This varicosity is naked and touches the glycocalyx of a smooth muscle cell (arrow). ×21,275.

DISCUSSION

There is general agreement that the large airways of several species have dual innervation by excitatory cholinergic and inhibitory adrenergic nerves [28]. There is, however, species variation in the quantity, distribution and function of the adrenergic terminals. In this study we have confirmed the presence in dog, monkey and baboon intrapulmonary airway smooth muscle of adrenergic terminals and other terminals having the fine structure of cholinergic terminals [7]. The presence in the dog of varicosities, some of which touch the glycocalyx of airway smooth muscle cells, suggests that there is local adrenergic influence on these cells. Effects of catecholamines on cholinergic neurotransmission may enhance peripheral adrenergic influence on airway smooth muscle [36]. The intertwining of adrenergic and cholinergic terminals establishes a morphologic basis for interactions between them. Also, studies of peribronchial ganglia [4,25] indicate adrenergic innervation

Fig. 12. Cholinergic terminal (C) lying in a depression of the sarcolemma of a baboon bronchial smooth muscle cell (br). Glycocalyx (g) covers the nerve terminal, muscle cell and intervening neuromuscular cleft (arrow). ×29,062.

Fig. 13. Cholinergic terminal containing a lamellar body (lb) and glycogen granules (gly) as well as large and small synaptic vesicles (sv) and mitochondria (mit). This baboon bronchial smooth muscle cell appears to enclose the nerve terminal, creating a nerve—muscle interval of about 18 nm (arrow). ×18,750.

of these neurons so that catecholamines may affect neurotransmission in the ganglia as well.

Consistent vagally-mediated bronchoconstriction with simultaneous supramaximal sympathetic stimulation [8] and the presence of numerous cholinergic terminals in close proximity to bronchial smooth muscle together indicate that neural regulation of these airways is predominantly cholinergic. Most of the bronchial smooth muscle cells in each bundle are separated from one another by glycocalyx and endomysium so that substances may diffuse from cholinergic varicosities between the smooth muscle cells throughout the muscularis. Transmitter release from the elongate terminals described above may enhance effective neurotransmission by uniformly distributing neurotransmitters in this system. Close neuromuscular contacts of the type found in monkey and baboon bronchial smooth muscle have been found in human airway smooth muscle and also in other autonomic neuroeffector systems [18,27,34]. If these are efferent terminals and if close contacts have any functional significance, then one can postulate both release of neurotransmitter into the neuromuscular cleft and the presence of complementary receptors (postsynaptic and possibly presynaptic) in the apposed membranes. It has been calculated that such narrow neuromuscular intervals as these can make high concentrations of intrasynaptic transmitter attainable [2]. Bennett [1] considered it likely that most smooth muscle systems are innervated by both small axon bundles and close-contact varicosities as is described in this paper in primate bronchial smooth muscle. It is suggested that mixed innervation by small axon bundles and close contacts may underlie the biphasic responses to nerve stimulation in some smooth muscle preparations, with the close contacts mediating the fast, drug-resistant component [3]. It is probable that many afferent nerve terminals were seen in the course of this study. King et al. [24] concluded that some enlarged axon profiles containing small mitochondria and agranular vesicles near avian airway smooth muscle cells may be sensory receptors.

In the species studied, those nerve varicosities that contain clusters of large dense-core vesicles and some small agranular vesicles resemble inhibitory (purinergic) terminals found in the amphibian lung and mammalian gut [5], but cannot be positively identified as such. Since the small vesicles do not have the dense-cores that are indicative of uptake of 5-hydroxydopamine, the terminals probably are not adrenergic. Purinergic terminals have been described as having many large opaque vesicles (80—200 nm) without prominent halos around the cores [31]. Such profiles in the lungs of dogs, monkeys and baboons may be purinergic terminals, but it is also possible that they are cholinergic terminals that have unusual proportions of large and small vesicles. Large and small vesicles are not uniformly distributed in nerve terminals and the large vesicles associated with many varicosities are clustered near one end of the enlarged axon segment so that a section through this portion would show mainly large vesicles [11]. Furthermore, large vesicles are known to be produced in the perikarya and transported along the axons to terminal regions. Hebb suggested that such axonal trans-

port supplies terminals with the structural units required for storage and release of transmitter [22]. It seems likely that these vesicles may aggregate at some point along the axon which could enlarge to establish a new site of transmitter release. Therefore, movement of vesicles in support of synaptic transmission may account for some unusual axon profiles, especially during growth or modification of the nerve plexus. It is uncertain whether the fine structure of purinergic terminals is characteristic enough to be used as the sole criterion for identification. Morphometric studies of vesicle distribution in these terminals are unconvincing because there is a continuum of profiles ranging from those with many small and few large vesicles to those with many large and few small vesicles [19]. Although distinctions (noted above) between large opaque vesicles of purinergic nerves and large dense-core vesicles of cholinergic nerves have been made, the original criteria have not been rigidly followed in identifying purinergic terminals. Some reported purinergic varicosities [30] are not axon enlargements with clusters of synaptic vesicles but are intervaricosity axon segments containing several large dense-core vesicles and microtubules. Nevertheless, there is evidence for nonadrenergic, neural inhibition of airway smooth muscle contraction in several species [6,10,12,26,29]. Burnstock has reviewed the evidence that nonadrenergic, inhibitory nerves release ATP [6], and VIP-containing nerves have been visualized in airway smooth muscle [37], but it has not been established what neurotransmitter is released by these nerves.

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