Adrenergic Innervation of the Parasympathetic Ciliary Ganglion in the Chick

Abstract. *The chick ciliary ganglion receives a nonvascular sympathetic innervation in addition to the well-known cholinergic one; fluorescent, varicose adrenergic fibers form pericellular baskets. Adrenergic fibers were identified electron microscopically in ganglia fixed with potassium permanganate. The fibers degenerate after injection of 6-hydroxydopamine. No true synaptic relationships involving adrenergic varicosities and ganglion cells or cholinergic terminals were demonstrable. The distribution of the adrenergic fibers suggests a kind of "distance à synapse" with the choroidal cells or with the preganglionic fibers (or both). The adrenergic innervation might provide a modulation of the cholinergic transmission.*

The ciliary ganglion of the chick is composed of about 4000 neurons. These are subdivided into two distinct cell groups, the so-called ciliary and choroid neurons, innervating the striated musculature of iris and ciliary body and the choroidal smooth muscle fibers, respectively (*1, 2*). Both types of cell receive preganglionic cholinergic fibers via nerve III (oculomotor) (*3*). Until a few years ago, the avian ciliary ganglion was thought to consist of cholinergic elements only (*2, 4*). Recently, however, Ehinger (*5*), using the Falck-Hillarp fluorescence technique, has demonstrated that adrenergic fibers are present in the ciliary ganglion and form baskets of synaptic character around the small cells. These correspond to the choroid neurons according to the terminology used now (*2*).

Our investigation was undertaken in order to answer the following questions: (i) Are there two classes of choroid neurons receiving adrenergic and cholinergic innervation, respectively, or do adrenergic and cholinergic fibers converge on the same target? (ii) If a convergence exists, which are the exact relations between adrenergic and cholinergic structures in this ganglion? (iii) To what extent does this model lend itself to functional experimentation?

It appears from our data that a convergence exists. However, as a difference from the cholinergic fibers, which form axosomatic and axodendritic synapses of the Gray type I, the varicosities of the adrenergic fibers do not establish true synaptic junctions with the choroid neurons and are found in more or

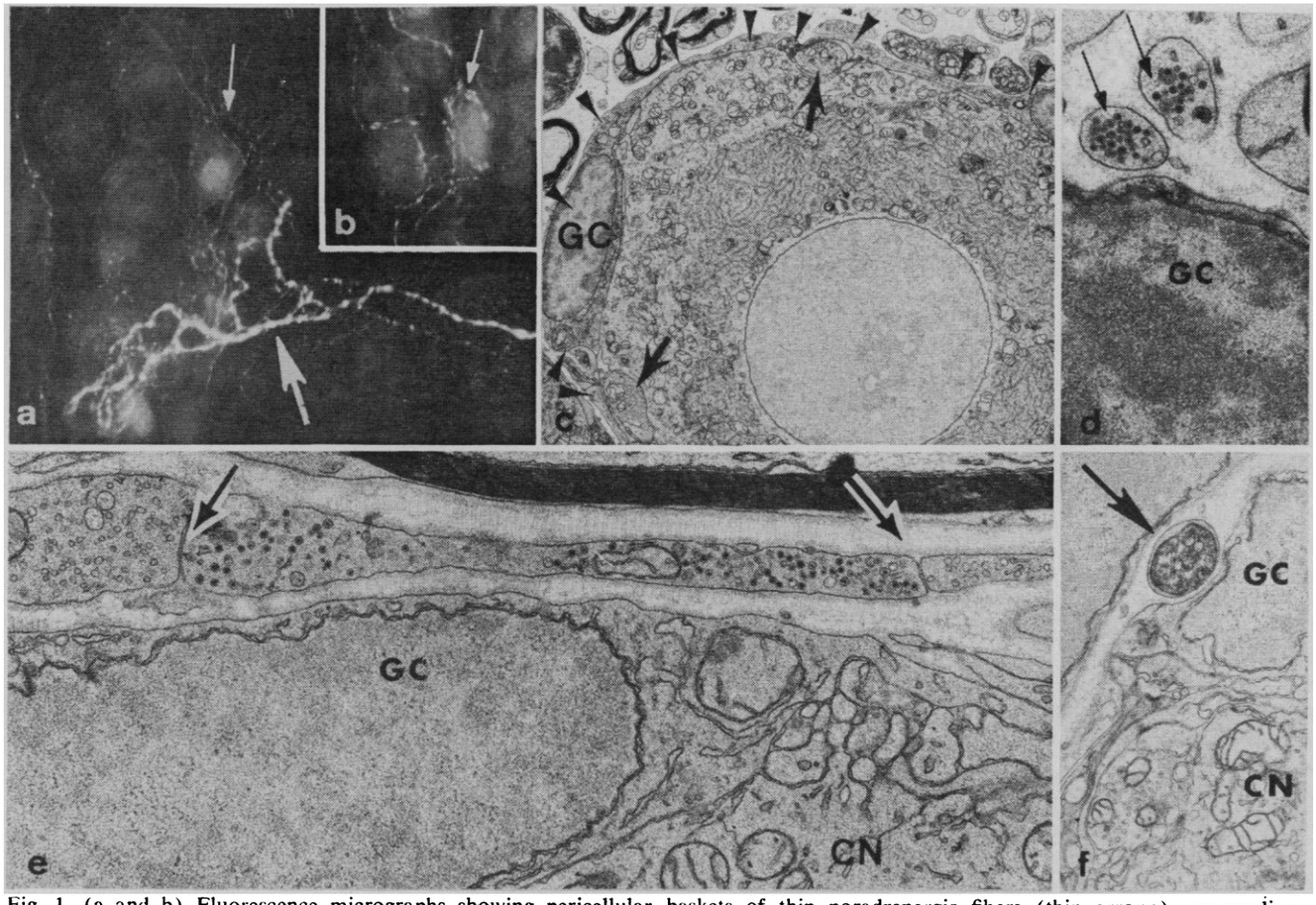

Fig. 1. (a and b) Fluorescence micrographs showing pericellular baskets of thin noradrenergic fibers (thin arrows) surrounding choroidal neurons in the chick ciliary ganglion, as well as coarse vasomotor fibers (large arrow) (× 200). (c to f) Electron micrographs from ganglia fixed in potassium permanganate. (c) A choroid neuron and its surrounding structures. Large arrows point to preganglionic cholinergic terminals synapsing on the perikaryal surface [see (f)] and containing nongranulated synaptic vesicles. Note the flattened satellite glial cells (*GC*) ensheathing completely the ganglionic neuron (arrowheads). Neighboring myelinated and unmyelinated axons are unlabeled (× 3000). (d) Two naked noradrenergic varicosities with granulated vesicles (arrows) facing a satellite cell (*GC*) (× 23,000). (e) A naked varicose fiber separated by a gap of only 2000 Å, including the two basal laminae, from the satellite cell (*GC*) of a choroid neuron (*CN*). The noradrenergic fiber is closely apposed to cholinergic boutons (at arrows) (× 21,000). (f) Degenerating varicosity (arrow) 2 hours after intravenous injection of 100 mg of 6-hydroxydopamine per kilogram of body weight (× 16,000).

less close apposition to preganglionic fibers and terminals. Since the ganglion is sizable (about 0.5 mg) as well as easily accessible, and since the preganglionic cholinergic and adrenergic fibers have different origins, the model seems also useful for electrophysiological and biochemical studies on the role of adrenergic innervation in synaptic functions.

White Leghorn hens, 5 weeks to 1 year of age, were used for this investigation. For fluorescence microscopy, specimens were prepared according to the newly introduced glyoxylic acid–or formaldehyde-Vibratome technique (*6*). For electron microscopy, ciliary ganglia were dissected out, divided in two halves, and immersed in potassium permanganate (*7*). The blocks were dehydrated in serial dilutions of cold methanol, treated with propylene oxide, and embedded in a mixture of TAAB (TAAB, England) embedding resin and Epon. Ultrathin sections, stained with uranyl acetate and lead, were studied by electron microscopy (Hitachi HU-12). Some of the animals were killed 1 to 96 hours after one to four intravenous injections of 6-hydroxydopamine (100 mg/kg) (*8*).

The pericellular baskets of fine varicose terminals display a formaldehyde-induced green fluorescence (Fig. 1, a and b), as already described by Ehinger (*5*). In our sections, prepared without prior pharmacologic treatment, the nerve cells exhibit fine gold-orange autofluorescent granules, which on occasion are prominent, but no aminergic neuronal perikarya are present in the ganglion (*9*). Vasomotor fibers are few, coarse, and easily distinguishable from the pericellular ones (Fig. 1a). While in the choroid cell area the adrenergic fibers are numerous and form a conspicuous network, in the area containing mainly ciliary neurons the adrenergic fibers are fewer, less branched, and have fewer varicosities. Some of the fibers in the ciliary neuron area pass from the ganglion to the postganglionic nerves. Thus, the adrenergic innervation seems to be mainly related with neurons of the choroid category.

In the electron microscope the choroid neurons can easily be distinguished from the ciliary neurons on the basis of four criteria: (i) they do not have compact perikaryal myelin; (ii) the nucleus is usually located in the center of the perikaryon; (iii) their Nissl substance is not restricted to the perinuclear region but may occupy the peripheral cytoplasm: and (iv) there are fewer axosomatic boutons, which can also be found opposite to the axon hillock. The choroid nerve cell body with its cholinergic boutons is surrounded (Fig. 1c) by flattened satellite cells, whose processes overlap extensively, forming a complete glial sheath around the perikaryon, penetrated only by the cholinergic preganglionic fibers, the exiting initial axon segment, and a few short dendrites. Between this glial sheath and the surrounding connective tissue, a basal lamina is present. Around the cells, profiles of dendrites and small myelinated axons are evident. These processes have a distinct glial sheath. At several places one can also recognize bundles of two or more unmyelinated axons, completely or partially covered by a Schwann cell, as well as free endings of cholinergic and adrenergic types. The adrenergic varicosities are easily recognized by their small dense core vesicles (Fig. 1, d and e) (*7*). More often they occur close to the glial sheath covering the choroid neurons, together with a preterminal axon devoid of synaptic vesicles, or with cholinergic boutons (Fig. 1e). They also occur free in the extracellular space (Fig. 1d) at a distance of 0.2 to a few micrometers from the surface of choroid neurons. Adrenergic varicosities were never found in direct contact with the choroid neuron beneath the glial satellite cells.

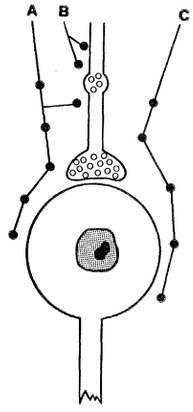

Fig. 2. Diagram of the possible functional relationships of the noradrenergic fibers (black dotted lines) with the cholinergic (with clear vesicles) fibers and the choroid neurons. Noradrenergic fibers might modulate (*A*) both preganglionic fibers and choroid neuron, (*B*) preganglionic fibers only, or (*C*) the choroid neuron. Typical synaptic junctions are absent in all three cases.

The anatomical situation does not allow us to conclude with certainty which is the exact target of the adrenergic fibers. The fact that the adrenergic varicosities lie closer to preganglionic cholinergic fibers than to the ganglionic neurons does not necessarily imply that the former and not the latter is provided with adrenergic receptors, since the proximity between adrenergic and cholinergic fibers might simply be due to the sharing of the same Schwann cell sheath. It seems that the catecholamine, if liberated from the varicosities, should easily reach possible receptors on the surface of the choroid neurons by diffusion, as glial sheaths are patent to small molecules (*10*). The conspicuous network of varicose adrenergic fibers surrounding choroid neurons suggests a functional role for the adrenergic innervation. On the basis of our observations, we present three possibilities as shown schematically in Fig. 2: (i) the adrenergic fibers interact with the preganglionic fibers and choroid neurons; (ii) the adrenergic fibers interact with the preganglionic fibers only; and (iii) the adrenergic fibers exert their effect on the choroid neuron exclusively through what could be called a "synapse à distance" (*11*).

From our sections, we see that most of the fluorescent adrenergic fibers reach the ciliary ganglion through the oculomotor nerve. However, they must join this nerve within the orbit since both the intracranial portion of the nerve and its root contain only a few vasomotor fluorescent fibers. The fibers presumably originate from the cranial cervical ganglion (the avian equivalent of the superior cervical ganglion in mammals). As the parent adrenergic neurons lie outside the ganglion, they can be removed surgically without damage to the cholinergic preganglionic fibers. On the contrary, it should be possible to selectively provoke hypertrophy of the adrenergic fibers with nerve growth factor (*12*). We have evidence that the adrenergic fibers of the ciliary ganglion degenerate after injection of 6-hydroxydopamine (Fig. 1f). Their peripheral origin, green fluorescence, electron microscopic appearance after potassium permanganate fixation, and degeneration after treatment with 6-hydroxydopamine (*13*) suggest that the fibers under consideration are noradrenergic, although definitive proof depends on further investigation.

The aminergic innervation of the parasympathetic ciliary ganglion in the chick (*14*) resembles, at least to some extent, that of ganglionic cells in the mammalian myenteric plexus and in Gasserian and spinal ganglia (*15*) [reviewed in (*16*)]. In the intestine, however, the adrenergic terminals may (*17*) or may not (*18*) form distinct synaptic junctions on the cholinergic neurons. The adrenergic effect seems to be mediated by an α receptor (*19*). In certain species, however, the anatomical situation in intestinal ganglia

appears more complicated than in the avian ciliary ganglion because of the presence of peripheral aminergic and "peptidergic" neurons (*18, 20*). The situation in Gasserian and spinal ganglia is still unclear (*15*).

A close relationship between adrenergic and cholinergic axons in the proximity of smooth muscles of several peripheral organs has been described (*21*), although the two types of axons, as in our case, do not form true synaptic junctions. There is circumstantial evidence for a peripheral mutual interaction between adrenergic and cholinergic terminals (*21*).

Obviously, pharmacological studies are necessary to establish the functional role of the adrenergic fibers in the ciliary ganglion as compared to the other tissues discussed here. Marwitt *et al.* (*2*) studied the effect of adrenergic blockers in the pigeon ciliary ganglion in vitro using extracellular recording. Only dibenzyline showed some effect in concentrations that did not impair nerve conduction. Knowledge gained by studies of our model might relate to situations in the central nervous system, for instance in the cerebellum (*22*).

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9. After treating the animals with nialamide and L-dopa, Ehinger (*5*) observed enhancement of the fluorescence of adrenergic baskets as well as the appearance of some fluorescent cells, whose pharmacohistochemical behavior is not typical for adrenergic neurons. During our extensive electron microscopic study, nerve cells with granulated vesicles comparable to the interneurons of sympathetic ganglia were never observed in the ciliary ganglion. We noticed, however, the presence of a considerable number of mast cells.
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14. The mammalian ciliary ganglion differs from the avian counterpart, as it appears to contain only rare adrenergic terminals under normal conditions. However, in slowly exsanguinated animals or after prior pharmacologic treatment, small fluorescent cells appear, as well as pericellular networks. The significance of these findings remains unclear [B. Hamberger, K. A. Norberg, U. Ungersted, *Acta Physiol. Scand.* **65**, 235 (1965); K. T. Huikuri, *ibid.* **69**, Suppl. No. 286 (1966); B. Ehinger and B. Falck, *ibid.* **78**, 132 (1970)].
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Cation Dependence of High-Affinity Angiotensin II Binding to Adrenal Cortex Receptors

Abstract. *The specific binding of monoiodinated angiotensin II by particulate receptors from the bovine adrenal cortex is enhanced by addition of sodium and potassium ions, but not other cations. In the presence of 140 millimolar sodium, increased uptake of angiotensin II by adrenal receptors is associated with the appearance of high-affinity binding sites with an association constant of* 2×10^9 *liters per mole.*

Specific receptors for angiotensin II have been described in particulate subcellular fractions from the bovine and rat adrenal cortex (*1, 2*). The majority of the angiotensin II receptor sites in bovine and rat adrenal cortex particles were found to be located in the plasma membrane fraction (*2*). Meyer *et al.* (*3*) came to a similar conclusion about the origin of the particulate angiotensin receptors derived from aortic smooth muscle cells. The octapeptide angiotensin II displays much greater avidity for adrenal cortex receptors than the decapeptide precursor, angiotensin I. Also, competitive binding studies performed in the presence of adrenal cortex particles and ^{125}I-labeled monoiodoangiotensin II show a close correlation between adrenal binding-inhibition activity and biological potency in a variety of angiotensin fragments and analogs (*1, 2*).

During an investigation of angiotensin receptors in various target tissues of the hormone, we noted that specific binding of ^{125}I-labeled angiotensin II to particulate fractions of bovine and rat adrenal cortex was influenced by the buffer composition, and especially by the cation content of the incubation medium. The experiments shown in Fig. 1 depict the basic finding, that sodium and potassium ions significantly increased the binding of angiotensin II to bovine adrenal cortex receptors, whereas the tris(hydroxymethyl)aminomethane ion showed no such effect. The increase in angiotensin II binding appeared to be saturable with respect to the cation concentration between 0 and 200 m*M*. At concentrations of sodium and potassium above 250 m*M*, the stimulation of binding gradually decreased and reached the original level, observed in the absence of both cations, at about 500 m*M*. The effects of sodium and potassium on angiotensin II binding were specific for these ions, and were not reproduced by rubidium, cesium, lithium, or magnesium; rather, some of these cations caused inhibition of angiotensin binding at high concentrations (Fig. 1B).

Particulate receptor preparations in-