Effects of Preganglionic Denervation and Postganglionic Axotomy on Acetylcholine Receptors in the Chick Ciliary Ganglion

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Abstract. The regulation of nicotinic acetylcholine receptors (AChRs) in chick ciliary ganglia was examined by using a radiolabeled anti-AChR mAb to quantitate the amount of receptor in ganglion detergent extracts after preganglionic denervation or postganglionic axotomy. Surgical transection of the preganglionic input to the ciliary ganglion in newly hatched chicks caused a threefold reduction in the total number of AChRs within 10 d compared with that present in unoperated contralateral control ganglia. Surgical transection of both the choroid and ciliary nerves emerging from the ciliary ganglion in newly hatched chicks to establish postganglionic axotomy led to a nearly 10-fold reduction in AChRs within 5 d compared with unoperated contralateral ganglia. The declines were specific since they could not be accounted for by changes in ganglionic protein or by decreases in neuronal survival or size. Light microscopy revealed no gross morphological differences between neurons in operated and control ganglia. A second membrane component of cholinergic relevance on chick ciliary ganglion neurons is the α-bungarotoxin (α-Bgt)-binding component. The α-Bgt-binding component also declined in number after either postganglionic axotomy or preganglionic denervation, but appeared to do so with a more rapid time course than did ganglionic AChRs. The results imply that cell-cell interactions in vivo specifically regulate both the number of AChRs and the number of α-Bgt-binding components in the ganglion. Regulation of these neuronal cholinergic membrane components clearly differs from that previously described for muscle AChRs.

MOTOR innervation of vertebrate skeletal muscle plays a major role in regulating the number and distribution of nicotinic acetylcholine receptors (AChRs)[1] in muscle tissue (for reviews see Fambrough, 1979, and Schuetze and Role, 1987). Electrophysiological studies have indicated that neuronal AChRs may also be regulated by cell-cell interactions. Focal iontophoretic application of acetylcholine (ACh) to the surface of frog and mudpuppy parasympathetic neurons during intracellular recording reveals a nonuniform distribution of ACh sensitivity consistent with the greatest receptor localization being at points of synaptic contact (Harris et al., 1971; Roper, 1976; Dennis and Sargent, 1979). Surgical denervation of the neurons causes an increase in the mean ACh sensitivity, a more uniform distribution of sensitivity, and a decrease in the mean rise time of the response. These results suggest the appearance of substantial numbers of extrasynaptic AChRs as a consequence of neuronal denervation (Kuffler et al., 1971; Roper, 1976; Dennis and Sargent, 1979). Recent studies with frog sympathetic neurons have led to a different conclusion: the neurons have a nonuniform distribution of ACh sensitivity, but denervation produces no change in the magnitude, distribution, or time course of the ACh response of the cells (Dunn and Marshall, 1985). The basis for the different results with frog denervated sympathetic and parasympathetic neurons remains unresolved. The effects of postganglionic axotomy on neuronal ACh sensitivity have been examined in parasympathetic ciliary ganglia of newly hatched chicks where surgical transection of the postganglionic nerves causes an eightfold reduction in sensitivity after 3–4 d (Brenner and Martin, 1976). Postganglionic axotomy may also produce a reduction in the ACh sensitivity of adult guinea pig sympathetic neurons (Purves, 1975). These findings suggest that the number and possibly the distribution of neuronal AChRs can be regulated by one or more types of cell-cell interactions, and that the underlying mechanisms may differ from those previously described for regulation of the well-characterized skeletal muscle AChR.

Recently probes have been identified that distinguish and permit the quantitation of AChRs in the chick ciliary ganglion. One of these is a monoclonal antibody, mAb 35, that was raised against AChR purified from *Electrophorus* electric tissue and recognizes the "main immunogenic region" of muscle and electric organ AChR α subunits (Tzartos et al., 1981; Barkas et al., 1987). Several lines of evidence indicated that mAb 35 cross reacts with the AChR of chick ciliary gan-

1. *Abbreviations used in this paper*: ACh, acetylcholine; AChR, nicotinic acetylcholine receptor; α-Bgt, α-bungarotoxin.

glion neurons (Jacob et al., 1984; Smith et al., 1985, 1986; Stollberg et al., 1986). This indication has now been confirmed by demonstrating that the α-neurotoxin Bgt 3.1, which specifically and completely inhibits the AChR response of ciliary ganglion neurons (Ravdin and Berg, 1979; Ravdin et al., 1981), can be used to identify AChRs on the neurons (Halvorsen and Berg, 1986) and that the receptors are the same as those recognized by mAb 35 on the surface of the cells (Halvorsen and Berg, 1987).

The availability of mAb 35 as a probe for neuronal AChRs allows one to examine for the first time in a quantitative manner the way in which cell–cell interactions may regulate the number and distribution of neuronal AChRs. We have used ^{125}I-mAb 35 to measure the levels of total AChR present in detergent extracts of ciliary ganglia from newly hatched chicks after preganglionic denervation or postganglionic axotomy in vivo. For comparison, the levels of α-bungarotoxin (α-Bgt)–binding component in the extracts were also determined, since the component is distinct from, though possibly related to, the functional AChR on the neurons. We have measured amounts of total ganglionic protein, number of surviving neurons, and mean neuronal soma and nuclear diameters to determine whether degenerative changes in the neurons might account for the observed changes in AChR levels. We show here that the total number of AChRs declines dramatically after axotomy as predicted by the electrophysiological studies on chick ciliary ganglion neurons. Denervation, however, produces a slow decline in the total number of ganglionic AChRs rather than an increase or maintained level of receptors as observed for ACh sensitivity after preganglionic denervation of frog and mudpuppy autonomic neurons (Kuffler et al., 1971; Roper, 1976; Dennis and Sargent, 1979; Dunn and Marshall, 1985). The total number of α-Bgt–binding components also declines after the operations, and the decline is more rapid than the decrease in AChRs.

Materials and Methods

Operations

Surgical preganglionic denervation or postganglionic axotomy was performed on ciliary ganglia in chicks 2–4 d after hatching by a modification of methods previously described (Pilar and Tuttle, 1982). Briefly, chicks were anesthetized with methoxyflurane followed by sodium pentobarbital. A small incision was made along the caudal portion of the lower eyelid. The eyeball was carefully retracted and the connective tissue attaching the eyeball to the orbital wall was gently separated to expose the posterior portion of the eyeball. Extraocular muscles were spread apart to reveal the ciliary ganglion. For preganglionic denervation, iridectomy scissors were used to cut the preganglionic input from the Edinger-Westphal (accessory oculomotor) nucleus. For postganglionic axotomy all of the ciliary and choroid nerves emerging from the ganglion were cut. Denervation or axotomy was performed on a single ciliary ganglion per chick, leaving the contralateral ganglion as a control. The lower eyelid incision was then sutured and the chicks allowed to recover from the anesthesia. There was occasional bleeding. Animals that bled heavily were not retained for the experiments. The use of alcohol-sterilized instruments and Betadine antiseptic solution to paint the skin around the lower eyelid appeared to be sufficient to prevent infection. Chicks were maintained in a heated brooder for 1–5 d after axotomy and 2–10 d after denervation. The complete absence of a pupillary light reflex was used as a criterion for a successful operation; the ganglion was examined at the end of the experiment to confirm that the appropriate nerves had been completely severed. The success rate of the surgery was ~85%.

AChR Assays

mAb 35 was purified and radioiodinated to specific activities of 2–3 × 10^{18} cpm/mol as previously described (Smith et al., 1985). Total ganglionic AChRs were assayed by determining the number of specific ^{125}I-mAb 35–binding sites present in detergent extracts prepared from ciliary ganglia as previously described (Smith et al., 1985). Briefly, both operated and control ganglia were dissected, trimmed free of connective tissue, and stored frozen at −70°C while accumulating stocks. The ganglia were then thawed and homogenized at 4°C by hand in 10 mM $NaPO_4$, pH 7.4, containing 50 mM NaCl and 0.5% Triton X-100. Particulate debris was removed by centrifugation for 1 min at 15,600 *g*. Aliquots of the extract were then incubated with 4 nM ^{125}I-mAb 35 for 30 min at room temperature, and bound ^{125}I-mAb 35 was separated from free antibody by ion exchange chromatography on DEAE cellulose. Nonspecific binding, determined by including a 30–40-fold excess of unlabeled mAb 35 in the binding reaction, was subtracted from total radioactivity retained by the column to calculate specific binding. Results were corrected for a mean efficiency of 64% for the column procedure (Stollberg and Berg, 1987).

Other Assays

α-Bgt was purified and radioiodinated by a modified chloramine T method as previously described (Ravdin and Berg, 1979; Lindstrom et al., 1981). Binding sites were assayed in detergent extracts by using a modification of the method of Meunier et al. (1974) as previously described (Smith et al., 1985).

Total ganglionic protein was measured in detergent extracts by the protein microassay (Bio-Rad Laboratories, Richmond CA).

Neuronal cell counts were carried out on serial sections of both operated and control ganglia as previously described (Landmesser and Pilar, 1974; Nishi and Berg, 1977). Briefly, day 5 axotomized and day 10 denervated ganglia along with contralateral control ganglia were fixed with 2% glutaraldehyde and 2% paraformaldehyde in 20 mM $NaPO_4$, pH 7.2, containing 60 mM sucrose. The ganglia were rinsed, trimmed, postfixed with osmium tetroxide, and processed for Epon embedment. Ganglia were serially sectioned at a thickness of 7 μm. The sections were mounted on subbed glass slides (Rogers, 1973) and stained with toluidine blue. All ciliary and choroid neurons possessing a nucleus with a distinct nucleolus were counted in each section of the ganglion. The cell counts were corrected for double counting by the method of Abercrombie (1946). Ciliary and choroid neurons were distinguished by morphological criteria (Landmesser and Pilar, 1974). Neuronal soma and nuclear diameters were measured with a light microscope using a calibrated reticule and eyepiece.

Materials

White Leghorn chick embryonated eggs were obtained locally from McIntyre Poultry (Lakeside, CA) and maintained and hatched at 39°C in a humidified incubator. The hybridoma cell line secreting mAb 35 was generously provided by Dr. Jon Lindstrom of the Salk Institute (San Diego, CA). *Bungarus multicinctus* venom was purchased from Miami Serpentarium (Salt Lake City, UT). Methoxyflurane was purchased from Pitman-Moore, Inc. (Washington Crossing, NJ), and sodium pentobarbital from Sigma Chemical Co. (St. Louis, MO).

Results

Changes in the Number of AChRs

Unilateral postganglionic axotomy of ciliary ganglia in newly hatched chicks caused a rapid decline in the total number of ganglionic AChRs as detected by specific ^{125}I-mAb 35 binding in ganglionic detergent extracts. No compensatory change was observed in the number of AChRs in the unoperated contralateral ciliary ganglion from the same animals (Fig. 1 *A*). 5 d after axotomy the ratio of AChRs in operated and control ganglia had declined ~10-fold (Fig. 1 *B*). Unilateral preganglionic denervation of ciliary ganglia in newly hatched chicks also produced a decline in ganglionic AChRs, though with a slower time course. Again, no change was observed in the number of AChRs in unoperated

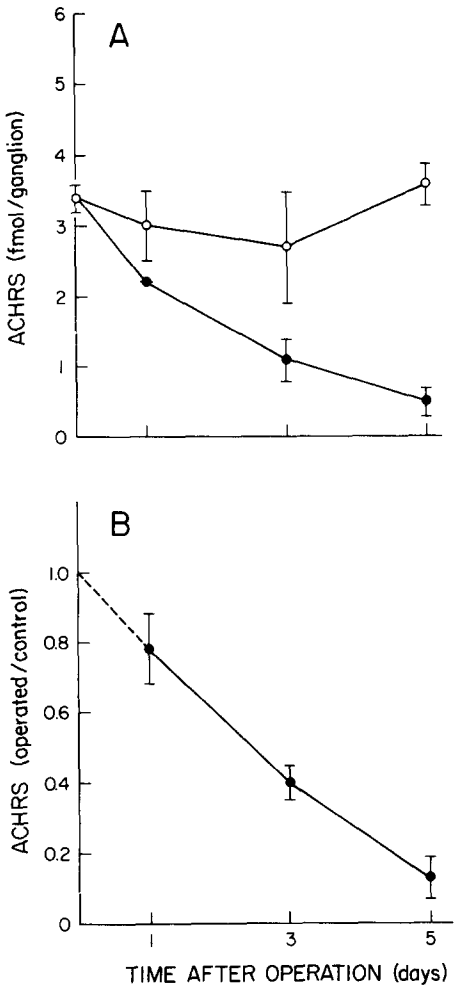

Figure 1. AChRs in axotomized ganglia. The ciliary and choroid nerves emerging from the ciliary ganglion were surgically transected in newly hatched chicks, and the axotomized ipsilateral and unoperated contralateral ganglia were taken at the indicated times and assayed for specific ^{125}I-mAb 35 binding to total AChRs in ganglionic detergent extracts. Each value represents the mean ± SEM of 2–4 determinations. (*A*) The number of AChRs per operated (*solid circles*) or unoperated (*open circles*) ganglion. (*B*) The ratio of AChRs in operated and control ganglia. AChR levels in 5-d axotomized ganglia are only about a tenth of those present in unoperated contralateral ganglia.

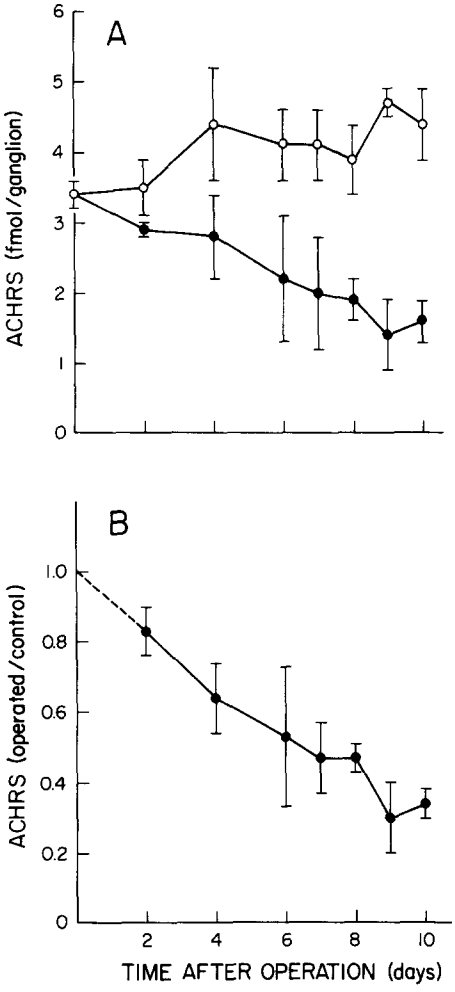

Figure 2. AChRs in denervated ganglia. The preganglionic nerve to the ciliary ganglion was surgically transected in newly hatched chicks, and the denervated ipsilateral and unoperated contralateral ganglia were taken at the indicated times and assayed for specific ^{125}I-mAb 35 binding to total AChRs in ganglionic detergent extracts. Each value represents the mean ± SEM of 2–4 determinations. (*A*) The number of AChRs per operated (*solid circles*) or unoperated (*open circles*) ganglion. (*B*) The ratio of AChRs in operated and control ganglia. AChR levels in 10-d denervated ganglia are about a third of those present in unoperated contralateral ganglia.

contralateral ciliary ganglia (Fig. 2 *A*). 10 d after denervation, AChR levels had fallen about threefold in denervated as compared with unoperated contralateral ganglia (Fig. 2 *B*). In these and subsequent experiments the number of AChRs was calculated assuming, for convenience, a 1:1 stoichiometry of mAb 35 bound to receptor. It is possible that two or more mAbs bind per receptor (Halvorsen and Berg, 1987). The exact stoichiometry is not important for the present studies, which instead depend only on a comparison of the relative amounts of receptor.

The AChR declines were specific in that they were not accompanied by decreases in total ganglionic protein. Axotomy produced a 62% increase in the amount of ganglionic protein in the operated side over a 5-d period while no change was observed in the contralateral side: values at 5 d were 37 ± 8 and 23 ± 3 μg/ganglion (mean ± SEM, n = 5) for operated and control ganglia, respectively. The increased protein probably reflected proliferation of satellite cells as previously described in autonomic ganglia after axotomy (Purves, 1975). Normalizing the levels of ganglionic AChRs for protein still yielded an ~10-fold difference between operated and control ganglia with respect to AChR levels 5 d after axotomy. No change in the amount of total ganglionic protein was detected as a consequence of denervation over the 10-d period examined even though most of the preganglionic terminals were likely to have degenerated within 3 d, assuming that pigeon (Giacobini et al., 1979) and chick are similar in this regard. Expressing AChR levels per

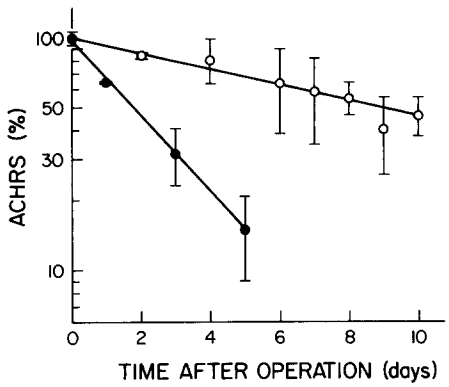

Figure 3. Decline in AChRs after axotomy and denervation. The number of AChRs per ganglion after axotomy (*solid circles*) or denervation (*open circles*) is presented as a percent of those present at the time of surgery. Values represent the mean ± SEM of 2–4 determinations and were calculated from the data presented in Figs. 1 *A* and 2 *A*.

ganglionic protein produced the same approximately threefold difference between operated and control side ganglia 10 d after denervation.

The declines in AChRs observed both after postganglionic axotomy and after preganglionic denervation represent reductions in the number of receptors in each case rather than decreases in the affinity of receptor for antibody probe: increasing the concentration of ^{125}I-mAb 35 threefold in the standard binding assay failed to detect additional specific binding sites in extracts of either denervated or axotomized ganglia (data not shown). The decline in AChRs after axotomy followed a single exponential rate of decay with a half-time for receptor loss of 43 h (Fig. 3). AChR loss after denervation also followed a single exponential rate of decay. The half-time for receptor loss in this case was 9 d (Fig. 3).

Neuronal Survival and Size

To determine whether the decreases in AChRs might reflect neuronal cell death after the operations, cell counts were performed on serial sections of operated and control ganglia. Cell morphology at the light microscope level was used to distinguish choroid and ciliary neurons (Landmesser and Pilar, 1974) so that separate counts could be carried out. 5 d after axotomy a decrease was observed in both populations of neurons (Table I). The magnitude of the decrease 5 d after axotomy (37%) was substantially smaller, however, than the magnitude of the decrease in ganglionic AChRs (90%). 10 d after denervation no difference was detected between control and operated ganglia either in the number of choroid or ciliary neurons (Table I). Light microscopic analysis revealed no gross degenerative changes in surviving neurons (Fig. 4), although no specific stains were used to detect changes in the distribution of Nissl substance in response to axotomy as previously described (Pilar and Landmesser, 1972). Measurements of neuronal soma and nuclear diameters revealed no degenerative changes or shrinkage of surviving neurons compared with control cells after either 5 d of axotomy or 10 d of denervation (Table II). These results demonstrate that the declines in AChR number produced by axotomy and by denervation did not reflect nonspecific degenerative changes in the neuronal population.

α-Bgt–binding Components

Chick ciliary ganglion neurons have a membrane component that binds α-Bgt. The component is distinct from the functional synaptic AChR on the neurons since it is not present in synaptic membrane, and it is different from the AChR detected by Bgt 3.1 and mAb 35 (Jacob and Berg, 1983; Smith et al., 1985; Halvorsen and Berg, 1986, 1987). The component is, nonetheless, likely to be involved in cholinergic signaling in some manner since nicotinic cholinergic ligands specifically block α-Bgt binding to the component, and the component is restricted in the neuron surface to areas surrounding preganglionic terminals. Accordingly, it seemed of interest to determine whether the α-Bgt–binding component was regulated in parallel with AChRs in the ganglion after postganglionic axotomy or preganglionic denervation.

Unilateral postganglionic axotomy produced a very rapid decline in the total number of ganglionic α-Bgt–binding sites as detected by specific ^{125}I-α-Bgt binding in ganglionic detergent extracts. Only about a quarter of control levels remained 1 d after the operation (Fig. 5 *A*). No further decline was observed at subsequent times. The number of α-Bgt–binding sites in the unoperated contralateral ganglion did not change over the 5-d test period. Unilateral preganglionic denervation also produced a rapid decline in the number of α-Bgt–binding sites in the ganglion such that about one-half of the original number was left at 2 d (Fig. 5 *B*). No further decrease was observed at later times after denervation. The number of α-Bgt–binding sites may have increased somewhat in unoperated contralateral ganglia over the same time course. Normalizing the α-Bgt–binding data for ganglionic

Table I. Neuronal Survival

Ganglion	Number of neurons			
	Ciliary	Choroid	Total	Percent
Control	1,080 ± 120	1,290 ± 220	2,370 ± 250	100 ± 11
Axotomy	650 ± 60	850 ± 80	1,490 ± 110	63 ± 5
Denervation	1,130 ± 70	1,270 ± 200	2,390 ± 210	101 ± 9

Ciliary ganglia were fixed, serially sectioned, stained, and counted separately for surviving choroid and ciliary neurons as described in Materials and Methods. Postganglionic axotomy for 5 d partially reduced the number of surviving neurons while preganglionic denervation for 10 d had no effect on the number of surviving neurons. Control: unoperated contralateral ganglia from chicks having the ipsilateral ganglion axotomized for 5 d. Axotomy: ganglia axotomized for 5 d by surgical transection of the postganglionic choroid and ciliary nerves. Denervation: ganglia denervated for 10 d by surgical transection of the preganglionic accessory oculomotor nerve. Each value represents the mean ± range of two ganglia that were serially sectioned and counted blind, separately.

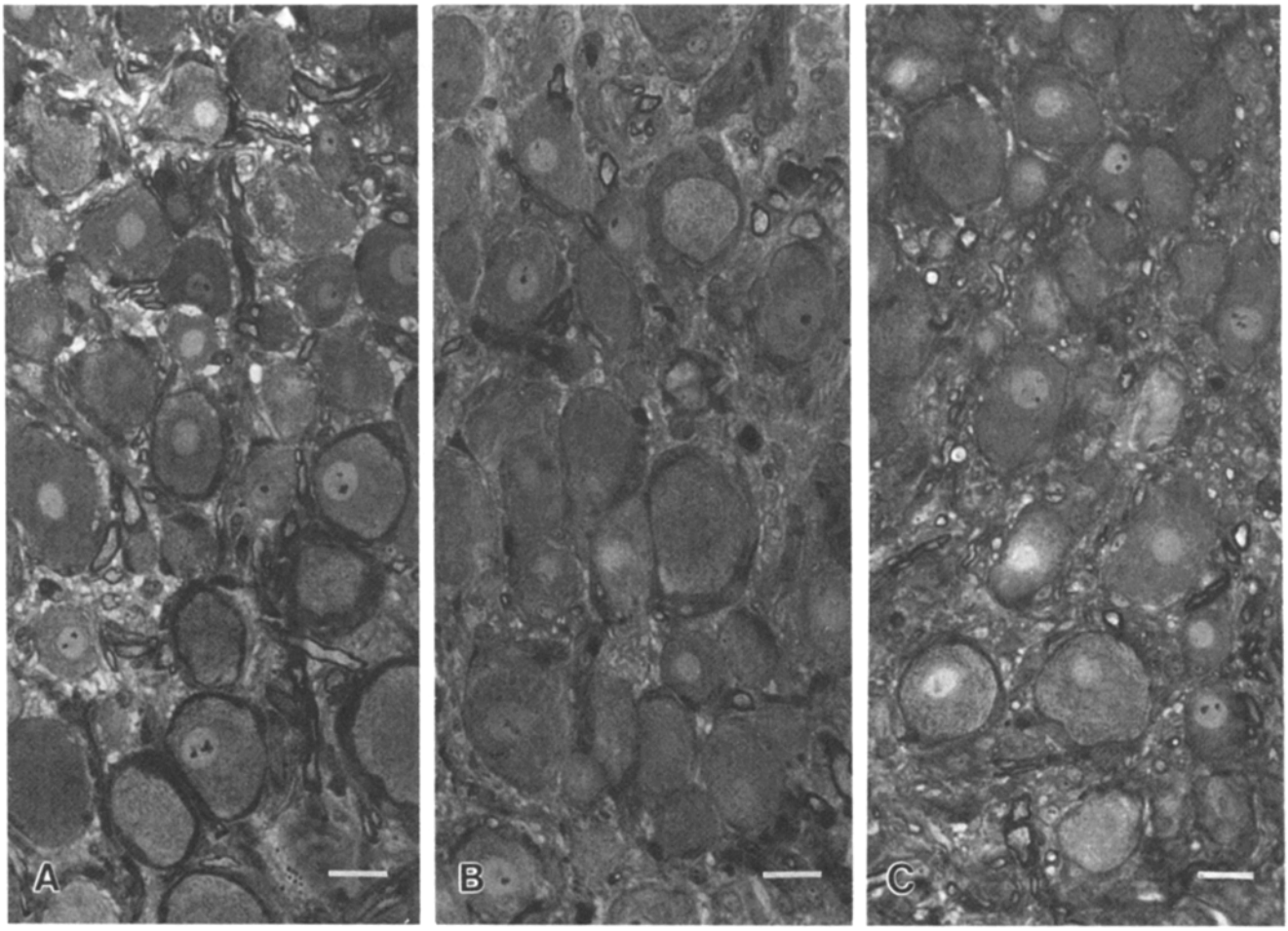

Figure 4. Neuronal morphology. Sections from operated and control ganglia were prepared as described in Materials and Methods and were viewed through a light microscope. No gross differences were observed in ciliary or choroid neuron morphologies for axotomized or denervated ganglia as compared with unoperated contralateral ganglia. (*A*) Unoperated contralateral ganglion from a chick having the ipsilateral ganglion axotomized for 5 d. (*B*) Ganglion 5 d after postganglionic axotomy. (*C*) Ganglion 10 d after preganglionic denervation. Bars, 50 μm.

protein after the operations did not substantially alter the patterns.

When comparisons are made between the declines that occurred after the operations in the number of α-Bgt-binding sites and the number of AChRs, it is clear that the two follow different time courses. α-Bgt-binding sites declined more rapidly, reaching a plateau value by the earliest time examined, both after axotomy and after denervation (Fig. 5). AChRs followed a more gradual but steady decline over the same time periods (Figs. 1 and 2). In all cases examined, the absolute number of α-Bgt-binding components exceeded by at least severalfold the number of AChRs, assuming a similar

Table II. Neuronal Soma and Nuclear Diameters

	Ciliary neurons				Choroid neurons			
Ganglion	Soma diameter	Percent	Nucleus diameter	Percent	Soma diameter	Percent	Nucleus diameter	Percent
Control	28.0 ± 1.3	100 ± 5	9.8 ± 0.5	100 ± 5	17.1 ± 0.9	100 ± 5	7.5 ± 0.4	100 ± 5
Axotomy	31.6 ± 0.8	113 ± 3	11.0 ± 0.3	112 ± 3	19.1 ± 0.8	112 ± 5	7.9 ± 0.4	105 ± 5
Denervation	28.9 ± 0.9	103 ± 3	11.2 ± 0.4	114 ± 4	17.2 ± 0.7	101 ± 4	7.7 ± 0.4	103 ± 5

The soma and nuclear diameters were measured for choroid and ciliary neurons in sections of ciliary ganglia prepared as described in Table I. Each value represents the mean ± range (in microns) of two ganglia. Approximately 40 ciliary and 40 choroid neurons were measured in different regions of each ganglion. No difference was observed in either parameter for operated ganglia compared with control ganglia. Control: unoperated contralateral ganglia from chicks having the ipsilateral ganglion axotomized for 5 d. Axotomy: ganglia axotomized for 5 d by surgical transection of the postganglionic choroid and ciliary nerves. Denervation: ganglia denervated for 10 d by surgical transection of the preganglionic accessory oculomotor nerve.

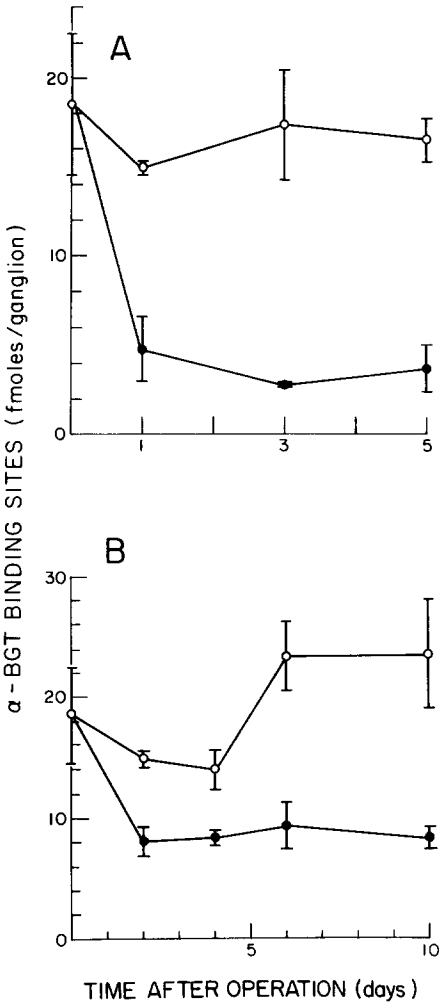

Figure 5. α-Bgt-binding components in operated ganglia. The total number of α-Bgt-binding components in detergent extracts prepared from operated ganglia (*solid circles*) were measured at the indicated times, and the values compared with those obtained from unoperated contralateral ganglia (*open circles*) from the same chicks. Each value represents the mean ± SEM of 3–4 determinations. (*A*) Axotomized ganglia. A sharp decline is observed in the number of α-Bgt-binding components per ganglion 1 d after axotomy; no additional change is observed over the next 4 d. (*B*) Denervated ganglia. The number of α-Bgt-binding components is decreased by day 2 of denervation and remains constant over the next 8 d. Note the different time scales in *A* and *B*.

stoichiometry of α-Bgt and mAb 35 binding to their respective membrane components.

Discussion

The decreases in AChRs and α-Bgt-binding components in the chick ciliary ganglion caused by postganglionic axotomy and by preganglionic denervation are specific consequences since they cannot be accounted for by changes in neuron number or size, or by changes in ganglionic protein. Morphological examination at the light microscopic level detected no gross degenerative consequences in neurons of operated ganglia. Only in the case of axotomy was a decrease in neuron number noted, and the extent of the decline 5 d after axotomy was not nearly as great as the decrease in AChRs or α-Bgt-binding components. Previous reports have suggested a similar resilience of the ganglion: axotomy of chick ciliary ganglia for 9 d was found to reduce total synaptic contact area on the neurons by only 37% (Brenner and Johnson, 1976) while denervation of adult pigeon ciliary ganglia caused no change in total ganglionic protein over 2 wk (Giacobini et al., 1979). Few, if any, changes were apparent in the membrane electrical properties of axotomized and denervated ganglion neurons compared with control neurons over the same time courses used in the present study (Kuffler et al., 1971; Purves, 1975; Brenner and Martin, 1976; Dunn and Marshall, 1985).

The decrease observed here in total ganglionic AChRs caused by axotomy is similar in rate and extent to the decrease previously reported for the mean ACh sensitivity of the neurons after axotomy (Brenner and Martin, 1976). The half-time of 43 h for receptor loss under these conditions is slower than the value of 22 h obtained for the half-life of AChRs on the neuron surface in cell culture (Stollberg and Berg, 1987). Either ciliary ganglion AChRs in vivo have a longer mean half-life or the receptors continue to be synthesized after postganglionic axotomy.

The loss of AChRs induced by denervation is much slower still, displaying a half-time of ~9 d. This decline differs both from the precedent provided by skeletal muscle AChRs and from the results of electrophysiological studies on neuronal surface AChRs. Surgical denervation or chronic paralysis of vertebrate skeletal muscle causes a large increase both in the number of extrajunctional AChRs as reflected in the appearance of ACh sensitivity in extrajunctional regions and in the total number of AChRs in the muscle tissue (for reviews see Fambrough, 1979, and Schuetze and Role, 1987). Denervation of frog and mudpuppy parasympathetic ganglia has also been reported to increase neuronal ACh sensitivity in the ganglia in a manner suggesting increased numbers of functional receptors on the neuron surface (Kuffler et al., 1971; Roper, 1976; Dennis and Sargent, 1979). No change in ACh sensitivity was found when frog sympathetic neurons were denervated (Dunn and Marshall, 1985).

The present finding that denervation of the neurons reduces the total number of AChRs may reflect differences between hatchling chick autonomic neurons and those of adult frog and mudpuppy with respect to AChR regulation. Alternatively, changes in ACh sensitivity may not be an appropriate measure of AChR number since recent studies show that the number of functional AChRs is substantially smaller than and regulated independently from the total number of AChRs on the neurons (Margiotta et al., 1987*a*, *b*). Another consideration is that measurements of total ganglionic AChRs as done here are influenced significantly by a large intracellular AChR pool in the neurons. Ultrastructural studies with mAb 35 have demonstrated that AChRs on the neuron surface in situ are located predominantly at synapses (Jacob et al., 1984). Substantial numbers of internal AChRs can also be identified, however, both in embryonic ganglia (Jacob et al., 1986) and in ganglia from newly hatched chicks (our unpublished observations). The intracellular receptors are associated with organelles known to be involved in the synthesis, processing, and transport of integral membrane proteins. In cell culture it has been shown that

nearly two-thirds of the total specific mAb 35–binding sites are intracellular, and that only a minor fraction of the internal sites is transported to the cell surface (Stollberg and Berg, 1987). The significance and fate of the large intracellular AChR pool is unknown. It is possible that denervation of the neurons causes a large decrease in the intracellular pool of receptor while having little effect on AChRs in the plasma membrane. In any case, it is clear that the regulation of AChRs by innervation in the chick ciliary ganglion is very different from that in vertebrate skeletal muscle since denervation reduces the total number of ganglionic AChRs but increases the number of muscle AChRs.

The relationship between the α-Bgt–binding component and the ganglionic AChR is unknown. The α-Bgt–binding component from chicken brain binds nicotinic cholinergic ligands and contains a subunit with partial amino acid sequence homology to the α subunit of chicken muscle AChR (Conti-Tronconi et al., 1985). Studies with the rat pheochromocytoma cell line PC12 (Patrick and Stallcup, 1977) and with chick ciliary ganglion neurons (Jacob and Berg, 1983; Jacob et al., 1984; Smith et al., 1985; Halvorsen and Berg, 1986, 1987) clearly indicate that the α-Bgt–binding component on the cells is distinct from the functional synaptic AChR. Even in chicken brain the α-Bgt–binding component has been shown to be separate from AChRs distinguished by mAb 35 and high-affinity nicotine binding (Whiting and Lindstrom, 1986*a*, *b*). Nonetheless, it is intriguing that the α-Bgt–binding component is influenced specifically and in a qualitatively similar way to the ganglionic AChR by the surgical disruption of cell–cell interactions, although the effect on the α-Bgt–binding component is more rapid. It seems unlikely that the α-Bgt–binding component serves as a precursor to AChRs on neurons: multiple brain components have been identified that bind nicotine with high-affinity but not α-Bgt (Whiting and Lindstrom, 1986*a*, *b*), and recent evidence suggests multigene families for neuronal AChRs rather than a precursor–product relationship among receptor subtypes (Boulter et al., 1986; Goldman et al., 1986, 1987). Moreover, ciliary ganglion neurons have large intracellular pools of AChRs as defined by mAb 35 binding that may in part serve as direct precursors to the surface AChRs (Jacob et al., 1986; Stollberg and Berg, 1987).

The early decline in α-Bgt–binding sites after ganglionic denervation raises the possibility that some of the sites may be preganglionic in origin. Cell culture studies clearly demonstrate that ciliary ganglion neurons have both α-Bgt–binding components and AChRs (Ravdin et al., 1981; Smith et al., 1986; Halvorsen and Berg, 1986, 1987). Ultrastructural investigations of α-Bgt and mAb 35 binding on the neurons in situ demonstrate a postsynaptic cell localization but cannot exclude the possibility that some of the sites may be associated with presynaptic terminals (Jacob and Berg, 1983; Jacob et al., 1984, 1986; Loring et al., 1985; Loring and Zigmond, 1987). The denervation studies described here indicate that about three-quarters of the AChRs and half of the α-Bgt–binding sites as lower limits are associated with postsynaptic cells. This follows from the expectation that most preganglionic terminals in the ganglion would degenerate within 3 d after denervation as shown for adult pigeon ciliary ganglia (Giacobini et al., 1979). At this time many AChRs and α-Bgt–binding sites are still present in the denervated chick ciliary ganglion. A previous report on adult chicken ciliary ganglia indicated that axotomy reduced ganglionic α-Bgt–binding levels while denervation had little effect (Fumagalli et al., 1978), possibly signaling a difference between newly hatched and adult chickens in this regard. It seems probable that all of the AChRs and α-Bgt–binding sites in the ganglion are associated with the ganglionic neurons.

The surgical manipulations used here are likely to have altered receptor number by isolating the neurons from required cell–cell interactions. Motor innervation of vertebrate skeletal muscle controls the number and distribution of AChRs on muscle cells probably both by direct nerve-muscle contact (Anderson and Cohen, 1977; Role et al., 1985) and by stimulation of muscle activity (Lomo and Rosenthal, 1972; Reiness and Hall, 1977). Factors have recently been purified from brain that increase the number and alter the distribution of muscle AChRs (Knaack et al., 1986; Usdin and Fischbach, 1986). Preliminary reports describe a stimulatory effect of spinal cord explants on the ACh sensitivity of sympathetic neurons in culture (Role, 1985), and suggest that before innervation the neurons have little or no ACh sensitivity (Schuetze and Role, 1987). As for postganglionic influences, isolating autonomic neurons from their peripheral targets has been shown to alter the synaptic properties of preganglionic contacts on the neurons (Purves, 1975; Brenner and Johnson, 1976), and muscle membrane fragments have been reported to be essential in sustaining the ACh sensitivity of ciliary ganglion neurons in culture (Tuttle, 1983). The underlying mechanisms of neuronal AChR regulation remain to be determined. Since neuronal and skeletal muscle AChRs are encoded by different genes (Boulter et al., 1986; Goldman et al., 1986, 1987), it is to be expected that they would be regulated differently.

We thank Alfredo Franco, Jr., and James Johnson for expert technical assistance.

Grant support was provided to M. H. Jacob by the National Institutes of Health (NS 21725) and to D. K. Berg by the National Institutes of Health (NS 12601), the Muscular Dystrophy Association, and the American Heart Association with funds contributed in part by the California Heart Association.

Received for publication 30 April 1987, and in revised form 5 June 1987.

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