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THE EFFECT OF AXOTOMY ON THE ACETYLCHOLINESTERASE OF THE SUPERIOR CERVICAL GANGLION OF THE CAT*†

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Abstract—The effects of chronic axotomy of postganglionic fibers on the AChE of sympathetic ganglia of the cat were investigated histochemically and quantitatively. Partial axotomy of the superior cervical ganglion was performed by sectioning the main postganglionic (internal carotid) branch or the external carotid branch, or by enucleation of the anterior contents of the orbit; the inferior mesenteric ganglion was partially axotomized by sectioning both hypogastric nerves. During the postoperative periods of 8 to 44 days, at the end of which the ganglia were studied, there was a marked but reversible loss of AChE from the perikarya of most of the axotomized, heavily and moderately stained sympathetic neurons; in the normal superior cervical ganglion of the cat, these groups together comprise less than 20% of the total ganglion cells, while in the inferior mesenteric ganglion the corresponding figure is even smaller; the remaining sympathetic neurons of both ganglia show only faint AChE staining. Although there was an initial impression of a concomitant loss of AChE from the preganglionic fibers, this was not borne out by more careful examination. Manometric determinations indicated that there was a loss of only 6% of the ganglionic AChE following chronic axotomy of the internal carotid branch of the superior cervical ganglion; after preganglionic denervation, there was a loss of approximately 75%. It was concluded that the fall in ganglionic AChE after postganglionic axotomy can be accounted for entirely on the basis of the loss of the enzyme from the axotomized ganglion cells themselves, and that the ensuing changes in synaptic transmission and drug sensitivity that have been reported for such ganglia are not due to this factor.

CHOLINERGIC neurons are characterized histochemically by the presence of high concentrations of acetylcholinesterase (AChE) throughout their axonal and dendritic processes and in the cytoplasm of their perikarya; at the latter location the enzyme occurs principally in the endoplasmic reticulum.[1–6] After axotomy of cholinergic motoneurons of the rat hypoglossal nucleus,[7, 8] amphibian spinal cord,[9] and cat and chicken ciliary ganglia,[8] there is a loss and subsequent restitution of their perikaryonal AChE at a time course which follows approximately that of the similar changes of the Nissl substance.

There have been numerous investigations of the effects of postganglionic axotomy on synaptic transmission and on the AChE content of various sympathetic ganglia,

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and a causal relationship between the two factors has been considered. For example, both Brown and Pascoe[10] and Acheson and Remolina[11] noted a gradual failure of synaptic transmission through the inferior mesenteric ganglion of the cat over the course of several days following postganglionic sectioning of the hypogastric nerve; during this period there were also marked changes in the sensitivities of the axotomized ganglion cells to various injected drugs. Although Acheson and Schwarzacher[12] studied the concomitant histological changes in the inferior mesenteric ganglion of the cat, other ganglia and species have been employed for studies of effects on acetylcholine (ACh) metabolism. The release of ACh during preganglionic stimulation of the superior cervical ganglion of the chronically axotomized cat was considered to be within normal limits.[10] The synthesis of ACh was unimpaired in the superior cervical ganglia of chronically axotomized rat in the presence of a normal concentration of K^+, although in contrast with control ganglia, synthesis was not increased with elevation of the K^+ level.[13] The total AChE activity of the latter ganglion was found to be reduced to 52% of the control value three weeks after postganglionic axotomy, and to 57% two weeks after preganglionic sectioning.[14] These values are consistent with the approximate distributions of AChE between the pre- and postsynaptic elements of the same ganglion as judged by histochemical examination.[15] On the other hand, histochemical studies have suggested that axotomy produces a partial loss of presynaptic as well as postsynaptic AChE in the superior cervical ganglion of the rat[16] and cat.[17] During the preparation of the present report, Härkönen[18] reported histochemical findings of a practically complete disappearance of both pre- and postsynaptic AChE from the rat ganglion from 1·5 to 7 days after postganglionic axotomy, followed by a return toward normal levels over the ensuing five months. Accordingly, in addition to their important physiological and pharmacological implications, the foregoing observations entail the question of whether degenerative changes in axotomized neurons can exert a retrograde, transneuronal influence on the stability of an enzyme, AChE, located presynaptically. That certain morphological features of axonal degeneration can extend transneuronally to the deafferented neurons has been demonstrated amply at a variety of sites.[19–23] Attempts to detect "transneuronal degeneration" in a retrograde direction have been generally unsuccessful.[24–26] The purpose of this investigation was to determine whether the preganglionic fibers are involved in the decreased AChE activity of axotomized ganglia and, if so, to eliminate hypoxia or trauma to the ganglion at the time of operation as the possible cause of such a phenomenon.

The superior cervical ganglion of the cat was chosen as the most suitable ganglion for both histochemical and quantitative study. Although its main postganglionic branch, the internal carotid, is of relatively short length prior to its entry into the tympanic bulla, axotomy is feasible with minimal damage to the blood supply; this branch contains approximately half the total postganglionic fibers, and apparently no significant number of preganglionic fibers.[27] The cholinergic preganglionic fibers and their terminals contribute most of the ganglionic AChE; of the ganglion cells, small percentages exhibit marked or moderate AChE activity, while the great majority, presumably the adrenergic neurons, contain only traces; pseudocholinesterase (butyro-ChE, BuChE) is confined to the Schwann sheath and capsular cells.[1, 2, 17, 28, 29] Because of the restricted length available for bisecting the internal carotid branch, the smaller external carotid branch was bisected in a few instances in order to avoid

any possible trauma to the ganglion or its blood supply. In addition, several enucleations of the anterior portion of the eyeball were performed to effect postganglionic section, at a considerable distance from the gangloin, of the neurons supplying the iris dilator. Only histochemical studies were performed after the latter two types of operation. The inferior mesenteric ganglion of the cat was also studied histochemically because of its ease of accessibility and its large postganglionic branches, the hypogastric nerves, which are of sufficient length for bisection 2 to 3 cm from the ganglion.

MATERIALS AND METHODS

A. Operative procedures

All operations were performed on fully grown cats under nearly aseptic conditions The animals were anesthetized with sodium pentobarbital, 30 mg/kg, i.p. Daily i.m. injections of 0·5 cc of Combiotin were given for 3 to 7 days postoperatively, depending upon the speed of recovery.

Partial axotomy of the superior cervical ganglion. Unilateral postganglionic section of the internal or of the external carotid branch of the superior cervical ganglion was performed successfully in a total of fourteen and three cats, respectively. Of the former, the ganglia of six were subsequently studied histochemically and the remaining eight were assayed for AChE manometrically. Bleeding was controlled throughout the operation by ligation, and care was taken not to touch either the preganglionic trunk or the ganglion itself. The internal carotid branch was ligated once and bisected distad to the suture and ganglion. With axotomy of the external carotid branch, it was possible to secure two ties around it and bisect between them. Unilateral enucleation of the anterior portion of the eyeball was performed in four cats. *Partial axotomy of the inferior mesenteric ganglion* was performed successfully in four cats by bilateral ligation and section of the hypogastric nerves 2 to 3 cm from the ganglion. *Preganglionic denervation of the superior cervical ganglion* was done in four cats. The vagosympathetic trunk was doubly ligated and bisected approximately 2 to 3 cm caudad to the ganglion.

After the majority of the axotomies, the third week postoperatively was selected as the most advantageous time to examine the ganglia, for it was during this period that most of the aforementioned physiological and pharmacological changes appeared. The time schedules are given in Table 1.

In the case of the superior cervical ganglion, on those days after the initial operation as tabulated above, both ganglia were removed and treated identically, the unoperated side serving as the control. The control for the partially axotomized inferior mesenteric ganglion was a ganglion from an unoperated, presumably normal, cat; both the control and experimental ganglia were likewise treated identically. After excision of the ganglia, the still anesthetized animals were sacrificed by bilateral, open pneumothorax.

B. Treatment of tissues

Histochemical and histological procedures. Immediately after removal from the cat, the control and experimental ganglia were dipped into cold 0·85% NaCl solution, then frozen with dry ice on the removable metal stages of the International-Harris cryostat. Sections of varying thickness were cut at $-15°$, placed on clean slides, and air dried. Both ganglia ware sectioned serially before being placed in the appropriate staining jars, and both were treated identically. In some cases, the control was sectioned

TABLE 1. TIME SCHEDULE FOR STUDY OF AChE CONTENTS OF AXOTOMIZED AND PREGANGLIONICALLY DENERVATED GANGLIA

Operation	Subsequent treatment	Days post-operatively when ganglion excised	Number of cats
Partial postganglionic axotomy			
1. Superior cervical ganglion			
Internal carotid branch	Histochemistry	14	1
		16	2
		21	1
		31	1
		35	1
	Manometric determination	13	1
		14	3
		15	1
		16	1
		18	1
		20	1
External carotid branch	Histochemistry	8	1
		11	1
		44	1
Enucleation of anterior ocular segment	Histochemistry	19	1
		21	1
		26	1
		37	1
2. Inferior mesenteric ganglion	Histochemistry	15	2
		16	1
		17	1
Preganglionic denervation	Manometric	16	2
Superior cervical ganglion	determination	18	2

before the experimental ganglion and in others the order was reversed. A typical experimental procedure was as follows: slide 1 : 20-μ section incubated 30 min for AChE; slide 2 : 10-μ section stained for Nissl substance; slide 3 : 20-μ section incubated 60 min for AChE; slide 4: 10-μ section stained for Nissl substance; slide 5: 20-μ section incubated 90 min for AChE; slide 6: 10-μ section stained for Nissl substance. The first six sections were placed on successive slides in this order; the next six were sectioned in the same way and placed on the same six slides next to the first six sections, until this set of six slides was filled and another set was begun. Interspersed were also 10 μ-sections to be stained for all ChE's (AChE + BuChE), and for BuChE alone at 60 min, 10-μ sections stained for AChE for 60 min and counterstained with cresyl fast violet, and 20-μ sections stained by silver impregnation.

Staining for AChE and BuChE was performed by the standard thiocholine method,[1, 2] with incubation periods ranging from 30 to 90 min. In this procedure, fresh frozen sections are placed on slides and incubated in a medium containing acetylthiocholine, copper glycinate, and maleate buffer. As thiocholine is released by the enzymatic hydrolysis of the substrate, it is precipitated as the mercaptide salt, copper thiocholine sulfate; the latter is converted subsequently to copper sulfide

by immersing the slides in ammonium sulfide solution. Butyrocholinesterase is inhibited by preliminary immersion of slides in 10^{-8} to $3 \cdot 10^{-8}$ M di*iso*propyl phosphorofluoridate (DFP), allowing selective staining of sites of AChE activity. For the selective localization of BuChE, butyrylthiocholine is used as substrate. *Nissl substance* was stained by a modification of the standard cresyl fast violet method.* *Silver impregnation* was performed by a modification of Bodian's[30] Protargol method.

Manometric determination of AChE activity was carried out in the Warburg apparatus by a modification of Ammon's method as described by Koelle.[1] It has been shown that the AChE activity of the superior cervical ganglion of the cat, expressed on a weight basis, varies considerably from animal to animal, but not by more than a few per cent between one ganglion and its contralateral counterpart from the same cat.[31, 32] The excised ganglia were placed immediately in cold 0·9% NaCl solution, trimmed of the pre- and all postganglionic trunks as close to the ganglia as possible, decapsulated, weighed, and homogenized by grinding with small amounts of powdered silica and 0·033 M $MgCl_2$ in a porcelain mortar. The homogenates were brought to a final concentration of 1 part wet weight of ganglion in 180 to 240 parts by volume with 0·033 M $MgCl_2$ solution, depending upon the size of the individual control ganglia. The chronically axotomized ganglia were invariably heavier than their controls by several milligrams. On the other hand, the chronically decentralized ganglia were lighter than their controls by 1 to 2 mg, owing presumably to the degeneration of the distal stump of the previously sectioned preganglionic nerve. Hence the homogenate of each experimental ganglion was brought to the same final volume as that of its unoperated, contralateral counterpart. Of the eight axotomies performed for Warburg determinations, three were done on the right side and the remainder on the left. The four preganglionic denervations were performed on the left side as were all other operations, i.e. for ganglia subsequently examined histochemically. All experiments were carried out in duplicate in the Warburg apparatus at 37° in an atmosphere of 5% CO_2–95% N_2 at a final pH of 7·4 after equilibration. In addition to the homogenates, the reaction mixtures consisted of 0·033 M $MgCl_2$, as an activator of ChE's, 0·037 M $NaHCO_3$, and 0·03 M acetyl-β-methylcholine (MeCh), as the selective substrate for AChE, with a final volume of 2·0 ml. Readings were taken 10, 20, 40, 70, and 100 min after the addition of the substrate. Figures were corrected for spontaneous hydrolysis of the substrate and for absorption or liberation of CO_2 by the homogenates.

RESULTS

Histochemical studies. In the normal superior cervical ganglion of the cat, AChE is present throughout the length of all entering preganglionic fibers and their terminals, and in high, moderate, or slight concentration in the various ganglion cells;[1, 2] it has been estimated that the percentages of ganglion cells exhibiting these relative concentrations of AChE are approximately 0·5, 18, and 82 respectively.[33] Only occasional postganglionic fibers, presumably those arising from the ganglion cells having high or moderate concentrations of AChE, show detectable staining for the enzyme. These distributions in a control ganglion from the present study are illustrated in Figs. 1–3 of Plate 1. They have been confirmed more recently with the gold

* C. Liu, personal communication.

thiocholine method, in which diffusion of the immediate histochemical reaction product, gold thiocholine phosphate, and the final conversion compound, gold sulfide, are not detectable by light microscopy.[34] In Fig. 4 is shown a section from a normal ganglion stained for Nissl substance. The BuChE activity in this ganglion is confined to the Schwann sheath and capsular satellite cells.

There was noted consistently in the axotomized ganglia a decrease or absence of heavily and intermediately stained neurons, corresponding with the regions showing mostly chromatolyzed cells, throughout the postoperative period of 8 to 44 days studied. This was more noticeable in the cat superior cervical than in the inferior mesenteric ganglion, since the latter contains even fewer heavily or moderately stained ganglion cells than the former. The neurons so affected did not lose their AChE completely, but generally displayed the very light staining normally encountered in the majority of the ganglion cells of cat sympathetic ganglia. In occasional cases, light or even moderate staining was present in neurons which showed chromatolysis in adjacent Nissl-stained sections (see below), but no instance of heavy staining in a chromatolyzed neuron was noted. It should be pointed out that an intrinsic limitation of a study of this type in the cat is the impossibility of comparing the AChE activity of a given neuron after axotomy with its normal level, since this might have been originally very light, moderate, or intense.

The disappearance of practically all heavily and moderately stained ganglion cells is illustrated in Figs. 5 and 9, which show AChE staining in regions of experimental ganglia containing mostly chromatolyzed cells (Figs. 6 and 10) as the result of prior section of the internal carotid postganglionic trunk at 16 and 21 days respectively. On the other hand, there are several moderately stained cells in Fig. 7, taken from another region of the same section as Fig. 5, where only about half the cells exhibit chromatolysis (Fig. 8). As mentioned above, there are a few lightly to moderately stained neurons in the lower left-hand portion of Fig. 7 which show chromatolysis in the corresponding Nissl-stained section. These observations are consistent with those reported by Fredricsson and Sjöqvist.[17]

The histochemical pictures of the various types of chronically axotomized cat superior cervical ganglia were suggestive on initial examination of a reduction in AChE activity in the preganglionic fibers and their terminals, as compared to the unoperated contralateral ganglia. This was the case particularly following section of the postganglionic internal carotid nerve, where the greatest number of ganglion cells was affected (compare AChE-stained Figs. 5, 7, and 9, and their corresponding Nissl-stained serial sections, Figs. 6, 8, and 10, with the AChE-stained controls, Figs. 1 and 2, and the Nissl-stained control, Fig. 4). The suggestive reduction in AChE activity persisted throughout the entire period, although the ganglia of the animals examined from the 31st day on were beginning to show signs of regeneration as evidenced by a decrease in the severity of chromatolysis. When the same sections were examined at higher power, however, the picture was suggestive more of a separation or shrinkage of the ganglion cells from the AChE-stained preganglionic fibers, rather than of a decrease in the intensity or extent of staining of the latter. This is illustrated in Figs. 11 and 12, which were taken at higher magnification from the same section as Fig. 9. The neurons shown exhibited chromatolysis in the corresponding Nissl-stained sections, as well as shrinkage and eccentric displacement of their nuclei.

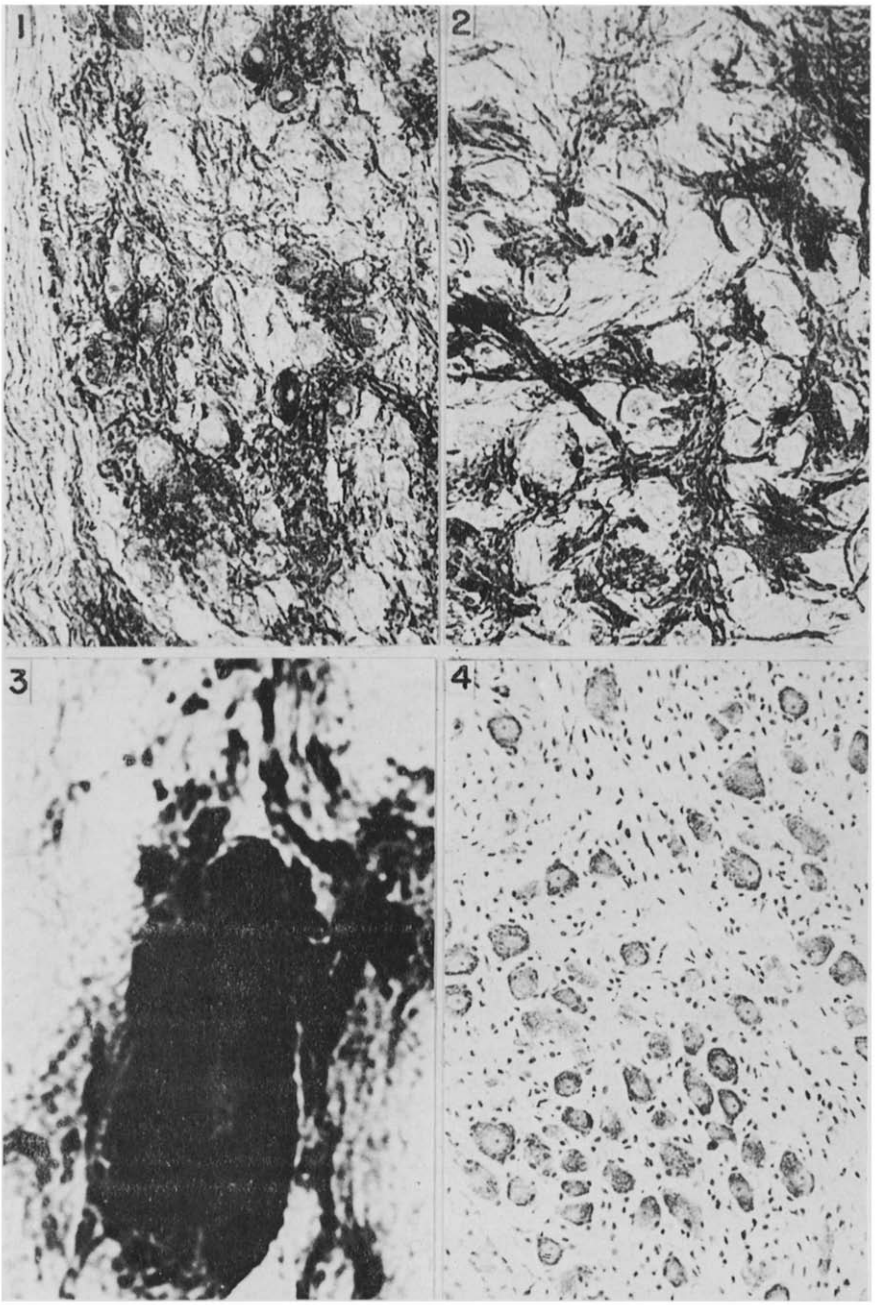

PLATE 1

All figures (1 to 16) in Plates 1 to 4 are shown at 144-fold magnification, and all AChE-staining was done at 60-min incubation, unless indicated otherwise.

Normal superior cervical ganglion of the cat.

FIG. 1. AChE. Note portion of preganglionic trunk on left and presence of occasional heavily and moderately stained neurons, with remaining neurons faintly stained; most of AChE activity associated with preganglionic fibers ramifying throughout the ganglion.

FIG. 2. AChE. Photomicrograph taken near postganglionic pole.

FIG. 3. AChE. Same section as in Fig. 2, × 970, showing a heavily stained ganglion cell; this type accounts for less than 1% of the total population of the superior cervical ganglion.

FIG. 4. Nissl stain (cresy violet); same ganglion as in Fig. 1.

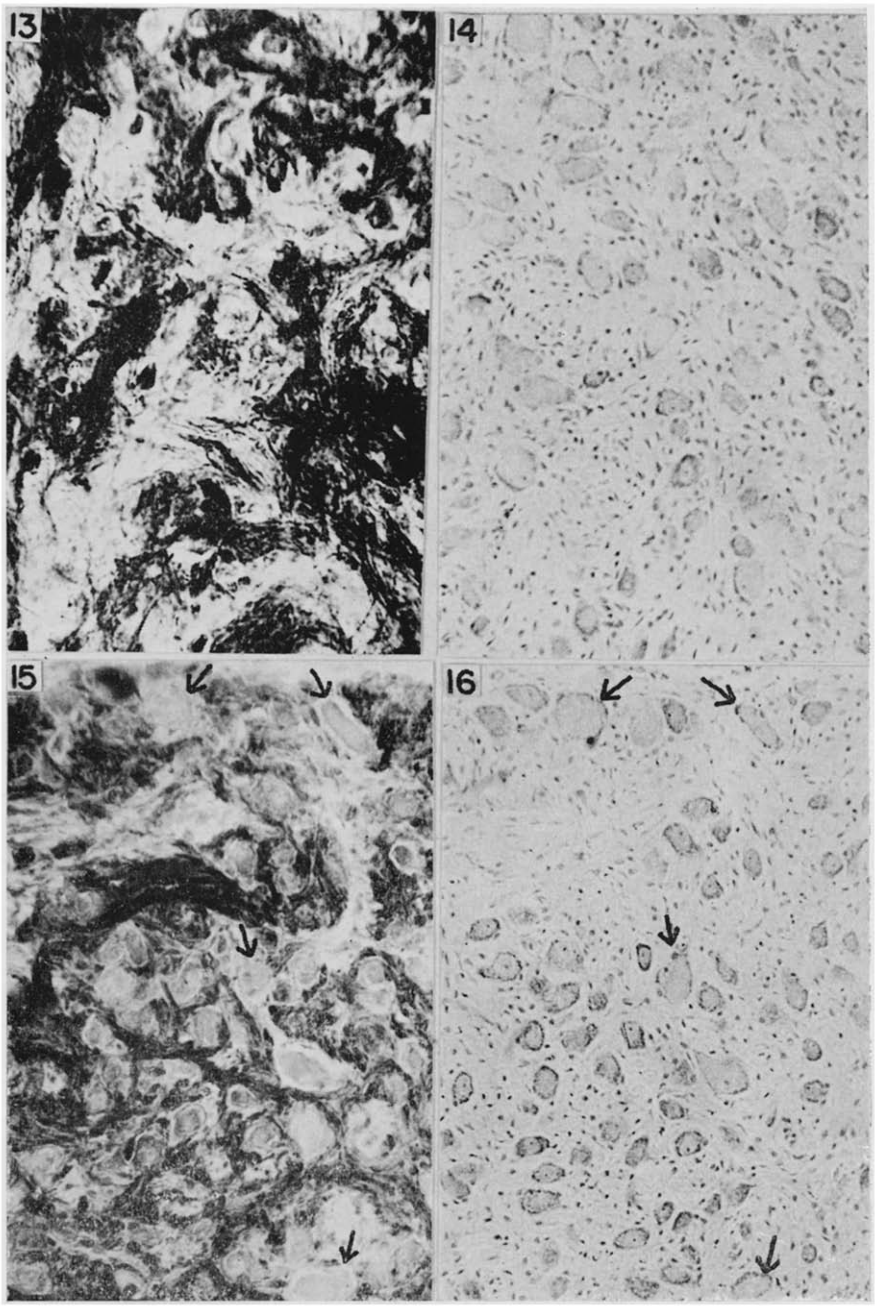

PLATE 4

FIG. 13. Axotomized (external carotid branch) superior cervical ganglion of the cat, 8 days postoperative. AChE-stained by 45-min incubation, counterstained with cresyl violet. Relatively few chromatolyzed ganglion cells are noted.

FIG. 14. Nissl stain. Serial section adjacent to that shown in Fig. 13.

FIG. 15. Axotimized (enucleation of anterior orbital contents) superior cervical ganglion of the cat, 21 days postoperative. AChE-stained by 35-min incubation, counterstained with cresyl violet. Note the four chromatolyzed neurons (arrows) which appear to be shrunken from the surrounding AChE-stained fibers.

FIG. 16. Nissl stain. Serial section adjacent to that shown in Fig. 15, with same four neurons indicated by arrows.

Another factor which contributed to the general impression of a decrease in fiber staining in the axotomized ganglia was an apparent "dilution" of the population of ganglion cells and preganglionic fibers by other elements (compare the AChE-stained controls, Figs. 1 and 2, with Figs. 5, 7, and 9, as well as their corresponding Nissl-stained sections, Fig. 4 and Figs. 6, 8, and 10 respectively.). That such a dilution actually occurred is indicated by the invariably greater weight of the internal carotid branch-axotomized ganglia in comparison with their contralateral control ganglia (Table 2). When the axotomized ganglia were removed, there was always a swelling

TABLE 2. EFFECTS OF POSTGANGLIONIC AXOTOMY (INTERNAL CAROTID BRANCH) AND OF PREGANGLIONIC DENERVATION OF THE AChE ACTIVITY OF THE CAT SUPERIOR CERVICAL GANGLION

Concentration of homogenate of experimental ganglion based on wet weight of corresponding control; substrate 0·03 M methacholine (MeCh); measurements of CO_2 release were made during 60-min. period starting 10 min. after tipping in substrate; L = left; R = right.

Cat no.	Nerve sectioned	Days postop.	Wts. of ganglia		AChE Activity	
			Control (mg)	Operated (% control)	Control (μmole MeCh/0·1 g/hr.)	Operated (% control)
23	Post-L	13	10·1	117	36·7	114·3
25	Post-L	14	9·8	142	39·2	92·8
26	Post-L	14	13·8	114	46·9	89·5
29	Post-R	14	8·2	116	49 8	103·7
22	Post-L	15	9·1	152	42·5	72·0
24	Post-R	16	11·5	124	28 9	96 3
33	Post-L	18	13·4	104	33·3	96·8
27	Post-R	20	10·8	140	46·1	89·6
Means ± S.E.*:				126 ± 6·0		94·4 ± 4·3
34	Pre-L	16	14·9	90·6	42·7	23·0
35	Pre-L	16	10·6	96·1	50·1	24·5
36	Pre-L	18	14·1	88·6	25·1	26·8
37	Pre-L	18	9·1	79·1	39·0	26·6
Means ± S.E.:				88·6 ± 3·5		25·2 ± 0·9

* S.E. $= \sqrt{\frac{\Sigma(d)}{n(n-1)}}$

at the point of previous sectioning that was suggestive of a neuroma, a glioma, or a combination of both. (As noted under Methods, such swellings were trimmed off, along with the trunks, prior to homogenization of the ganglia for quantitative determinations.) Regenerating nerve is known to undergo retrograde degeneration to the adjacent node of Ranvier and subsequently to send out sprouts from that point.[35] The possibility exists that there was axonal sprouting within the ganglion itself, proximal to the point of section. After axotomy of the superior cervical ganglion of the rabbit, Causey and Barton[36] noted an increase in its content of fine, unmyelinated interneuronal fibers, which they presumed to be postganglionic; such an alteration was not observed after chronic preganglionic section.

In those experiments in which scattered sections were stained selectively for BuChE,

there was no detectable difference between the experimental and control tissues, suggesting that the changes noted in the ganglionic AChE activity were not due to nonspecific effects of injury to the ganglion or its blood supply. These observations do not preclude the possibility of a small increase or decrease in total BuChE activity, which would not be apparent from histochemical examination.

The histochemical changes produced by axotomy of the external carotid branch were similar to those described above, but less extensive. Thus, in Fig. 13 there appears to be a diminution of AChE-stained fibers in the immediate vicinity of the chromatolyzed neurons, indicated more clearly in the Nissl-stained serial section, Fig. 14, as compared with the regions surrounding normal neurons in the same section.

In the superior cervical ganglia of the enucleated cats, chromatolyzed neurons were infrequent and somewhat scattered. The four seen in Fig. 16 appear in the corresponding AChE-stained section, Fig. 15, to be surrounded by clear halos devoid of enzymatic staining, adjacent to which are stained fibers.

Contrary to reports in the literature,[11, 37] the inferior mesenteric ganglion was found to be unpaired in each of the thirteen cats examined. Grossly, the ganglion appeared to be a fusion of several bodies of ganglionic tissue with fibers fanning into an inferior mesenteric ganglion proper, which is in turn connected to several ganglionic swellings, designated the accessory ganglia, from which arise the right and left hypogastric nerves. The complicated connections of the ganglion and its branches have been described recently.[38] Histochemical examination after section of both hypogastric nerves revealed an apparent reduction in the AChE activity surrounding the chromatolyzed ganglion cells, which were confined mainly to the caudal pole of the ganglion complex. However, the same phenomenon of dilution of the ganglion cells, as observed in the partially axotomized superior cervical ganglion, was apparent here.

Quantitative measurements. Each of the eight experimental ganglia in this series, which had been partially axotomized by section of the internal carotid postganglionic trunk 13 to 20 days previously, showed an increase in weight over its corresponding contralateral control ganglion, which ranged from 4% to 52%, so that the mean value for the axotomized ganglia was 126 $\perp$ 6% of the controls (Table 2). This finding adds confirmation to the impression, obtained from the histological examinations, of a "dilution" of the ganglion cells and preganglionic fibers, due possibly to gliosis, axonal sprouting, increase in fluid content, or a combination of these factors. The mean AChE activity of the axotomized ganglia was 94·4 $\pm$ 4·3% of that of the corresponding controls, with a range of 72% to 114%.

In contrast to the former group, each of the four preganglionically denervated ganglia showed at 16 to 18 days postoperatively a decrease in weight, with the mean value 88·6 $\pm$ 3·5% of that of the controls. The AChE activities fell quite uniformly, to a mean value of 25·2 $\pm$ 0·9% of the control activities; this value approximates those reported previously from similar measurements.[28, 29] Since it has been shown by histochemical examination that practically all the AChE activity of the preganglionic fibers and their terminals has disappeared at this time,[1] the residual value may be assumed to represent approximately the normal contribution of the postsynaptic elements to the total AChE of the ganglion. Accordingly, the mean loss of less than 6% which followed section of the major postganglionic trunk can readily be accounted

for entirely on the basis of a decrease in the AChE of the ganglion cells and their axons.

DISCUSSION

Both the histochemical and the quantitative results of the present investigation indicate that the loss of ganglionic AChE which follows partial postganglionic axotomy of the superior cervical and inferior mesenteric ganglia of the cat is confined to the postsynaptic elements (i.e. the ganglion cells and their axons), and does not involve that portion of the enzyme which is associated with the preganglionic fibers and their terminals. Accordingly, these findings provide no evidence of retrograde transneuronal degeneration with respect to the particular enzyme studied. It can also be concluded that the previously mentioned impairment of synaptic transmission and changes in sensitivity to various drugs that have been noted in cat sympathetic ganglia after postganglionic axotomy are probably not due to the concomitant decrease in ganglionic AChE activity. This conclusion follows from evidence that in the sympathetic ganglia of the cat, only the presynaptic AChE is oriented externally with respect to the neuronal plasma membrane, and this portion alone participates in the hydrolytic inactivation of ACh released during transmission. [32, 39]

The initial impression of a loss of AChE from the preganglionic fibers which was noted at the beginning of the present investigation and in a previous study of the axotomized superior cervical ganglion of the cat by Fredriccson and Sjöqvist[17] may be attributable to a combination of the aforementioned possible factors of shrinkage of the ganglion cells, axonal sprouting of postganglionic fibers, and proliferation or swelling of the capsular satellite and Schwann sheath cells. In conjunction with the last item, axotomy of frog sympathetic ganglia has been shown by electron microscopy to produce proliferation of the Schwann cell cytoplasm, so that the width of the original synaptic cleft changes from a normal value of approximately 200 Å to a severalfold increase, with the interposition of the satellite elements.* In assessing the present results, it should also be noted that in the superior cervical ganglion of the cat the overwhelming majority of synapses are of the axo-dendritic rather than axo-somatic type;[40] hence any effect of postganglionic axotomy on the presynaptic terminals would be more likely to occur in the protoplasmic tracts than in the immediate vicinity of the ganglion cells.

The considerably greater loss of AChE which was found both histochemically by Brown[16] and quantitatively (approximately one half) by Dhar[14] after postganglionic axotomy of the superior cervical ganglion of the rat can be readily accounted for by the presence of moderate to high concentrations of the enzyme in essentially all the sympathetic ganglion cells of this species.[8, 15] More difficult to explain is the practically complete loss of both pre- and postsynaptic AChE noted histochemically by Härkönen[18] during the week after postganglionic section of the same ganglion. One possibility is that with the limited space available for exposure and sectioning of all postganglionic nerve trunks in the rat, the blood supply to the ganglion may have been compromised, leading to nonspecific, reversible, degenerative changes. In an earlier quantitative study, Sawyer and Hollinshead[28] obtained a total loss of both the AChE

* W. K. Riker, personal communication.

and BuChE of the superior cervical ganglion of the cat after stripping the entire postganglionic innervation; silver-stained sections of such ganglia showed complete degeneration of all preganglionic terminals. Another factor to be considered is that in the ganglion of the rat, axotomy might result in a conversion of presynaptic AChE from a fixed "desmo" to a soluble "lyo" form; the latter might then be lost to histochemical detection but remain measurable by the quantitative assay of homogenates.

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