J. Physiol. (1976), **254**, *pp.* 455–473
With 13 *text-figures*
Printed in Great Britain

THE ACETYLCHOLINE SENSITIVITY OF THE SURFACE MEMBRANE OF MULTIPLY-INNERVATED PARASYMPATHETIC GANGLION CELLS IN THE MUDPUPPY BEFORE AND AFTER PARTIAL DENERVATION

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(*Received 9 April 1975*)

SUMMARY

1. The surface chemosensitivity to iontophoretically applied acetylcholine (ACh) of single nerve cells in the cardiac ganglion of the mudpuppy was examined.

2. Some synapses on the neurones can be recognized in the living preparation with differential interference contrast optics. Identified synaptic regions of the ganglion cells were more sensitive to ACh than were other areas. The mean sensitivity of synaptic areas was 509 mV/nC, but that of random spots on the cell surface (which were mainly non-synaptic) was only 190 mV/nC. The mean rise time of ACh responses at synapses was 23 msec and at random spots was 36 msec. These data suggest that the density of ACh receptors is highest under the synapses on the postsynaptic membrane.

3. When some, but not all, of the presynaptic terminals on the ganglion cells are destroyed by cutting the vagus nerve, the sensitivity of the entire surface membrane to applied ACh increases. This increase in sensitivity reaches a maximum about 4–6 weeks after the operation.

4. Synaptic transmission at excitatory collateral synapses which remain after vagal degeneration is not altered by this hypersensitivity.

5. Neurones from ganglia which have been isolated and maintained in organ culture also become hypersensitive to applied ACh. This heightened chemosensitivity develops much faster *in vitro*; hypersensitivity in cultured ganglia becomes manifest within 4–5 days, in contrast with 4–6 weeks after vagus degeneration *in vivo*.

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INTRODUCTION

When muscle cells are deprived of their nerve supply, a profound increase in the sensitivity of the fibres to the neuromuscular transmitter, ACh, occurs. This denervation hypersensitivity is not unique to muscle fibres, but also occurs to some extent in neurones, such as those in autonomic ganglia (Kuffler, Dennis & Harris, 1971). Indeed, denervation supersensitivity is thought to be a widespread phenomenon, occurring in brain and spinal cord as well as in autonomic ganglia and muscle tissues (Cannon & Rosenblueth, 1949; Trendelenburg, 1963).

The detailed mechanisms which underlie this denervation hypersensitivity have been studied most thoroughly at the vertebrate neuromuscular junction. Axelsson & Thesleff (1959) and Miledi (1960) showed that, after denervation, the restricted sensitivity of the muscle surface to applied ACh spread from the end-plate regions to normally insensitive extrasynaptic regions of the fibre surface.

Although denervation hypersensitivity is believed to occur in the central nervous system when neurones are deprived of some, but not all, of their synaptic input (Cannon & Rosenblueth, 1949), it has not been possible to study this problem at a cellular level. For example, one might expect that spreading of chemoreceptors over the surface of partially deafferented nerve cells, if it occurs, would have an affect on synaptic transmission at the remaining intact terminals. The question whether hypersensitivity occurs after partial denervation may bear directly on alterations in neuronal transmission which occur after damage in the brain and spinal cord (cf. Nieto & Escobar, 1972).

The present paper reports the results of experiments on parasympathetic ganglion cells in the mudpuppy which receive synapses from at least three different sources. Taking advantage of the fact that single ganglion cells and their synaptic endings can be visualized in the living isolated ganglion, chemosensitivity of small portions of the surface of nerve cells was tested using techniques described by Dennis, Harris & Kuffler (1971). The results show that ACh sensitivity is normally highest under the synaptic areas of neurones which have a heterogeneous population of synaptic endings. Furthermore, it was possible to test the effect of *partial* denervation on post-synaptic chemosensitivity and on synaptic transmission at remaining intact synapses.

METHODS

Methods for anaesthetizing the animal, isolating the ganglion, and recording intracellularly from nerve cells are described in the previous päper (Roper, 1976).

Microapplication of acetylcholine. ACh was applied from glass micropipettes which

had tips below the limit of resolution of the light microscope. These micropipettes were drawn from glass capillary tubing which contained a few glass fibres; pipettes were filled by injecting 2 M acetylcholine into the shank with a 30 gauge hypodermic needle. The micro-electrodes were left overnight at 5° C and inspected under the microscope at 400 × the next day in order to select those which were completely filled. ACh electrodes had resistances from 400 to 800 MΩ. If the resistance was below 400 MΩ, the electrode was discarded.

ACh was applied to the cell surface by passing 0·5–5 msec current pulses through the acetylcholine pipette. Current was monitored with the bath clamp described in the previous paper; no attempt was made to square the current pulse. An excess braking current (about $1{\cdot}5 \times 10^{-9}$ A) was impressed initially on the drug pipette at each position and then reduced to the optimal level as described below. ACh pipettes were discarded if they required over 10^{-9} A braking current. Typically, braking currents were under $0{\cdot}5 \times 10^{-9}$ A.

Current pulses were applied to the ACh pipette via a 500 MΩ resistor which was mounted very near the shank of the drug pipette. This location minimized capacitative losses in the current records. Braking current was provided by a variable d.c. source via a 9000 MΩ series resistance.

Preliminary experiments established that for the same amount of charge delivered, ACh pulses greater than 5–10 msec duration produced smaller responses than brief pulses. Also, it was difficult to measure the amplitude of current pulses lasting less than 1 msec due to capacitative losses introduced by the high-resistance ACh pipettes. On the other hand, 2 msec iontophoretic pulses were found to give the most consistent results, and consequently were adopted as the standard.

Quantitative measurement of the ACh responses. After positioning the ACh pipette (see Results), the braking current on the drug pipette was reduced while delivering repeated 2 msec doses of ACh until there was a barely detectable depression and prolongation of the ACh response. The brake was then increased just slightly to restore the response to its maximum value. Next, current intensity was adjusted so that the ACh depolarization was about 4 mV and records were photographically averaged by superimposing several traces. The ACh pipette was then withdrawn from the surface and this procedure was repeated at a different position on the principal cell.

The rise time and sensitivity were the two quantities measured for each response. The rise time of the ACh response was measured from the onset of the current pulse to the peak of the response. Sensitivity was expressed in mV/nC (cf. Miledi, 1960).

It proved useful to plot the sensitivity (mV/nC) against the rise time (msec) for each point tested on the cell surface in order to exclude aberrant responses. Fig. 1 illustrates such a plot for responses from many experiments. There is a roughly inverse linear correlation between log mV/nC and the rise time. Fixed upper and lower limits (interrupted lines) were selected after inspection of many experiments and the points which lay outside these limits were not included in the analysis. It was found that this procedure excluded responses where the ACh pipette had partially penetrated the cell, where the resting potential had rapidly deteriorated, where the resistance of the ACh electrode had suddenly decreased, or when there was a change in the braking current from the optimum level. If in any one experiment the majority of ACh responses fell outside these limits, all those data were excluded from the final analysis.

Identifying synapses. Some synaptic boutons could be recognized under Nomarski differential interference contrast optics (McMahan & Purves, 1976). The ACh pipette was directed either specifically on to synaptic regions or was placed at random sites (which include some synaptic areas, see Results).

Partial deafferentation of the cardiac ganglion. Principal cells were partially denervated by cutting both vagus nerves near their exit from the skull in animals anaesthetized with 0·5 % MS 222. This operation left the intrinsic ganglionic synaptic connexions intact (McMahan & Purves, 1976; Roper, 1976). In the mudpuppy, the vagus nerve passes near the base of the gills and can be exposed by making a small incision there. The overlying musculature was spread with blunt forceps, and the nerve trunks freed from the large blood vessels with which they are closely associated.

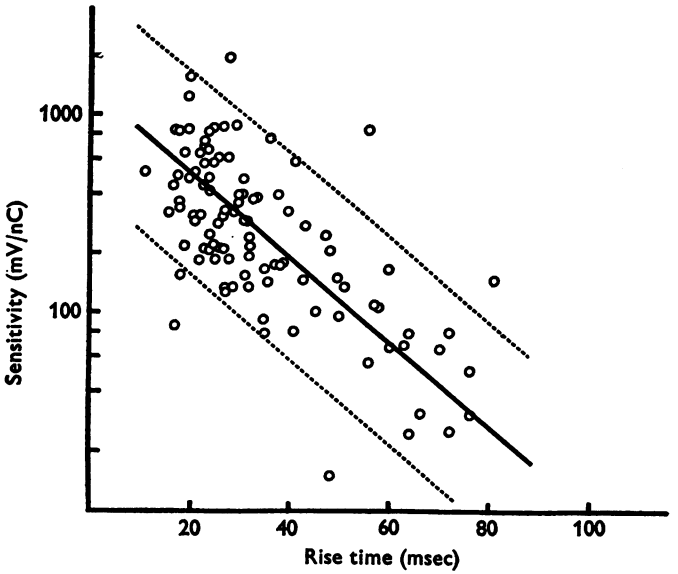

Fig. 1. Graph of sensitivity (mV/nC) *vs.* the rise time (msec) for ACh responses from several experiments. Abscissa is msec and ordinate is log (mV/nC). The continuous line was drawn through the points and limits drawn to either side (interrupted line). Only responses which fell within the interrupted lines were analysed (see text). For example, the six ACh responses which fell outside these limits were excluded from further data analysis. The experimental data from each experiment were treated in the same manner.

About 5–8 mm of the nerve were removed to retard regeneration, and in no instance was reinnervation of the ganglion detected, either by visual inspection or by physiological studies. When animals were killed, the wound was reopened to inspect the operation and to confirm that the vagus nerves had been completely severed.

After the operation, animals were returned to an aerated tank containing 5 % frog Ringer solution and kept at room temperature.

RESULTS

Principal cell responses to applied ACh

The extracellular ACh micropipette was placed against the surface of the principal cell so that it caused a slight indentation and ACh was

expelled with a brief pulse of current. A rapid depolarization was recorded intracellularly after a short delay (Fig. 2). The size of the ACh response increased if the current pulse was increased. If the depolarization was large enough, an impulse was triggered (Fig. 3).

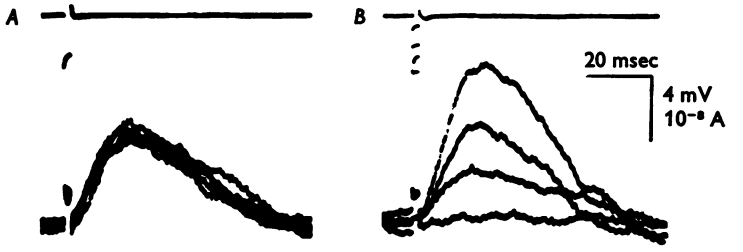

Fig. 2. Intracellularly recorded responses of principal cells in the cardiac ganglion to iontophoretically applied ACh. ACh responses are shown in lower traces and iontophoretic current pulses in the upper traces. *A*, superimposed responses to identical ACh pulses. *B*, responses were graded according to amount of current passed through the ACh pipette.

It soon became apparent that the response to ACh application varied depending on the location of the iontophoretic pipette. The following experiments were devised to map the regional chemosensitivity on the surface of the principal cell. The first section of this paper reports findings which compare the ACh sensitivity of synaptic areas with that of random areas on the principal cells in normal ganglia. Subsequent sections examine the chemosensitivity after partial denervation of the ganglion.

Regional chemosensitivity of the principal cell surface

Current response. In order to compare the ACh responses of various parts of the cell, the ACh pipette had to contact the surface membrane at each point, and not be located outside the Schwann cell layer which covers part of the soma (McMahan & Purves, 1976). The criterion for establishing the placement of the ACh pipette on the neuronal surface was the 'current response' described by Harris, Kuffler & Dennis (1971). Briefly, the current response is evoked by increasing the current passed through the extracellular pipette. Above a certain intensity, a depolarizing potential change which is clearly different from the ACh response is recorded intracellularly. The current response lasts only for the duration of the pulse, is graded with the current strength, and appears to be the result of positive charge being injected directly into the cell interior from the *external* micropipette (Harris *et al.* 1971). The presence of this response was taken to mean that the micropipette was in intimate contact with the neuronal surface.

Following such a current response, the intensity of iontophoretic pulses was reduced. If the drug pipette had not penetrated the surface membrane (which was sometimes the case), the ACh sensitivity of that location was recorded.

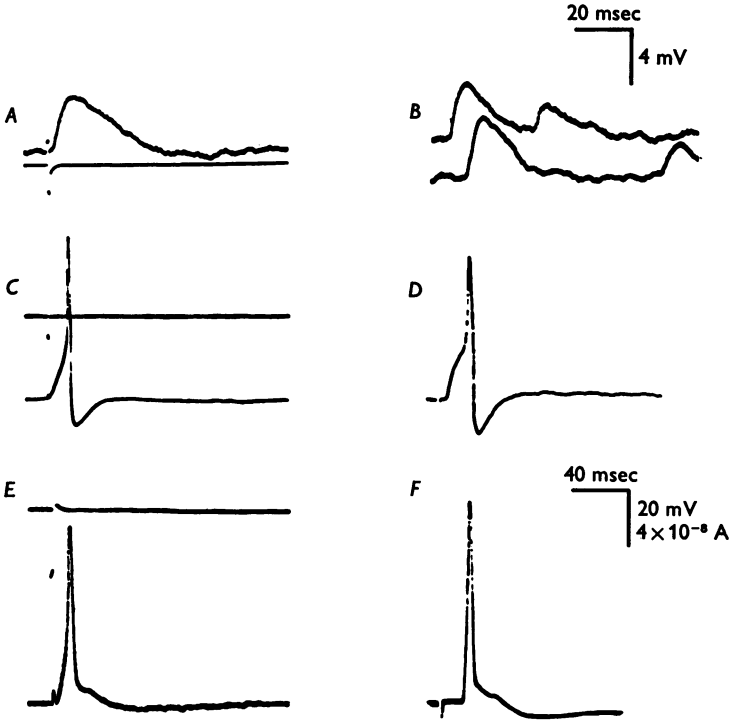

Fig. 3. Responses evoked by iontophoretically applied ACh (left) compared with responses evoked by nerve-released transmitter (right). In *A*, the response to a subthreshold dose of ACh at an identified synapse is compared with spontaneous miniature excitatory post-synaptic potentials (*B*) evoked by mechanical irritation of the same synapse with the extracellular pipette. Suprathreshold doses of ACh in *C* and *E* are compared with responses evoked by vagal stimulation in *D* and *F*. Note the change of gain and time scales between *A*, *B*, and *C*, *D*, *E*, *F*. The iontophoretic current pulses are shown in the bottom trace of *A*, and in the upper traces in *C* and *E*.

ACh sensitivity of synaptic areas. The first question studied was whether the subsynaptic region had a high ACh sensitivity. Synapses were identified with Nomarski optics under 400 × magnification and ACh was applied to these areas. The onset of these responses was typically very fast, often starting to rise before the end of the iontophoretic pulse and reaching a peak in less than 20 msec. The mean rise time for ACh responses from synaptic areas was 23 msec. The corresponding sensitivities were high,

sometimes as much as 2000 mV/nC, with a geometric mean value of 509 mV/nC.

Another characteristic of the response to ACh when the iontophoretic pipette was located near an identified synapse was the close resemblance of ACh responses to those produced by nerve-released transmitter, as shown in Fig. 3. Immediately after the records were photographed in record *A* of Fig. 3, the synapse directly under the ACh electrode was mechanically irritated with the drug pipette. A burst of spontaneous miniature excitatory post-synaptic potentials ensued (Fig. 3*B*). There is a close resemblance between the iontophoretic response (Fig. 3*A*) and the spontaneous potentials (Fig. 3*B*).

ACh responses from randomly chosen sites. In contrast with the high sensitivity and fast rise times of responses when ACh was applied to the synaptic regions, the responses produced at randomly selected positions were usually smaller and more prolonged. There sometimes was a noticeable delay before the onset of the response, the rise time was increased, and the sensitivity was low. The mean rise time of responses from randomly selected sites was 36 msec and (geometric) mean sensitivity 190 mV/nC. Fig. 4 illustrates this difference between ACh sensitivity of identified synaptic spots and randomly chosen points on one principal cell. Because not all the synapses on a cell can be visually identified, the random positions include some synapses. Examples of highly sensitive random positions are shown in Fig. 4 (middle left and right). Data from many experiments such as this one are compiled in the form of a histogram in Fig. 5.

The histograms for responses to random application of ACh are skewed toward high sensitivities and short rise times presumably because the ACh pipette is sometimes placed near synaptic regions. Nevertheless, it is clear that the identified synaptic spots are much more sensitive on the average than other parts of the surface.

If the probability of placing the drug pipette directly over a synapse at randomly selected positions is roughly proportional to the fractional area of the membrane surface occupied by synapses, one can calculate the approximate area on the neurone affected by the iontophoretic application of ACh assuming only synaptic areas are highly sensitive. If the maximum rise time for ACh responses at synapses is taken as 28 msec, i.e. 1 S.D. (5 msec) greater than the mean value (23 msec) at identified synaptic spots, then the proportion of responses at *random positions* with a rise time equivalent to that of identified synaptic spots (i.e. 28 msec or less) was about 40 % (cf. Fig. 5). However, the boutons occupy only about 5 % of the surface membrane (McMahan & Purves, 1976). Thus, from the initial assumption, the ACh pipette was producing a maximal response at 40 ÷ 5, or 8 times the surface area occupied by the synapse. In terms of absolute dimensions, if a bouton occupies a roughly circular post-synaptic area of 7 μm^2 (McMahan & Purves, 1975), the effective resolution of the ACh application is an area with a radius of about 4 μm. These figures are necessarily very approximate. Nevertheless, such resolution is experimentally found

when ACh is applied to the end-plate region on skeletal muscle (Peper & McMahan, 1972).

Chemosensitivity of the axon. In a few instances it was possible to follow the axon for about 100 μm from the principal cell body and to apply ACh on to it. It was not possible, however, to map the sensitivity of the axon in any detail because it was difficult to ascertain that the ACh pipette was

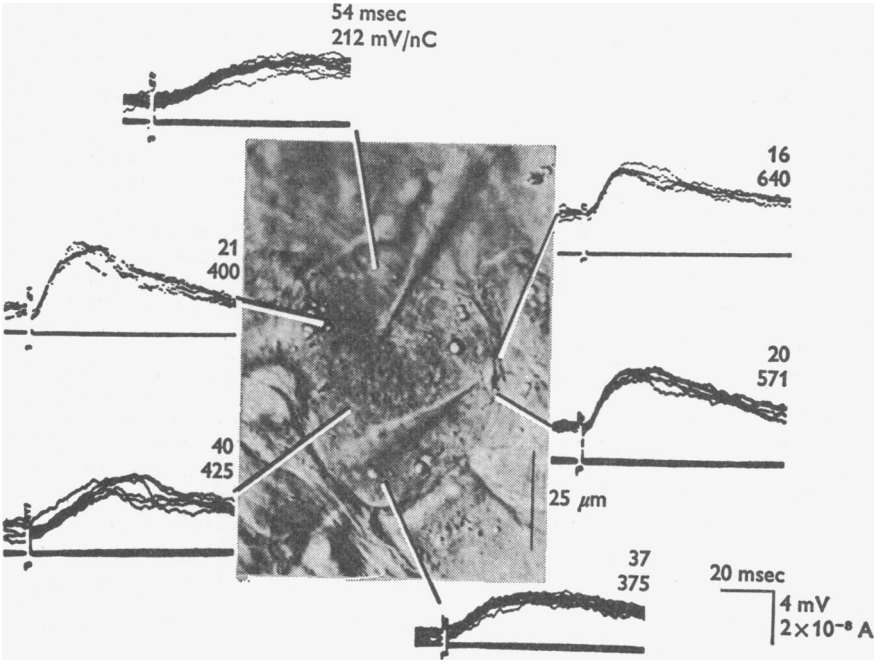

Fig. 4. Application of ACh to random positions and to identified synapses on a principal neurone in the ganglion. The photomicrograph shows the ganglion cell, the intracellular recording pipette (above) and the extracellular ACh pipette (below). The tip of the ACh pipette is located near a cluster of synapses on the far right side. Spherical inclusions in the cytoplasm are pigment granules. The responses to the applied ACh are shown to the side of the cell; dotted lines show the position of the ACh pipette tip. To the right of each record are printed the values of the rise time (top) and sensitivity, mV/nC (bottom). ACh was applied to two different positions near the identified cluster of synapses and the sensitivities (640 and 571 mV/nC), and rise times (16 and 20 msec) contrasted with those of the responses to randomly selected positions (ranging from 212 to 425 mV/nC and from 21 to 54 msec).

in good apposition everywhere along the axonal membrane. Nevertheless, at some positions the responses to ACh were quite fast-rising (Fig. 6). Slight vertical displacement (*ca.* 1–3 μm) of the drug pipette abolished the

response, eliminating the possibility that the ACh was diffusing back to sensitive parts of the cell body. The responses were graded with ACh dose and resembled in all respects the ACh responses at the cell body.

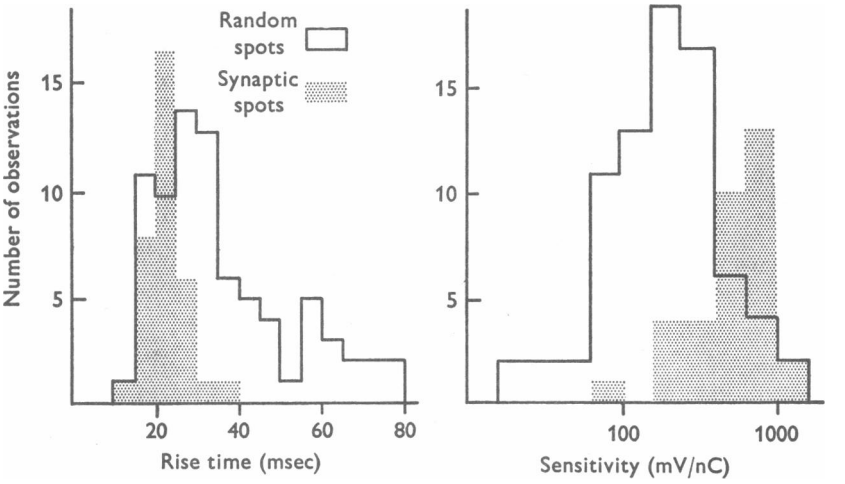

Fig. 5. ACh responses from random spots compared with those from synaptic regions. Responses from random positions are shown in the open figures and those from identified synapses are shown in the shaded figures in the histograms. The rise times are briefer and sensitivities higher for responses at identified synapses than for random positions, indicating that the chemosensitivity of the post-synaptic membrane is highest under the nerve terminals. Note the abscissa for the sensitivities (mV/nC) is a logarithmic scale.

The axonal responses, even up to 80–90 μm from the cell body, were nearly as fast rising as those recorded at synapses on the soma (Fig. 7). It was expected that spatial decrement of the potential would have prolonged and reduced the potentials. To check the 'electrotonic distance' of the axonal positions from the cell body, the following experiment was done. Intracellular hyperpolarizing current pulses of about 200 msec duration were delivered through a micro-electrode which was in the cell body. ACh responses were then superimposed with the ACh pipette touching the cell body or 80–90 μm from the cell along the axon. When ACh was applied to the cell body, hyperpolarization increased the amplitude of the response, as expected for an excitatory response caused by an ionic permeability increase. Hyperpolarization of the cell body when ACh was being applied 80–90 μm away on the axon also increased the amplitude of the ACh response, and to a similar extent (Fig. 7). Thus, the cell body and the axon were nearly isopotential over this distance. Synapses located

tens of microns from the cell body along the axon would be expected to be as effective as those located directly on the soma in producing post-synaptic potentials recorded in the soma. Synapses located as much as 100 μm from the cell body in the mudpuppy ganglion have been observed by McMahan & Purves (1976).

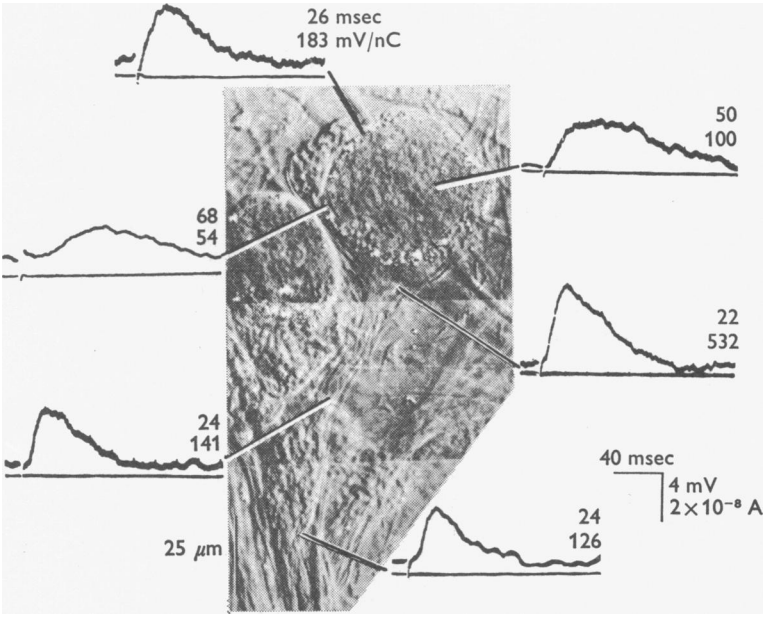

Fig. 6. ACh sensitivity of random positions on the axon and cell body of a principal cell. Responses are shown to the sides of the cell as in Fig. 4. The initial 50 μm of the axon are clearly visible, but a Schwann cell nucleus obscures the distal part. ACh, applied to two points along the axon (continuous lines), produced responses with rise times comparable to those from the most sensitive positions on the cell body.

ACh sensitivity of partially deafferented principal cells

Nerve-evoked responses after denervation. When the vagus nerve is severed, vagal boutons degenerate but the full complement of other synapses – post-ganglionic collateral synapses, interneurone synapses, and electrically transmitting contacts between principal cells – remain structurally intact (McMahan & Purves, 1976). Stimulation of the preganglionic nerve evoked normal vagal excitatory post-synaptic potentials for 12–14 days following the operation; at longer intervals no vagal responses could be produced. It was not possible to find a period when abnormal responses were evoked. This abrupt failure of synaptic transmission is

similar to that observed in skeletal muscle after denervation (Miledi & Slater, 1970).

On the other hand, excitatory post-synaptic potentials continued to be evoked in the principal cells by antidromic excitation of post-ganglionic axon collateral synapses. These collateral synapses, which are cholinergic, are described in the preceding paper (Roper, 1976).

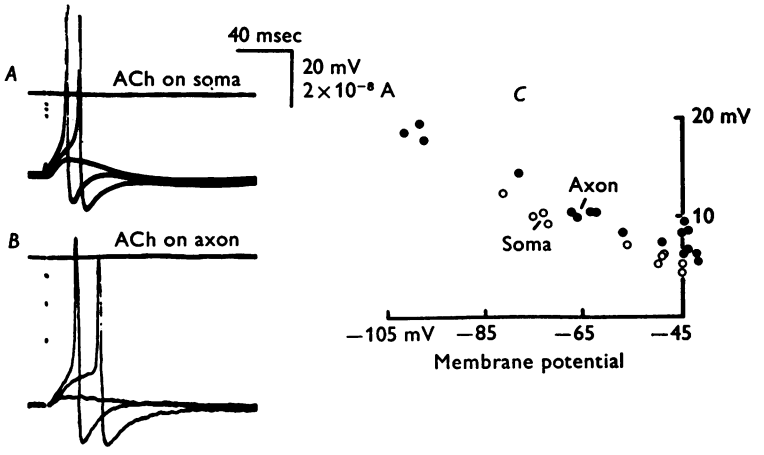

Fig. 7. Comparison of ACh responses from sensitive positions on the cell body (*A*) and the axon (*B*), about 90 μm from the soma. These data are from same experiment as illustrated in Fig. 6. The recording electrode was in the cell body. Increasing the dose of ACh at the soma (*A*) evoked responses similar to those evoked when ACh was applied to the axon (*B*). *C*, when the cell body was hyperpolarized by current passed through the electrode inserted in the cell body, ACh responses at the soma and as well as at the axon were increased, and to the same extent. The abscissa is the membrane potential at the soma; the ordinate is the peak response (mV) to constant doses of ACh. Open circles are the responses recorded when ACh was applied to the soma and closed circles are responses when applied to the axon, 90 μm from the soma.

Increase in ACh sensitivity after partial deafferentation. When the vagus nerve degenerated, the distribution of chemosensitivity over the cell surface gradually changed. Over a period of about 4 weeks after denervation the average sensitivity of the membrane to randomly applied ACh increased; by the 5th week highly sensitive places on the membrane were usually found wherever the ACh pipette was positioned. Fig. 8 shows records of ACh responses from a cell which had been partially denervated 5 weeks prior to the experiments. The data from several animals are compiled in the form of histograms for various times after severing the vagus nerve, and show the time course of the spread of sensitivity (Fig. 9).

A puzzling finding was that the distribution of chemosensitivity gradually returned to normal after about 9–10 weeks (Fig. 9). It was, therefore, important to check that there were no signs of reinnervation of the ganglion. Stimulation of the cut preganglionic nerve stump did not evoke responses at any time after the nerve fibres had degenerated, as long as 10 weeks after the operation. Moreover, regenerating synapses were not seen in the electron microscope over 10 weeks after vagotomy (McMahan & Purves, 1976).

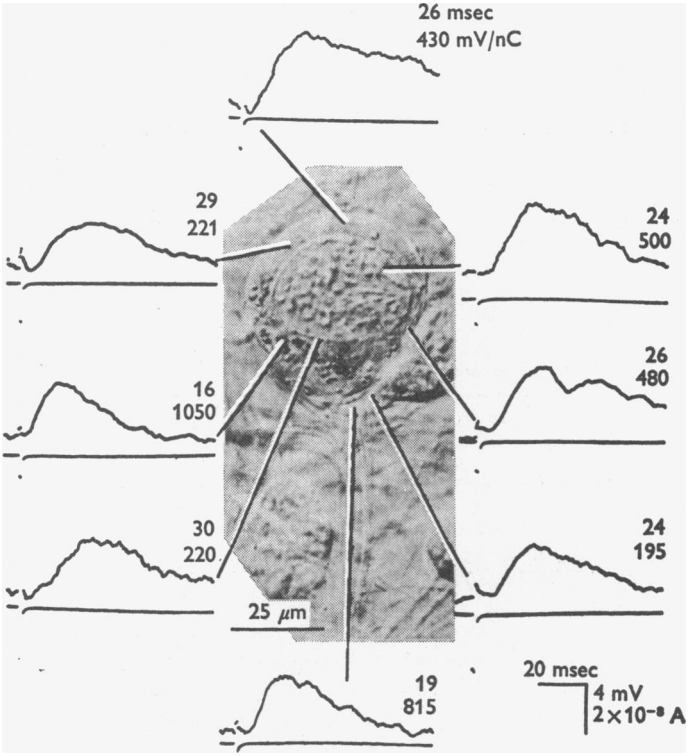

Fig. 8. Sample records of ACh responses from randomly chosen points on a partially denervated ganglion cell. The vagus nerve had been cut 5 weeks before, but the collateral synapses and other synapses were intact. To the right of each response are shown the values of the rise time (msec, top) and sensitivity (mV/nC, bottom).

Chemosensitivity of ganglion cells maintained in vitro

In order to test some of the factors which contribute to the spread o ACh sensitivity after partial denervation in the mudpuppy, the chemosensitivity in ganglia isolated in organ culture was mapped at various times after incubation in sterile culture medium (80% Leibovitz medium

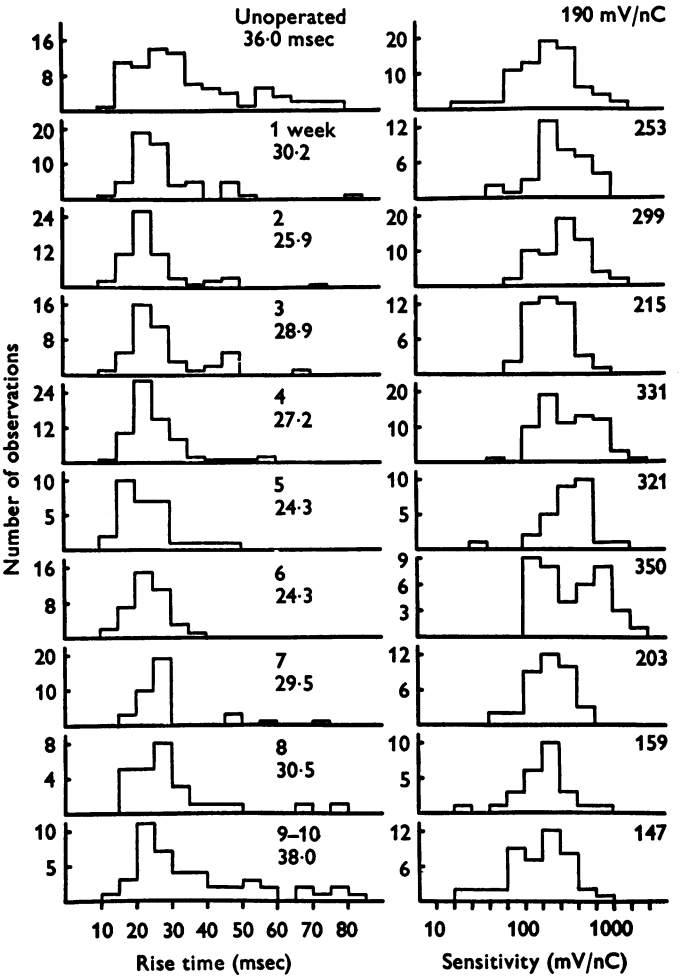

Fig. 9. Development of hypersensitivity after partial denervation. Analysis of the rise times and sensitivities of ACh responses at *random* spots from cells which had been partially denervated for different times. The rise times are shown on the left, and sensitivities (mV/nC) are shown on the right from several experiments at each stage of denervation. The top pair of histograms shows data from unoperated animals (cf. Fig. 5). To the right in each rise time histogram are numbers showing the length of time after the vagus nerve was cut (top) and the average rise time, in msec (bottom). To the right of each sensitivity histogram is the (geometric) mean value in mV/nC. Note, the abscissae for the sensitivities are logarithmic scales. The mean rise time decreases from 36 msec to about 24 msec during the first 5 weeks after the operation and returns to 38 msec after 10 weeks. The (geometric) mean sensitivity increases from 190 to 350 mV/nC during the first 5–6 weeks and then declines to 147 mV/nC after 10 weeks.

with 2 mM-$CaCl_2$ and glucose) at room temperature (cf. McMahan & Kuffler, 1971).

Ganglia with the heart attached could be maintained *in vitro* for periods of at least 1 month. The heart beat spontaneously even after 4 weeks in organ culture. The connective tissue surrounding the principal cells became oedematous after 2·5–3 weeks, but the principal cells appeared

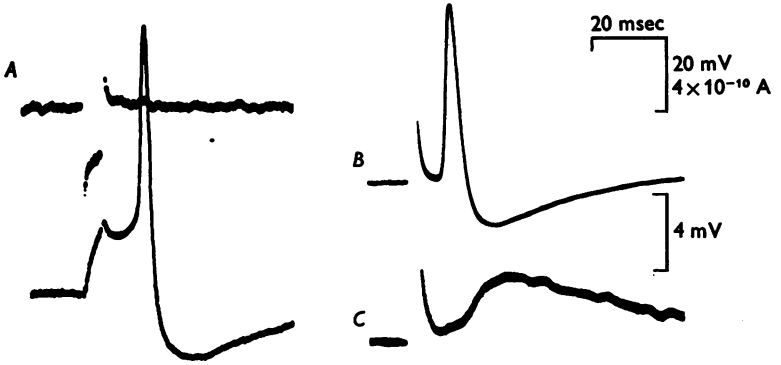

Fig. 10. Examples of neuronal activity in principal ganglion cells maintained in culture for 20 days. *A*, direct stimulation of a cell by brief current pulses applied through the recording electrode (upper trace). *B*, an antidromic impulse in a different ganglion cell, evoked by stimulation of the post-ganglionic nerve. *C*, same cell as in *B*. Altering the stimulus current to the post-ganglionic nerve excited axon collateral synapses.

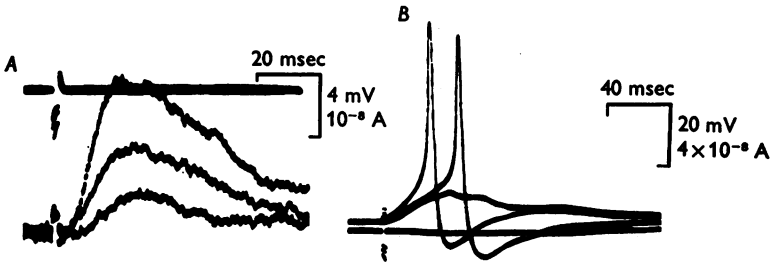

Fig. 11. ACh responses in ganglion neurones maintained in culture for 2 weeks. *A*, increasing the dose of ACh (upper trace) produced graded responses. *B*, different cell, ACh doses of sufficient size evoked impulses. Current pulses shown in bottom trace.

normal under the light microscope. Cells from cultured ganglia could be penetrated with micropipettes and normal resting potentials, action potentials, and synaptic potentials were recorded for some weeks (Fig. 10 and preceding paper).

Preganglionic terminals appeared to degenerate much more rapidly in tissue culture than after denervation *in vivo*. After only 3–4 days, stimula-

tion of the vagal stump failed to evoke responses in the neurones. On the other hand, spontaneous miniature potentials and evoked synaptic potentials originating from the axon collateral synapses were still present.

Increased ACh sensitivity of principal cells in cultured ganglia

When ACh was applied to ganglion cells maintained for some days in organ culture, the responses resembled those seen in partially denervated ganglia (*in vivo*) and from identified synapses in normal ganglia (Fig. 11). Wherever it was tested, the ACh sensitivity of the membrane of cells in organ culture was high.

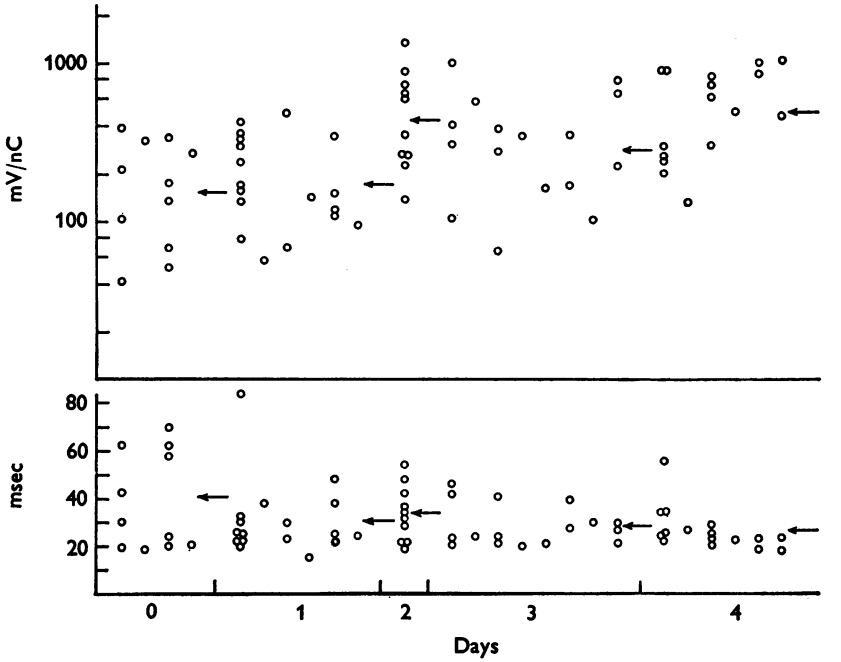

Fig. 12. Development of hypersensitivity after partial denervation *in vitro*. Sensitivities and rise times of responses to ACh at randomly chosen spots are shown for twenty-five different ganglion cells in culture. In each vertical column are measurements from a single cell. The arrows show the mean values of sensitivity and rise time of the responses. Cells are grouped from left to right according to the number of days the isolated ganglion was kept in culture, from 0 to 4 days.

Regional ACh sensitivity in cultured ganglia was mapped for several cells taken from different preparations at varying intervals after isolation in order to study the time course of developing hypersensitivity. The onset of denervation supersensitivity occurred much more rapidly *in vitro* than

it had after vagotomy (Fig. 12). In addition, after 4 days in organ culture the ACh sensitivity of ganglion cells was quantitatively indistinguishable from that of principal cells which had been denervated for 4–6 weeks. These data are summarized in Fig. 13. It is apparent that quantitatively similar changes occur in culture and *in vivo* after cutting the vagus nerve.

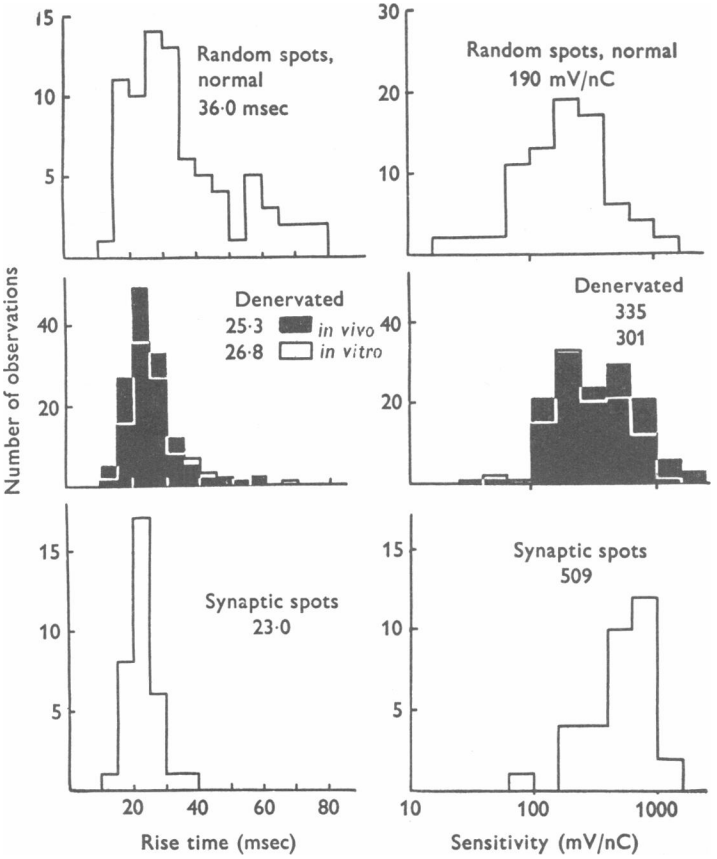

Fig. 13. ACh responses compared for unoperated animals, partially denervated animals, and isolated ganglia kept in organ culture. The histograms show the rise times (left) and sensitivities (right, mV/nC) for random ACh application on normal ganglion cells (top), on denervated and cultured ganglion cells (middle) and for ACh application to identified synapses (bottom). The denervation sensitivity changes *in vitro* and *in vivo* are quantitatively very similar when the periods of maximal chemosensitivity changes are compared (4–6 weeks after cutting the vagus nerve *in vivo*; 4–20 days *in vitro*). Numbers to the right of each histogram are the mean values for rise time (msec) and geometric mean sensitivities (mV/nC). The sensitivity histograms are plotted with logarithmic scales.

DISCUSSION

The results of the present investigation indicate that nerve cells with synaptic endings from different sources have discrete patches of heightened transmitter sensitivity on the surface membrane under nerve terminals. The relatively close spacing of synaptic boutons and the inability to identify visually all the boutons on the living principal cell prevents an estimate of the true chemosensitivity of *non*-synaptic membrane. Certainly the difference in sensitivities between synaptic and non-synaptic membrane must be greater than the values reported here since many of the random spots where ACh was applied included some boutons. Furthermore, it is likely that the responses at these latter spots may be due in part to diffusion of ACh to nearby boutons. This is suggested by the increased time to peak of these responses (cf. Harris *et al.* 1971, p. 551).

Also, after partial denervation either *in vivo* or *in vitro*, the transmitter receptors spread over the cell surface. In other words, the synapses which remain after the majority of the endings (roughly 90%, cf. McMahan & Purves, 1976), have been destroyed cannot substitute for the loss of innervation; the original distribution of chemosensitivity is not retained.

Significance of denervation supersensitivity in multiply-innervated ganglion cells

Although not quantitatively analysed, certainly there were no gross differences in the post-ganglionic axon collateral responses between cells which were maximally chemosensitive (e.g. 4–6 weeks after denervation), and those which had a normal chemosensitivity distribution (intact ganglia and ganglia denervated for 9–10 weeks). This suggests that the spreading of transmitter receptors *per se* after partial denervation did not greatly affect the remaining intact synaptic inputs on nerve cells. The functional importance of spreading chemosensitivity is still obscure.

The role of neuronal activity in denervation supersensitivity

Several interesting problems remain unsolved, for example whether the change in chemosensitivity after partial denervation in the ganglia was due to decreased activity of the post-synaptic cell or to loss of some 'trophic' influence which had been provided by the vagal synapses, or both. Vagotomy removes all excitatory drive for the principal cells. Therefore, it is possible that cessation of neuronal activity in the ganglion cells caused the chemosensitive area to increase.

Experiments on dually innervated frog sartorius muscle fibres are relevant to this problem. When these cells were partially denervated by surgically destroying one of the two nerve terminals on a muscle fibre,

Miledi (1960) found that the chemosensitivity around the denervated end-plate increased even though muscle activity was not abolished. However, recent investigations of the ACh sensitivity of denervated mammalian muscle fibres when stimulation was applied directly to the muscle indicate that activity *per se* can influence the receptor distribution (Lømo & Rosenthal, 1972; Purves & Sakmann, 1974).

The present experiments do not resolve this problem concerning the importance of impulse activity in neurones. However, it is curious that the heightened ACh sensitivity of the partially denervated ganglion cells declined after some 7–10 weeks without any signs of reinnervation or the onset of spontaneous activity. In the ganglion cells which had been partially deafferented for several weeks, the return of normal sensitivity was not caused by an over-all deterioration of the surface chemosensitivity; spots with high sensitivity still were found after 10 weeks, and in fact, the distribution of ACh sensitivity after partial denervation for 9–10 weeks closely resembled that found in the unoperated animals (Fig. 10). Similar results have been obtained at the neuromuscular junction. For example, the increase in chemoreceptive area which is caused by cutting the nerve supply to muscles appears to decline after prolonged denervation (Albuquerque & McIsaac, 1970; Hartzell & Fambrough, 1972). The present data suggest that the denervation supersensitivity is a transient phenomenon, not completely associated with cessation of impulse activity.

I am grateful to Dr S. W. Kuffler who participated in some of the early experiments and who, along with Drs U. J. McMahan and D. Purves, gave advice and encouragement during the experiments and the preparation of the manuscript. In addition, I am indebted to the valuable criticism of the manuscript by Drs E. Furshpan, D. Potter and J. Diamond. I also thank Mr R. B. Bosler for his invaluable assistance in instrumentation.

This work was supported by USPHS grant no. 1F02 NS54024 and 1R01 NS11505.

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