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Original Article

Time-course of post-denervation changes in calcitonin gene-related peptide and substance P immunoreactive nerve fibres in superior cervical ganglion in rat

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ABSTRACT

In rat sympathetic ganglia decentralization by preganglionic denervation leads to intraganglionic sprouting of adrenergic nerve and also in an intraganglionic increase of peptidergic fibres immunoreactive (IR) for substance P (SP) and calcitonin gene-related peptide. We explored the time-course of the post-denervation changes in intraganglionic nerve fibres immunoreactive for calcitonin gene-related peptide and substance p in superior cervical ganglia of normal rats and of rats at intervals from 2 days to 70 days following preganglionic denervation with prevented reinnervation. This study has indicated that many if not all fibres of the networks arise, in two phases, by sprouting from small bundles of similarly immunoreactive nerve fibres which traverse the normal ganglion, entering or leaving it at many points. They survive preganglionic denervation and are thus probably sensory. It is suggested that activity in such new sensory collateral branches may contribute to the low levels of ganglionic activation observable in the autonomic failure of multiple system atrophy in man.

1. Introduction

Long-term preganglionic denervation of the rat superior cervical sympathetic ganglion(SCG) results in intraganglionic sprouting of adrenergic nerve fibres (Ramsay & Matthews,1985) and also in an intraganglionic increase of peptidergic fibres immunoreactive (IR) for substance P (SP) and calcitonin gene-related peptide (CGRP; Zaidi & Matthews, 2011). These newly formed nerve fibres form synapses, which are capable of stimulant triggered exocytosis, upon the ganglionic neurones (Zaidi & Matthews 1999).

In order to establish at what time the increase in the numbers of intraganglionic SP-IR and CGRP-IR fibres begins and to discover the rate and pattern of this phenomenon, we performed timed series of preganglionic denervations, in pairs of rats with survival intervals ranging from 2d to 70d.

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2. Materials And Methods

SCGs of Wistar rats were used in this study. All rats were obtained from an approved breeder (Harlan, UK). All animal were maintained and handled according to Home office guidelines for the care and use of experimental animals. Animals were housed individually in wire bottomed cages (65× 40× 45cm) in a facility with controlled conditions of temperature (21-22◦C) and illumination 12 hours light –dark cycle with water and food available at libitum. All surgical procedures were performed under chloral hydrate anaesthesia (350 mg/kg, intraperitoneally). The animals lay quietly and unresponsive throughout the operation. The depth of anaesthesia was assessed by the abolition of pedal withdrawal reflex.

In 16 male Wistar rats, weighing 150-200 g, the left cervical sympathetic trunk (CST) was exposed and cut 5 mm below the lower pole of the superior cervical ganglion. The proximal end of the CST was sutured into the sternomastoid muscle with 7/0 silk to prevent the regeneration of preganglionic fibres back to the ganglion. The skin was sutured by interrupted everting sutures, and the rats were allowed to survive for (n = 2 each) 2, 7, 14, 21, 31, 42, 56, 70 days. The success of the operative procedure was confirmed by observing maintained ptosis on the left side,

immediately following recovery from the operation and again just before the final operation, at which both right and left SCGs were removed under terminal anaesthesia. The ganglia were fixed in Zamboni's fixative (Stefanini et al. 1967) at 4°C for 4-6 h.

After fixation ganglia were rinsed at 4°C in several changes of 0.1M sodium phosphate buffer solution, pH 7.4, until all yellow coloration had disappeared, placed in 10% sucrose in phosphate buffer at 4°C for 2-3h, then transferred into 30% sucrose in phosphate buffer containing 0.1% sodium azide (Sigma) and left overnight at 4°C.

For light microscope studies longitudinal sections 10µm thick were cut on a cryostat (Bright) and mounted sequentially two to a slide, well separated, on glass slides which had been pre-coated by dipping in a solution of 0.05% chromalum (BDH Chemicals) and 0.5% gelatin (BDH Chemicals) in double-deionized water and dried in a dust-free atmosphere. Mounted sections were stored at 4°C before incubation.

SP and CGRP were detected separately by peroxidase immunohistochemistry in pairs of consecutive 10µm cryostat sections mounted on the same slide, at 60µm intervals throughout each ganglion. In a few cases co-localization was explored by double immunofluorescence histochemistry of single 10µm sections.

Quantitative immunohistochemical assessment

Pairs of consecutive sections incubated for separate detection of SP and CGRP immunoreactivity were scanned at x400 under a Zeiss light microscope fitted with an eyepiece graticule engraved with 100 small squares. The numbers of intersections formed with the graticule by SP-IR and CGRP-IR fibres were counted in successive frames throughout each 10µm section of a 1 in 6 series for each peptide in each ganglion. Intersections formed by immunoreactive fibres with the leading edges of the graticule were excluded during counting to avoid repetition. Counts were expressed as mean numbers of intersections per graticule area per ganglion, ± S.E.M., for comparison.

Counts were also made of sites in each section where SP-IR or CGRP-IR fibres were seen forming basket-like wrappings around neuronal cell bodies (perineuronal baskets), and the mean numbers of baskets per graticule area per ganglion, ± S.E.M., were compared.

In all the immunochemical processing one section from each set was processed without treating with primary antibody for assessment of the extent of background nonspecific labelling.

3. Results

In the younger animals of this series, including the normal and contralateral ganglia, both CGRP-IR and SP-IR nerve fibres were consistently observed. CGRP-IR fibres were in all cases the more abundant. The level or incidence of SP-IR in fibres was very low, at or below the level of detection. Immunoreactive fibres of either type were both non-varicose and beaded, varicose in appearance. (Fig. 1 a).

Denervated ganglia

At 2d post-denervation all the finely varicose CGRP-IR intraganglionic trails, networks and basket formations had disappeared. At no subsequent time did these reappear in exactly the same form. In one of the two experiments a few fragments of degenerating CGRP-IR fibres were found in the stump of the CST. Within the ganglion a few coarser, non-varicose intrafascicular CGRP-IR fibres remained intact, including some with branches entering the neuropil and giving sparsely varicose loops and twigs to encircle one or two neurones (Fig. 1 b). These were rendered conspicuous by the loss of the finer networks. Fine varicose perivascular CGRP-IR fibres were still present, and small bundles of coarser non-varicose CGRP-IR fibres, sometimes paravascular, were still found entering or leaving and traversing the ganglia, as in the older normal and contralateral ganglia. From the neighbourhood of a few of these bundles one or two finer varicose CGRP-IR fibres, resembling new sprouts, extended for short distances into the adjacent neuropil.

At 7d post-operatively both the denervated ganglia showed similar persistence of perivascular CGRP-IR fibres and coarser CGRP-IR bundles, and these remained consistently present throughout the timed series of denervated ganglia. Fine varicose trails and baskets were completely absent but in one of the two experiments numerous CGRP-IR fibres of moderate to fine calibre, some varicose but mostly non-varicose, were seen running in parallel courses along the ganglionic stump of the CST (Fig. 1 e). In the same ganglion many CGRP-IR fibres of similar character were found running longitudinally, singly or in twos and threes, branching occasionally, in the intraganglionic nerve fascicles and apparently entering the ganglion near to or along the CST (Fig. 1c, d). In some places these fine-calibre fibres appeared to originate from coarser fibres in the compact bundles (Fig. 1 c). Similar, but fewer, intrafascicular CGRP-IR fibres were found here and there in the other 7d post-operative ganglion near its cranial end, in fascicles adjacent to a bundle of coarse CGRP-IR fibres, one of which appeared to give origin to one of the finer intrafascicular fibre. This ganglion also showed a few coarser longitudinally-running solitary CGRP-IR fibres of the type seen in normal ganglia, and in places these also were seen apparently giving rise to fine, varicose intrafascicular fibres, some of which were observed to enter the neuropil and encircle neurones there.

At 14d post-operatively both denervated ganglia showed many moderately fine, mostly varicose CGRP-IR nerve fibres crowding the ganglionic stump of the CST and culminating in rows of discontinuous points of IR at and near to its cut end (Fig. 1f); and, more markedly than at 7d, similar fibres were found running in the intraganglionic nerve fascicles throughout the ganglion

(Fig. 1g). Immunoreactive nerve fibres were much more frequent in the fascicles than in the neuropil. A few varicose nerve fibres were seen leaving fascicles to enter the neuropil. Within the neuropil CGRP-IR nerve fibres of moderate calibre, with scanty, coarse varicosities, were found here and there, running longitudinally or contouring, and occasionally branching, among the neurones.

By 21d and also at 31d post-operatively the CGRP-IR nerve fibres in the ganglionic stump of the CST were still numerous. Intrafascicular fibres of similar character extended into the ganglion from this same region and at 21d were still the most frequent class of immunoreactive fibres throughout the ganglion (Fig. 1h). In addition, and more often than at 14d, moderately coarse CGRP-IR nerve fibres were seen contouring among the neurone somata, sometimes encircling individual neurones and showing occasional coarse varicosities (Fig. 1h, j). These fibres lay singly or in patches in the neuropil and tended to be more abundant in cranial than in caudal regions. The perineuronal networks so formed resembled those seen following long-term denervation and differed markedly from the fine, richly varicose networks and perineuronal baskets of normal and contralateral ganglia, having a coarser calibre and a wider mesh, with varicosities which were larger but much less frequent. In these ganglia perivascular CGRP-IR fibres were conspicuous along the intraganglionic blood vessels. In one ganglion at 21d post-operatively finely varicose nerve fibres were seen branching directly into the neuropil from the neighbourhood of intraganglionic arterioles. Non-varicose branches emerging similarly from peri- or paravascular locations in this ganglion, and in another at 31d, were observed to give rise to perineuronal varicose fibres. In the other 21d ganglion moderately fine varicose CGRP-IR nerve fibres with long intrafascicular courses were found passing cranialward toward a region of branching, coarsely varicose immunoreactive fibres in the neuropil. At 31d intrafascicular fibres were still a conspicuous feature (Fig. 1j) but in places fibres in the neuropil tended to be more abundant. The first basket-like formation round a neurone was seen at 31d but was unlike the basket of a normal ganglion, resembling rather a close net or grille, composed of coarse sparsely varicose fibres.

By 42d and increasingly thereafter, at 56d and 70d post-operatively, such 'baskets' were becoming more frequent, and the newly-formed perineuronal CGRP-IR networks in the neuropil were ceasing to be patchy and becoming progressively more pervasive and diffuse (Fig. 1k, l, m). CGRP-IR fibres had now largely disappeared from the CST stump. Intrafascicular CGRP-IR fibres were becoming relatively fewer and were less often varicose and of fine calibre, coming increasingly to resemble in calibre the fibres in the neuropil networks. Instances were seen of CGRP-IR nerve fibres apparently entering intraganglionic fascicles from extraganglionic nerve bundles, then passing on into the perineuronal networks. Fig. 2 a shows examples of all three classes of fibres. The CGRP-IR fibres forming the networks were moderately coarse and markedly wavy, with a few large varicosities (Fig. 1k, m). They often showed quite long courses in the neuropil, contouring, crossing, branching and changing direction (Fig. 1l, m) and sometimes encircling individual neurones (Fig. 2 b, c). In contour and calibre these fibres quite closely resembled the CGRP-IR nerve fibres innervating the carotid body, which lies immediately adjacent to the SCG at the base of the ECN (Fig. 2 d). 'Baskets' of the unusual grille or tangle form first seen at 31d (e.g. Fig. 1k) occurred with a low incidence which tended to increase with survival interval, and by 70d this had reached levels comparable with those found in normal and contralateral ganglia. The difference between the ganglia at 70d and at 210d post-denervation was that at 210d the neuropil networks tended to have become more abundant and to permeate the ganglion more completely.

Changes observed in contralateral ganglia

In some ganglia contralateral to ganglia denervated for between 14d and 42d, signs were seen of a possible intraganglionic sprouting reaction of CGRP-IR fibres. In the 14d contralateral ganglia unusual fine-calibre, branching, varicose CGRP-IR nerve fibres were seen in intraganglionic nerve fascicles, occasionally in one ganglion and more often in the other, where up to 3 or 4 per fascicle were found in places in the mid- to cranial region of the ganglion. These were additional to the coarser, non-branching CGRP-IR nerve fibres occasionally seen in fascicles in all ganglia. Similar fine, finely varicose intrafascicular fibres were seen in both contralateral ganglia at 21d post-operatively. In one ganglion at 31d intraganglionic fascicles were seen to contain both varicose and non-varicose CGRP-IR fibres of fine calibre. A few such fibres were also present in fascicles in both ganglia at 42d. Few or no intrafascicular fibres of this character were seen in contralateral ganglia after 56d or 70d survival.

In all the ganglia of this series, taken together, with one or two single exceptions, no neurones showed more than occasional low levels of putative CGRP-IR, and no neurones showed any SP-IR.

Counts of CGRP-IR fibres

The incidences of CGRP-IR fibres, represented as mean numbers of intersections with an eyepiece graticule, for sets of normal, denervated and contralateral ganglia over this series are shown in the histogram of Fig. 3a. The normal ganglia, taken from rats of the same ages and litter-groups as the experimental animals at 14d, 56d and 210d post-operatively, show constant levels. Denervated ganglia show a sharp fall at 2d, representing a 75% loss of CGRP-IR fibre intersections from the normal or 2d contralateral level. This is followed by a rise to supranormal numbers, which continues with some fluctuation toward the high levels observed in the longer term. The histogram of Fig. 3a is plotted without a strict time-scale, but in Fig. 3b the counts for individual denervated ganglia are shown along a representative time-scale, together with mean values for normal ganglia for comparison. Fig. 3b shows that that the early rise is evident in one ganglion by 7d and in both ganglia by 14d. This early rise is

transient, partly subsiding by 21-31d. It is succeeded from 31-42d onward by a more sustained rise which continues thereafter, with a possible pause at about 56d, toward the high level already described for 210d. The early rise corresponds with the early appearance of fine-calibre new intrafascicular fibres in the ganglia, and the later steep rise at and after 31d coincides with the progressive establishment of new, coarser, sparsely varicose networks in the neuropil. In each case there are signs of an overshoot, with some subsequent regression of earlier-formed fibres.

The contralateral ganglia show at 2d no change from the normal, but the counts are slightly elevated in both ganglia at 7d and in one ganglion at 14d and show a second rise at 21-31d, subsiding thereafter (Fig. 3a; results for individual ganglia not shown). These quantitative changes reflect and confirm the apparent sprouting reaction in the contralateral ganglia, described above. They slightly precede each of the two phases of sprouting in the denervated ganglia to which they are contralateral, and in each case they are transient rather than sustained.

Substance P

In all categories of ganglia, normal, contralateral and denervated, very little SP-IR was detected in the younger ganglia which formed the earlier part of this series. Faint traces of SP-IR were found in occasional nerve fibres in paravascular locations, or in bundles which in adjacent sections contained CGRP-IR nerve fibres, in young normal ganglia and in contralateral ganglia before 42d, at which stage and subsequently such fibres became more frequent, trails and patches of finely varicose SP-IR nerve fibres began to appear in the perineuronal neuropil, and a few fine solitary SP-IR nerve fibres were found running longitudinally in intraganglionic nerve fascicles. In denervated ganglia, however, already at 14d post-operatively a few fine varicose nerve fibres were found branching in the cranial neuropil of one ganglion. Similar varicose SP-IR fibres were observed at 21d post-operatively, in the cranial ganglionic neuropil and in a few fascicles which in adjacent sections contained CGRP-IR fibres. By 31d fine varicose fibres in the neuropil were more frequent and the first faint suggestion of a perineuronal coarse, net-like basket was seen, as for CGRP. At 42d and subsequently the SP immunoreactivity of fibres was stronger, and widely-scattered wavy, mostly varicose SP-IR fibres were seen branching and contouring within the neuropil. The distribution of these fibres was patchy and locally dense, being more prevalent cranially than caudally (Fig. 2 e, f). At 42d and 56d coarser, non-varicose intrafascicular fibres, some with long courses, were seen giving finer, varicose branches which entered the neuropil (Fig. 2 g).

From 31d onward occasional perineuronal basket-like formations were seen, of the same grille-like coarse texture found for CGRP-IR fibres in these denervated ganglia (Fig. 2 h). Peri- and paravascular SP-IR nerve fibres were also occasionally observable in these ganglia from 42d onward.

Thus, in the denervated ganglia SP-IR fibres first appeared at 14d post-operatively, later than the sprouts of CGRP-IR fibres, and unlike these were from the beginning found in the neuropil rather than in fascicles. In the neuropil they increased in numbers as the CGRP-IR neuropilar networks increased, and began to show perineuronal basket-like formations at the same stage. This pattern, taken together with the later-developing distribution of SP-IR fibres in the normal and contralateral ganglia, suggests that a certain stage of maturation of SP-IR fibres in terms of peptide content must be attained before they can be visualized by the present technique, and illustrates the presumptive time-course of such maturation in the denervated ganglia.

Counts of SP-IR fibres

The mean numerical incidences of intersections with SP-IR fibres in this series of ganglia are represented in the histogram of Fig. 3c. A logarithmic scale has been used, in view of the very low values in the younger animals and in the early post-operative stages. As in Fig. 3a, the time-scale is not strictly proportional. Fig. 3c shows that the mean counts for SP-IR fibres in the denervated ganglia rise steeply from 14d onward to 42d and are at all times greater than those for the normal and contralateral ganglia. The rise is approximately logarithmic from 14d to 42d, with a pause thereafter.

In the normal and contralateral ganglia the rise in counts approximately parallels the rise in the denervated ganglia but at a much lower level, suggesting that a similar process of maturation may be involved in each case, even though some of the SP-IR fibres will be from a different, preganglionic source. The time-course of the changes in SP-IR fibre counts for individual denervated ganglia is shown in Fig. 3d, together with the mean values for the groups of normal ganglia. This shows wide divergences in the counts within pairs of denervated ganglia in the earlier part of the series.

3. Discussion

In the present study a timed series of shorter-term denervations has provided evidence suggesting that the new immunoreactive fibres arise at least to some extent, if not entirely, by sprouting from small nerve bundles containing similarly immunoreactive nerve fibres, present in the normal ganglion, which enter and traverse the ganglion at several sites.

Observations made at shorter intervals after denervation in the present experiments suggest that this partial substitution for the absent preganglionic nerve fibres occurs in two phases. There is an initial sprouting, well established by 7-14 days, into and along pathways occupied by the Schwann cells of the lost preganglionic nerve fibres. This is seen most concentratedly into and along the stump of the cervical sympathetic trunk (CST), in a cranio-caudal direction which is the reverse of the pathway followed by the original fibres, and also entering the ganglion from the same region close to the entry point of the CST, and spreading out cranialward into the ganglion along the distributing nerve fascicles. Other, but less abundant, points of entry and spreading of early sprouts are seen, notably near to the point of exit of the intrenal carotid nerve (ICN), which is close to the lower pole of the nodose or inferior vagal ganglion. The sprouting seems to occur from fibres of the presumed sensory nerve bundles associated with the ganglion. Some apparent sprouts have also been seen to arise from immunoreactive nerve fibres accompanying the intraganglionic blood vessels. The major sprouting reaction into and along the CST, and into the ganglion from the region of the CST entry point, is likely to be provoked by growth factors released by the Schwann cells of the degenerated preganglionic nerve fibres, distal to the point of nerve section. The lesser levels of sprouting at more distant locations in the ganglion may well represent responses to the same growth factors, present in lesser concentrations where the denervated Schwann pathways are fewer and more dispersed. Rat Schwann cells isolated in culture synthesize and release into the medium nerve growth factor-like neurite promoting factors (Assouline et al. 1987; Yamamoto et al. 1993), and these are not specific to single neuronal types or pathways. Nerve growth factor (NGF) itself promotes outgrowth of neurites from the smaller neurones of dorsal root ganglia, as well as from sympathetic neurones. It has been found to increase in the rat iris following either sensory or sympathetic denervation (Ebendal et al. 1980). Extirpation of the rat SCG has been shown to lead to increase of SP-like IR in the iris (Cole et al. 1983, Kessler et al. 1983) and of CGRP level in the iris and pial arteries (Schon et al. 1985).

The second phase of sprouting of IR fibres begins later, during the second or third post-operative week, and builds up much more slowly. It involves the gradual establishment of nerve networks in the ganglionic neuropil and culminates in the formation of synaptic contacts with the ganglionic neurones. The fibres of these networks do not become more prevalent than the new intrafascicular fibres until approximately 6 weeks post-denervation. They are slightly coarser than the new intrafascicular fibres, and they show occasional varicosities; but nevertheless they appear to arise by sprouting from the IR intrafascicular nerve fibres which arose in the first phase. It is only as the second phase develops that fibres with clear evidence of SP-IR first begin to be at all numerous in the denervated ganglia. In the second sprouting phase there is a rapid, almost exponential, build-up of SP-IR in fibres in the newly forming neuropil networks. This slightly precedes the onset of appearance of SP-IR in fibres of the normal ganglia of rats of the same age, and in the contralateral ganglia. The delay in onset and slow build-up of the second phase of sprouting of CGRP-IR fibres, and the differential response of the SP-IR, suggest that this phase involves a different stimulus and, or, a later stage of maturation of the developing nerve fibres, if not also actually a different source such as another class of sensory nerve fibres, or the ganglionic neurones themselves.

Since in the longer term CGRP and SP have been found to co-exist in many fibres of the networks it is possible that the same nerve fibre can contain detectable levels of CGRP from the earliest stage of intrafascicular sprouting but detectable SP only at the stage when it has extended into the neuropil, into the immediate environment of the neurones and their satellite cells, and is in a position to make functional contacts with the neurones. This difference might originate in, or be correlated with, differences of function between the two peptides. The even later appearance of detectable levels of SP-IR in the normal and contralateral ganglia suggests, however, that the explanation is not necessarily simple, since here the functional innervation by the preganglionic nerve fibres, including the few which are SP-IR, will have been fully established from an early stage. Here it seems that the level of SP is dependent on the chronological age of the rat, with lower concentrations prevailing in younger animals. As was noted earlier, however, the demonstrable level of a peptide does not necessarily indicate its rate of turnover. It may be that the relative sensitivities of the two detection systems are playing a part here, since the special divalent anti-SP, anti-HRP monoclonal antibody which was used requires no second or third antibody steps and therefore lacks the additional amplification which these would provide. This highly specific antibody thus appears to have highlighted a quantitative difference in expression or turnover of the peptide between the first and the second phases of sprouting. This, together with the neuropilar location and synaptic nature of the networks formed in the second phase, which establish presumably functional connexions with the ganglionic neurones, reinforces the impression that this phase is likely to be elicited by a different stimulus for sprouting. This stimulus, like the earlier one, is evidently not specific to a particular class of nerve fibres. New adrenergic synapses also form, progressively, in the denervated ganglion at a stage corresponding to this second phase (Ramsay & Matthews 1985). The nerve terminals of these synapses are discriminable by 5-OHDA labelling from those of presumed sensory nerve fibres which contain large dense-cored vesicles not labelled by 5-OHDA (Zaidi & Matthews 1999). These adrenergic synapses most probably arise from the ganglionic neurones, either of the same ganglion or from other ganglia via axons innervating the ganglionic vascular smooth muscle (Ramsay & Matthews 1985). Both types of new synapses appear to be functionally capable of release (Zaidi & Matthews 1999).

The reason for supposing that the stimulus for the second phase is different is that this phase involves the entry of new neurites into the neuropil and the eventual formation of synapses. The nature of this stimulus might be neurite-promoting factors released by the supporting satellite cells of the denervated neurones, as has been shown for glial cells (Varon & Somjen 1979) and, or, by the neurones themselves. The former is the more probable: ganglionic neurones isolated in culture show some capacity for survival (Acheson et al. 1995; Orike et al. 2001a, b), but this may not include release of growth-promoting factors into the medium. Remote axotomy of dorsal root ganglion sensory neurones by cutting of the sciatic nerve is followed by sprouting of adrenergic, evidently postganglionic sympathetic, nerve fibres into the relevant sensory ganglia (McLachlan et al. 1998). These fibres were presumed to arise from the sympathetic innervation of blood vessels supplying the ganglia. Additional, peptidergic sprouts also arose locally in the ganglia from small SP-IR and CGRP-IR neurones. The secretion of growth factors in these ganglia is most likely to have come from the neuronal satellite cells, through an interaction between themselves and the remotely-injured neurones.

Further evidence suggesting the secretion of growth factors from satellite cells of ganglionic neurones comes from the use of guanethidine, which produces widespread postganglionic sympathetic denervation. Following such postganglionic denervation, non-sympathetic nerve fibres including CGRP-IR and, or, SP-IR fibres expand their territories not only in the peripheral target organs (Evans et al.1979; Carvalho et al. 1986) but also in the sympathetic ganglion (SCG: Aberdeen et al. 1990, 1992; Benarroch et al. 1992). The injury in the ganglion is to the ganglionic neurones and not to the preganglionic nerve fibres, and few of the neurones survive, thus the stimulus for sprouting of peptidergic fibres is likely to have arisen to a large extent from neuronal satellite cells.

The neuronal satellite cells may have rather specific roles in the sympathetic ganglion. They completely envelope the neurones and their dendrites, except at points of synapse or of non-synaptic apposition or attachment to other neurites, and at occasional sites exposed to the ganglionic tissue space. Synapses consistently have complete wrappings. Sites at which neural elements are exposed to the ganglionic tissue space are relatively few and inextensive, but can involve small parts of the surfaces of neuronal somata, dendrites and near-terminal axons. They are thought to include points of non-synaptic release and, or, of effector action of substances such as long-acting neurotransmitters or neuromodulators which are capable of action at a distance after diffusion (Matthews et al. 1987; Kiraly et al. 1989). Little is known of possible metabolic or functional differences between the satellite cells of the ganglionic neurones, which envelop the neuronal somata and their dendrites, and the Schwann cells which enwrap the preganglionic nerve fibres or the postganglionic axons. It is not clear whether sites of synapse are regularly enclosed by the Schwann cells of the preganglionic axons or by neuronal satellite cells, or whether this role is variable or interchangeable. Synapses in adult rat ganglia are rarely axo-somatic, but axo-dendritic synapses may be located within the satellite sheath of a neurone or of the dendritic shaft, and there the local satellite cells could be exposed directly to the end-products of nerve terminal degeneration, which might recruit them to secrete specific neurotrophins. Alternatively, the denervated neurones might interact with the satellite cells to induce a similar response, distinct from that of the Schwann cells of degenerating axons. It is indeed uncertain whether these two cell types, the satellite cell and the Schwann cell, are really distinct except in their differing relationships with the neural elements. These per se might however impose upon them different secondary characteristics, through differing functional requirements. Observations made by Chamley et al. (1972) by time-lapse cinematography in tissue culture suggest furthermore that neuronal satellite cells of sympathetic ganglia may be differentiated from Schwann cells by their types of movements, as well as by differences in shape and tissue relationships. Some specific interaction between the satellite cells and the denervated but uninjured neurones, consequential upon the degeneration of nerve terminals impinging upon the neurones, may thus have provoked the secretion and release in the second sprouting phase of neurite-promoting factors which would elicit the formation of functional contacts upon the neurones from any sources capable of responding. In the situation of the denervated ganglion these would presumably include the sprouts formed in the first phase, which appear to provide the SP-IR and most or all of the CGRP-IR fibres of the neuropil networks, and the dendrites or axons of the uninjured ganglionic neurones, which apparently give rise to most of the adrenergic nerve terminals, augmented by potential sprouts from the adrenergic innervation of the blood vessels (Ramsay & Matthews 1985), and possibly also to some of the CGRP-IR nerve terminals found in the present study.

It is concluded that extrinsic, presumptively sensory neurones contribute to the degree of substitutive re-innervation which occurs in chronically decentralized sympathetic ganglia and that activity in these neurones could account at least in part for the moderate level of excitation of ganglionic neurones which may occur in such ganglia. The synaptic effect of such activation is not necessarily completely random, since there is evidence to suggest some selectivity in the establishment of the new connexions, but there may in addition be a wider influence resulting from non-synaptic release. The actions of the released peptides might moreover play an important role in the maintenance of the denervated neurones, pending possible reinnervation by regenerating preganglionic nerve fibres.

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