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A comparative study by retrograde neuronal tracing and substance P immunohistochemistry of sympathetic preganglionic neurons in spontaneously hypertensive rats and Wistar–Kyoto rats

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ABSTRACT

A comparative morphological study of the sympathetic preganglionic neurons that innervate the superior cervical ganglion (SPN-scg) was made in spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar–Kyoto (WKY) rats. The cytoarchitectonics and dendroarchitectonics of the SPN-scg were studied following retrograde transport of choleragen subunit B horseradish peroxidase conjugate (CB-HRP) and Fluorogold. Significant differences were observed in the maximum and minimum diameters of neurons of the nucleus intermediolateralis pars principalis (ILp) and in the minimum diameter of neurons in the nucleus intermediolateralis pars funicularis (ILf) between SHR and WKY rats ($P < 0.01$). These diameters were decreased in neurons of SHR. The distribution patterns of dendrites of SPN-scg also showed differences between SHR and WKY rats. The dendritic distribution patterns showed the following changes in SH rats: (1) the mediolaterally oriented dendrites were reduced in number, (2) the ladder-like configuration of the medially oriented grey-matter dendrites was less prominent, (3) the medially oriented dendrites formed a triangular or dome-like configuration, and (4) the white matter dendritic plexuses and subependymal plexuses were reduced. Similar differences between SHR and WKY rats were also observed in our immunohistochemical study of substance P-like fibres. In addition, the SP study has also shown a close association of SP fibres with the central canal both in SHR and WKY rats; some of the SP fibres penetrated the ependymal lining to run longitudinally up or down the central canal. This finding suggests the presence of substance P-positive neurons contacting the cerebrospinal fluid.

Key words: Hypertension; sympathetic neurons; substance P.

INTRODUCTION

It has long been thought that the sympathetic nervous system may play a critical role in initiating and/or sustaining essential hypertension (Judy et al. 1976; Abboud, 1982). These neurogenic abnormalities may originate in 3 main parts of the sympathetic nervous system: (1) in the efferent pathways of the sympathetic nervous system, i.e. the adrenergic terminal and effector cells as indicated by hypertrophy of blood vessels (Folkow, 1971; Hart et al. 1980), abnormalities in active and passive sodium transport of vascular muscle (Hermsmeyer, 1976; Abel & Hermsmeyer, 1981; Campbell et al. 1981), increase of the adrenergic postjunctional $\alpha 1$ receptors (Amann et al. 1981) and decrease of prejunctional $\alpha 2$ receptors (Takeshite & Mark, 1978); (2) in the afferent or sensory endings, as shown by an impairment or resetting of arterial baroreceptors in various models of hypertensive animals before the onset of hypertension (Gordon et al. 1981), an exaggerated sensory input from the cardiac endings and increased renal afferent nerve activity which might also be implicated in the maintenance of high sympathetic tone (Katholi et al. 1981; Mark & Kerber, 1982); and (3) in the central nervous system (Reis & Doba, 1974; Fein, 1982;

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Brody et al. 1988, 1991; Gomez-Sanchez, 1991; Scharer et al. 1993) where central cardiovascular control centres such as the central amygdaloid nucleus (Galeno et al. 1982), the hypothalamus (Yamori & Okamoto, 1969; Krukoff, 1988; Komatsu et al. 1992), the nucleus local coeruleus and nucleus tractus solitarius (Kawamura et al. 1976) have been shown to play an important role in the development of hypertension.

Of all these 3 parts of sympathetic nervous system, whether from central cardiovascular control centres to the peripheral efferent pathway (Saper et al. 1976; Amendt et al. 1979; Loewy et al. 1979; Swanson & Mekellar, 1979; Saper & Loewy, 1980) or from visceral sensory endings to the central cardiovascular control centres (Kuo & de Groat, 1983; Kuo et al. 1984), sympathetic preganglionic neurons play a critical role, especially for the former. Using the method of retrograde transport of choleragen B subunit conjugated horseradish peroxide (CB-HRP) and Fluorogold, the present study compared the morphology of the sympathetic preganglionic neurons of spontaneously hypertensive rats with age-matched normotensive Wistar–Kyoto rats. To confirm and complement the findings in the retrograde neuronal tracing studies, a substance P (SP) immunohistochemical study was carried out. Furthermore, it has been shown that the distribution patterns of substance P immunoreactive products were similar to those of the sympathetic preganglionic neurons (Oldfield et al. 1985).

MATERIALS AND METHODS

Retrograde tracing procedure

Thirteen spontaneously hypertensive rats (SHR) and 13 correspondingly age-matched normotensive Wistar–Kyoto (WKY) controls aged 4–5 months were used in this study. These rats were obtained from the Animal Resource Centre, Murdoch, Western Australia. All SH strain rats develop hypertension after 2 months and 4 months later, the blood pressure persists between 160 and 200 mmHg (Okamoto, 1969). The rats were anaesthetised with chloral hydrate (0.40 g/kg), after which the right superior cervical ganglion (SCG) was exposed through a midline incision in the neck. 5 μl of 1% CB-HRP (List Biological Laboratories, Inc., USA) were then slowly injected into the right SCG of 6 SHR and 6 WKY rats. A similar amount of 4% Fluorogold (Fluorochrome, Inc., USA) was injected into the right SCG of 3 SHR and 3 WKY rats. Both CB-HRP and Fluorogold were used for neuron labelling. This is because with CB-HRP and under the dark-field microscopy, labelled neurons are clearly demonstrable, especially their dendritic distribution patterns. In rats given Fluorogold injection, only the neuronal cell bodies were labelled; this method was used to study the orientation of the sympathetic preganglionic neurons. Four rats were given 10 μl of the tracers to ensure a maximum labelling of the cells and their processes. No noticeable difference in the labelling of cells was observed between rats given 5 μl and those given 10 μl of tracer. All injections were made with a glass micropipette connected to a Hamilton syringe. To ensure a maximum labelling of the sympathetic preganglionic neurons that innervate the superior cervical ganglion, 5 or 10 μl of HRP was gradually injected over a period of 30 min. Following each injection, the injection side was dabbed with a cotton bud. To avoid spillage or leakage, a very fine glass micropipette was used.

After 72 h, the animals were perfused transcardially with 50 ml of saline initially, followed by 500 ml of a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) over 30 min. After that, the rats were perfused with 500 ml of 10% sucrose in 0.1 M phosphate buffer (pH 7.4) for 30 min. The spinal cord was then cut into 5 blocks at the following levels: C7–C8, T1–T2, T3–T4, T5–T6 and removed. All blocks were immersed in 30% solution of sucrose overnight before serial cryostat sectioning at 30 μm thickness in the horizontal plane. For rats which had been injected with Fluorogold, the sections were mounted on gelatin-coated slides, air-dried, xylene-cleared, cover-slipped and photographed under a fluorescence microscope. These were used for study of orientation of cell bodies. Tissues from rats which had been injected with CB-HRP were processed according to the procedure of Mesulam & Rosene (1979), following which they were counterstained with neutral red and then photographed under bright and dark-field illuminations. These were used for measurements of cell bodies or for examining the orientation of dendrites. Qualitative studies of the dendritic distribution patterns of SPN-scg were made from photographs taken under dark-field illumination.

Although the spinal cord was cut into 5 blocks, our study showed that the highest concentration of labelled neurons was distributed in T1–T3 segments of the spinal cord. In view of this, only neurons from these segments were selected for measurement. Incomplete neuron profiles, i.e. neuronal profiles which did not contain a nucleus, were avoided. Quantitative

studies of cell bodies of the sympathetic preganglionic neurons that innervated the superior cervical ganglion (SPN-scg) were made from photographs magnified at ×270, but the strain of rats was not known (i.e. 'blind') to the investigator who did the measurement. A total of 600 neurons in the nucleus (n.) intermediolateralis pars principalis (ILp), 120 neurons in n. intermediolateralis pars funicularis (ILf), 60 in n. intercalatus spinalis (IC) and 60 in n. intercalatus pars paraependymalis (ICpe) were randomly selected from each group of SHR and WKY rats, i.e. 100 CB-HRP labelled neurons from ILp, 20 neurons from ILf, 10 neurons from IC and 10 neurons from ICpe from each SHR or WKY rat. Only neurons counterstained with neutral red and showing an identifiable nucleus were studied. The mean values of the maximum and minimum diameters and the ratio of the maximum to minimum diameter from each group of SPN-scg in SHR and WKY rats were calculated. The significance of the difference of means in the 2 groups of rats was determined using Student's t test. The ratios of the maximum to the minimum diameter indicated the shape of the neuronal profile, a ratio of 1.0 to 1.3 indicating a round profile, that of between 1.3 and 2 indicating an oval profile and that of > 2 as fusiform (Zhang et al. 1993).

In addition to the above, all Fluorogold-labelled neurons extending from C8–T5 were counted in 3 SHR and 3 WKY rats. Cell counting was carried out directly on sections under a fluorescence microscope. The results were analysed statistically using Student's t test.

Immunohistochemical procedure

Eight rats (4 SHR and 4 WKY) were used for immunohistochemical study. They were perfused with 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The spinal cord was cut into blocks at the following levels: C8–T1, T2–T3, T4–T5, then removed. The tissues were postfixed in the same fixative for 4 h and kept in 30% sucrose in phosphate buffer (0.1 M, pH 7.4) overnight. Horizontal sections (30 μm) were then cut and mounted on gelatinised slides. The sections were treated in 4% normal goat serum for 2 h at room temperature, after which they were washed in 0.1 M phosphate-buffered saline (PBS) containing 0.2% Triton X-100 and placed overnight in primary rabbit anti-substance P (SP) antiserum (Incstar, Minnesota) diluted 1:800 in PBS/Triton X-100. The next day, the sections were washed in PBS and placed for 1 h in biotinylated goat antirabbit IgG antibody (Vector Laboratories, Burlingame, CA, USA) diluted 1:2000 in PBS/Triton X-100. After 2 washes in PBS, the sections were placed in ABC reagent (Vector Laboratories, Burlingame, CA, USA) in PBS/Triton X-100 for 1 h. The sections were then washed in PBS and reacted in a solution of 0.002% H_2O_2 and 0.076% 3,3′-diaminobenzidine (Sigma) in Tris buffer for 15 min. The sections were dehydrated, coverslipped, and photographed under dark-field illumination.

For control immunohistochemical incubation, some sections were incubated in 1% normal goat serum without anti-substance P antibody.

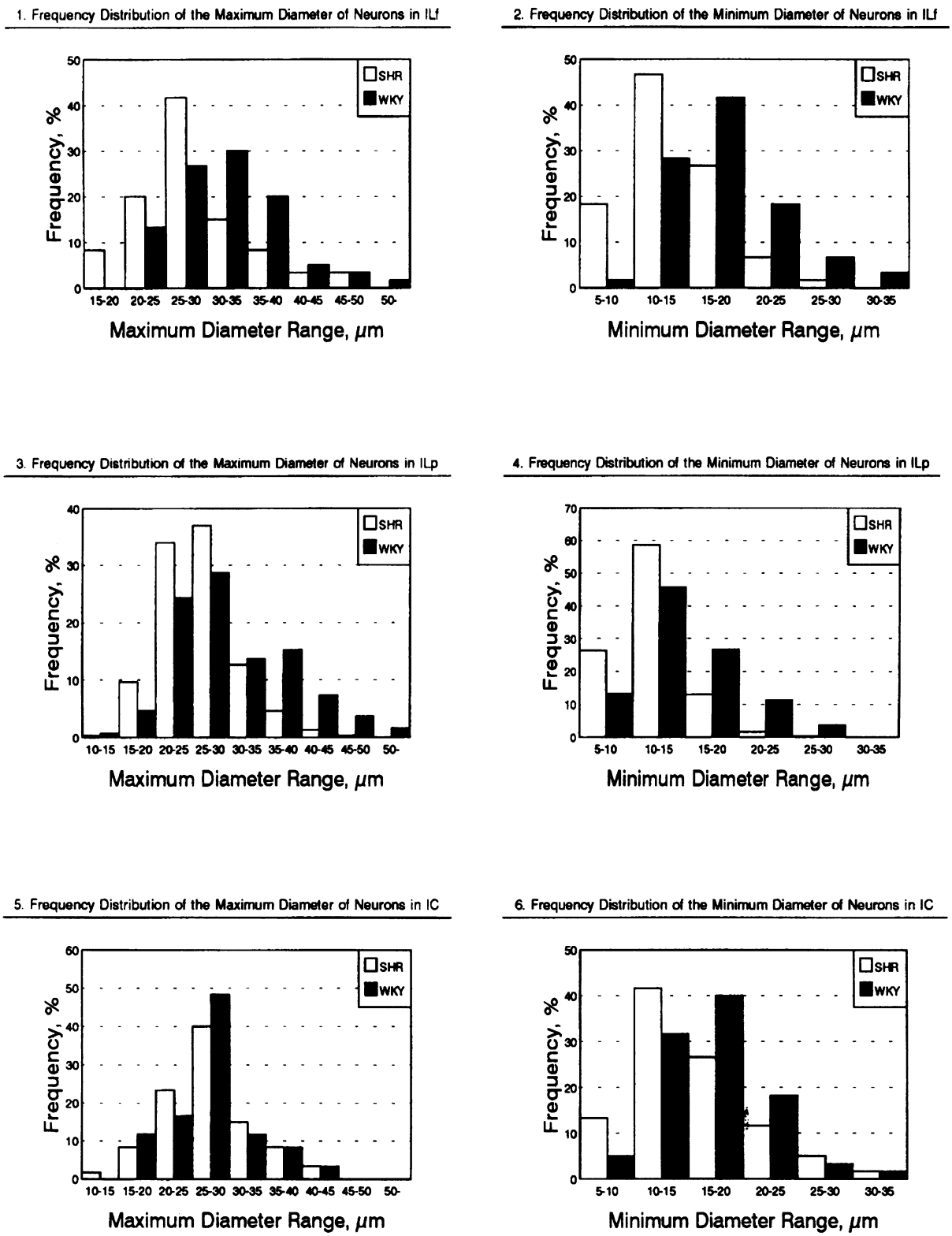
RESULTS

Comparison of the sizes of the SPN-scg in SHR and WKY rats

Table 1 shows the mean maximum and minimum diameters of 4 groups of SPN-scg in SHR and WKY rats. ILp neurons of SHR were significantly smaller than those of WKY rats in both their maximum and minimum diameters ($P < 0.01$). For ILf neurons, only in their mean minimum diameter were the neurons in SH rats significantly smaller than those of WKY rats. In IC and ICpe, there was no significant difference in the diameter of the neurons in the SHR and WKY rats. Only 1% of the sympathetic preganglionic neurons in rats occurred either in the n. intercalatus spinalis (IC) and n. intercalatus pars paraependymalis (ICpe), i.e. out of a total of 1600 sympathetic preganglionic neurons examined, only 16 neurons were found to be present in each of IC and ICpe. It is possible that the small population of these two groups of neurons may have accounted for the lack of

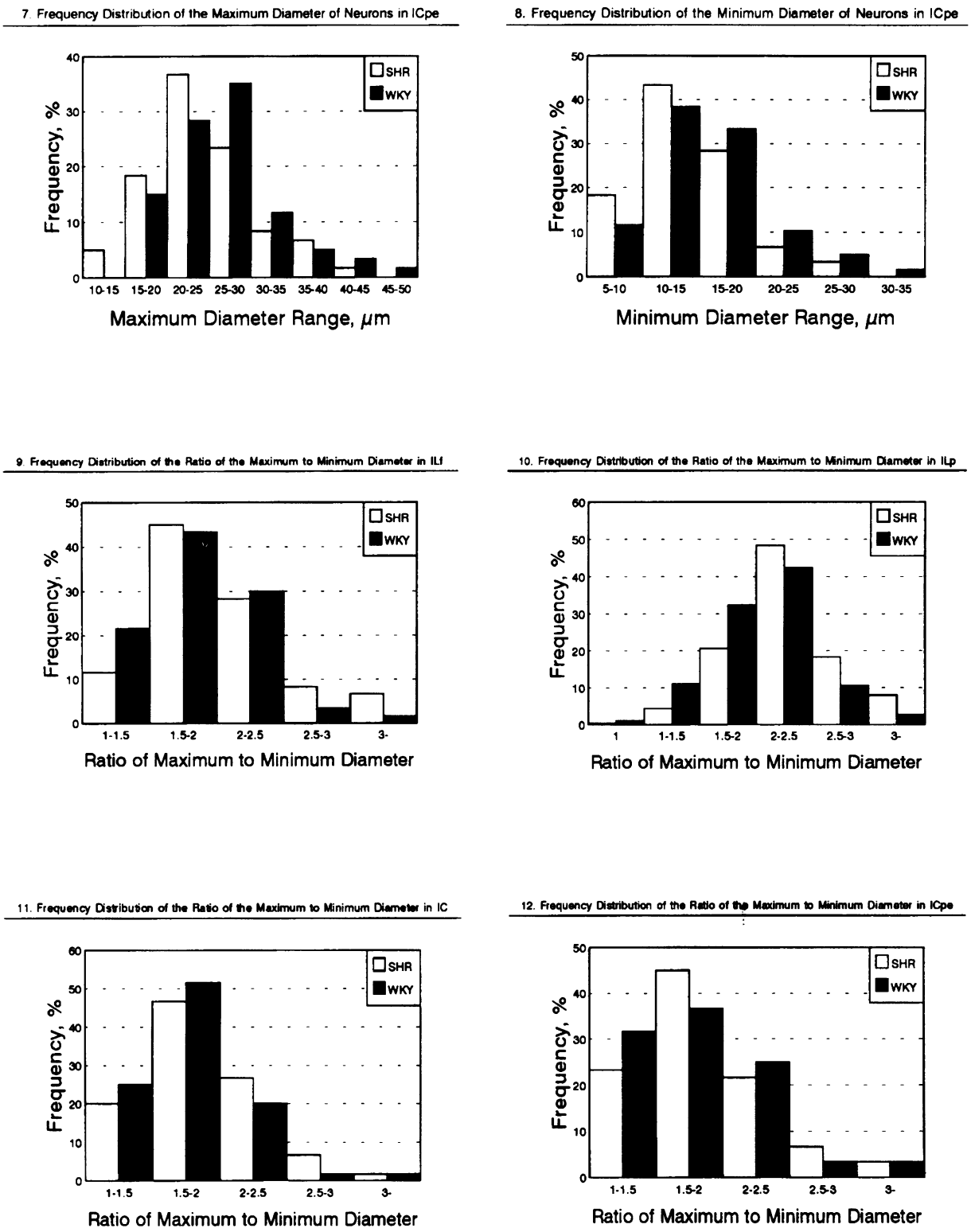
Table 1. *Mean maximum (Max) and mean minimum (Min) diameter of the 4 groups of SPN-scg in SHR and WKY rats*

SPN-scg		SHR Mean ± S.D. (μm)	WKY Mean ± S.D. (μm)	t test
Ilf	Max	28.84 ± 7.73	31.06 ± 6.41	$P > 0.05$
	Min	14.27 ± 3.49	17.16 ± 3.69	$P < 0.01$
ILp	Max	25.90 ± 5.38	29.84 ± 7.96	$P < 0.01$
	Min	12.16 ± 3.05	15.52 ± 4.52	$P < 0.01$
IC	Max	27.30 ± 6.64	27.00 ± 5.54	$P > 0.05$
	Min	14.93 ± 4.51	16.51 ± 3.82	$P > 0.05$
ICpe	Max	24.69 ± 7.08	26.17 ± 6.03	$P > 0.05$
	Min	13.61 ± 3.21	15.62 ± 4.27	$P > 0.05$



Figs 1–8. Histograms showing the frequency distribution of the maximum diameters and minimum diameters of 4 groups of SPN-scg in SHR and WKY rats.

significance in statistical analysis. The histograms in Figures 1–8 show the frequency distributions of the maximum and minimum diameters in the 4 groups of SPN-scg. The frequency distributions of all groups were continuous and unimodal. The modal maximum diameter varied between 20 μm and 35 μm and the modal minimum diameter varied between 10 μm and 20 μm in all the 4 groups of SPN-scg. Figure 3 and



Figs 9–12. Histograms showing the frequency distribution of the ratio of the maximum diameter to minimum diameter of 4 groups of SPN-scg in SHR and WKY rats.

Figures 2 and 4 show that with the increase in the maximum and minimum diameters respectively, the ratios of the distribution frequencies of SHR to WKY in the same diameter group decrease, that is, in SHR, neurons with longer diameter are reduced. Figure 9 shows that from the ratio groups of 2–2.5 to 3−, the total distribution frequency is 43.3% in SHR and 35% in WKY rats. Figure 10 shows that from the

Table 2. *Mean ratios of the maximum diameter to the minimum diameter of the 4 groups of SPN-scg in SHR and WKY rats*

	SHR	WKY	
SPN-scg	Mean ± S.D.	Mean ± S.D.	t test
ILf	2.07 ± 0.52	1.81 ± 0.35	$P < 0.01$
ILp	2.17 ± 0.45	1.94 ± 0.43	$P < 0.01$
IC	1.80 ± 0.39	1.69 ± 0.36	$P > 0.05$
ICpe	1.84 ± 0.43	1.75 ± 0.44	$P > 0.05$

Ratios of 1.0–1.3 round; 1.3–2.0 oval; > 2.0 fusiform.

ratio groups of 2–2.5 to 3−, the total distribution frequency is 70.6% in SHR and 55.6% in WKY rats. This means more neurons are fusiform in SHR than in WKY rats.

Shape of the SPN-scg in SHR and WKY rats

Table 2 shows the mean ration of maximum diameter to minimum diameter of 4 groups of SPN-scg in SHR and WKY rats. It shows that neurons in ILf and ILp tended to be fusiform in SHR but oval in WKY rats. In IC and ICpe, the neurons in both SHR and WKY rats were mostly oval.

Cell counts

The total number of labelled neurons in SHR was 1610 ± 88.79 and that in WKY was 1605 ± 94.32. The 2 cell counts showed no significant difference ($P > 0.05$).

Orientation of SPN-scg in SHR and WKY rats

The general orientation of SPN-scg in both SHR and WKY rats appeared to be similar. In the ILf, most neurons were observed to be transversely or mediolaterally oriented, although some were longitudinally or obliquely oriented (Figs 13,14). In ILp, the neurons did not show any preferential orientation and their long axes were observed to be oriented longitudinally, transversely or obliquely (Figs 13, 14) in both SHR and WKY rats. In the IC, the long axes of neurons were observed to be transversely or obliquely oriented. A series of transverse cellular bands (Figs 17, 18) were also observed. Around the central canal, most of the cells in the ICpe were obliquely oriented; a few were transversely or longitudinally oriented (Figs 15, 16).

Dendritic distribution patterns of SPN-scg in SHR and WKY rats

Figures 19 and 20 show the dendritic distribution patterns of SPN-scg in WKY rats. Besides the traditional longitudinally directed dendritic bundles, there were also mediolaterally oriented dendrites. Medially oriented grey matter dendrites showed a ladder-like configuration and formed subependymal plexuses around the central canal (Fig. 19). Laterally oriented white-matter dendrites formed white-matter dendritic plexuses (Fig. 20).

In SHR, the dendritic distribution patterns showed some differences (Fig. 21). First, the ladder-like configuration of the medially oriented grey-matter dendrites was less obvious, the 'rungs' of the 'ladder' in this instance forming a dome-like or triangular configuration. Secondly, the number of mediolaterally oriented dendrites was reduced. Thirdly, the white matter dendritic plexuses and subependymal plexuses were diminished.

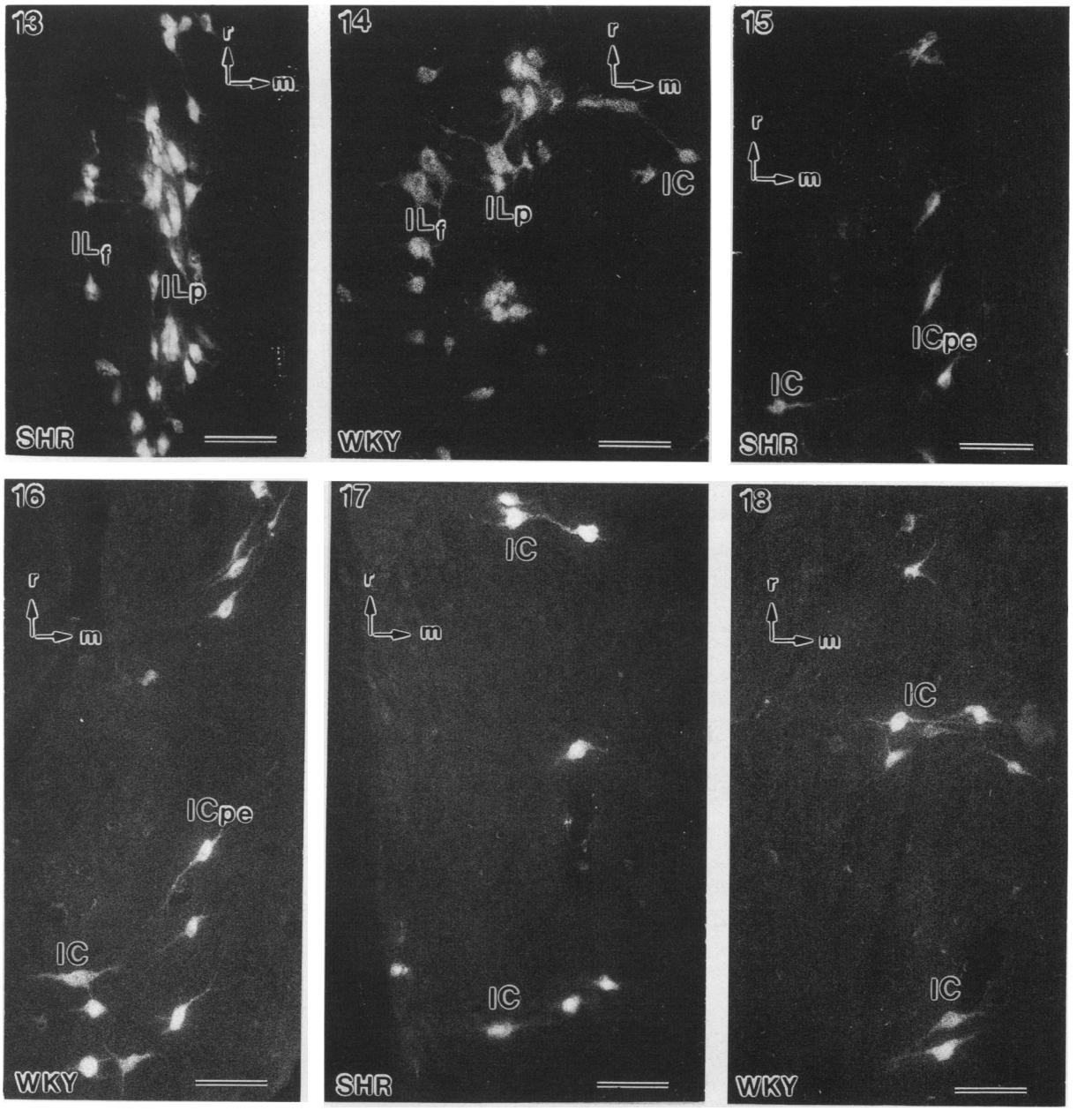
In all the rats injected with tracer, no labelling was found in neurons in the ventral horns or in the intermediolateral column contralateral to the injected SCG.

Immunohistochemical study

Figures 22, 23 and 24 show the distribution patterns of SP positive fibres in T2 segment of the spinal cord of SHR. SP fibres or terminals were observed to run longitudinally and mediolaterally and in close association with (1) longitudinally oriented dendrites (Figs 22, 23), (2) clusters of sympathetic preganglionic neurons (Figs 22, 23), and (3) medially oriented dendrites (Figs 22, 23). The medially oriented fibres and terminals gathered together to form a dome-like configuration, similar to those observed in the CB-HRP experiments.

A great number of SP fibres were observed adjacent to the central canal (Fig. 23, CC). We were able to confirm after counterstaining with neutral red that some SP immunoreactive nerve fibres also ran inside the central canal (Fig. 24).

Figure 25 shows the distribution patterns of SP terminals or fibres in T2 segment of the spinal cord of a WKY rat. Most of the observations made in WKY rats were similar to those in SHR. The only difference was that in WKY rats the medially oriented dendrites showed a ladder-like configuration similar to those observed in the CB-HRP retrograde tracing study (see above). SP immunoreactive cell bodies of sympathetic



Figs 13–14. Horizontal sections at T2 showing Fluorogold labelled neurons of the ILf and ILp in SHR and WKY rats respectively. Bars, 100 μm in all Figures from 13–25; r, rostral; m, medial.

Figs 15–16. Horizontal sections at T1 showing Fluorogold-labelled neurons of the ICpe in SHR and WKY rats, respectively. Bars, 100 μm.

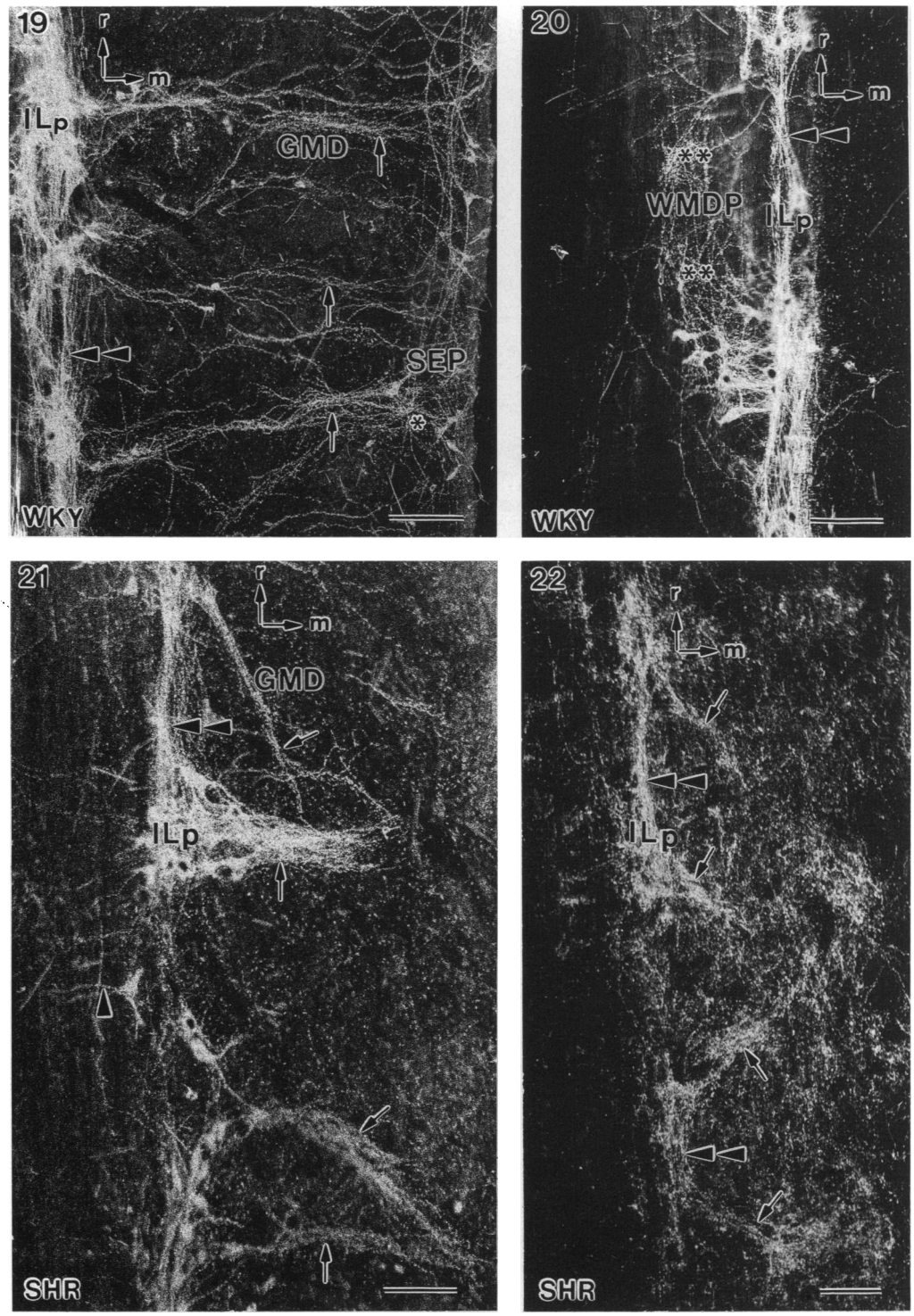
Figs 17–18. Horizontal sections at T2 showing Fluorogold-labelled neurons of the IC in SHR and WKY rats respectively. Bars, 100 μm.

preganglionic neurons were also observed, but the reaction products were barely detectable.

In all cases, the control sections failed to show any SP positive end-product.

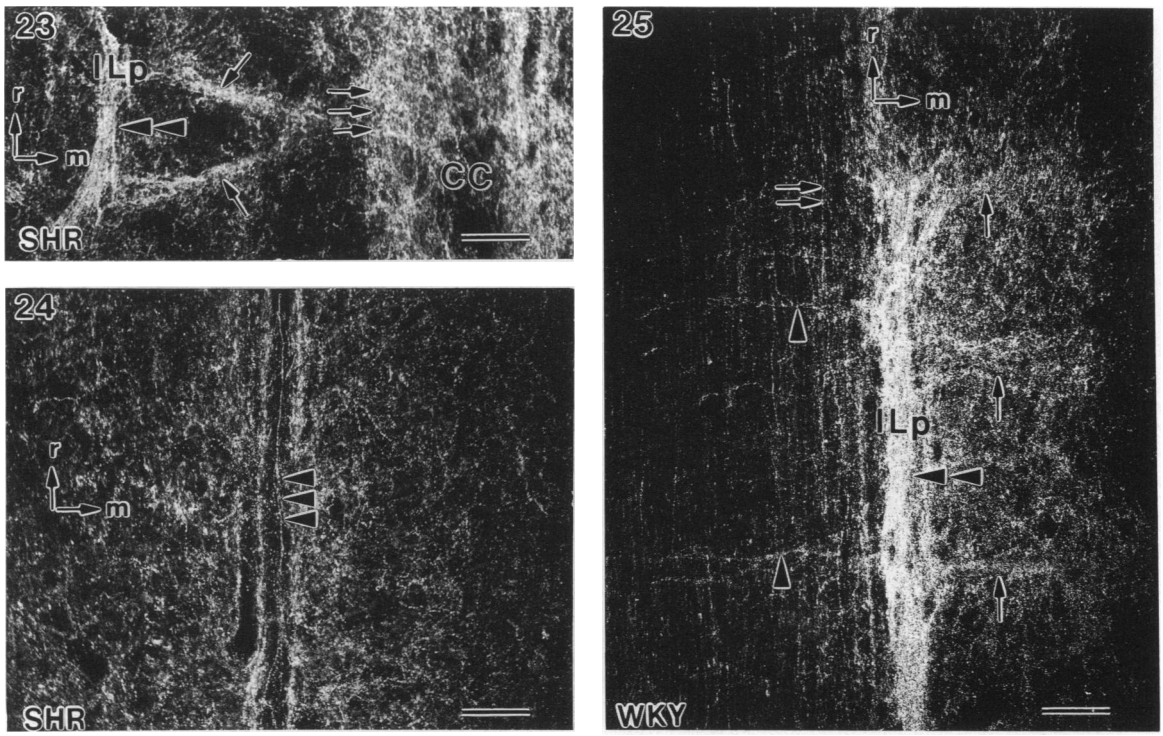
DISCUSSION

Quantitative studies of particular groups of sympathetic preganglionic neurons (SPN) (Petras & Cummings, 1972; Chung et al. 1975; Rando et al. 1981; Barber et al. 1984) are few and cursory. The results of these studies also differ from each other considerably. In the rhesus monkey, Petras & Cummings (1972) reported that the fusiform sympathetic preganglionic neurons in the IC group measured from 26 × 12 μm to 43 × 13 μm. In the cat, Oldfield & McLachlan (1981) found that cells in the IC group of sympathetic preganglionic neurons often exceeded 60 μm in their long diameter while the short diameter was usually between 15 and 25 μm. In rat, Barber et al. (1984) reported that the medium-sized neurons of the IC group of sympathetic preganglionic



Figs 19–22. For legend see opposite.

neurons measured 15 × 21 μm. These differences may be related to the methods used to measure the neurons. The present study has shown that in normal WKY rats, the neurons tended to be largest in ILf and smallest in ICpe (Table 1). This was not emphasised in the study of Barber et al. (1984). The results of the present study suggest that there are 2 possible circuits which regulate the activities of SPN-scg in WKY rats. One is a core circuit, consisting of cell bodies and longitudinal dendritic bundles of neurons of the ILp. The other is a paralateral circuit consisting of the white-matter dendrites, grey-matter dendrites, white-matter dendritic plexuses and subependymal plexuses. But in SHR, several differences in the dendritic



Figs 19–20. Horizontal sections at T2 showing CB-HRP labelled SPN-scg in WKY rats. GMD, grey-matter dendrites (single arrows); WMDP, white-matter dendritic plexuses (double asterisks); SEP, subependymal plexuses (single asterisk); longitudinal dendritic bundle (double arrowheads). Bars, 100 μm.

Fig. 21. Horizontal sections at T2 showing CB-HRP labelled SPN-scg in SHR. GMD, grey-matter dendrites (single arrows); white-matter dendrites (single arrowhead); longitudinal dendritic bundle (double arrowheads). Bars, 100 μm.

Figs 22–23. Horizontal sections at T2 showing the distribution patterns of SP positive fibres around sympathetic preganglionic neurons in SHR. Medially oriented SP fibres (single arrows) associated with medially oriented dendrites; these SP fibres emerge to form a triangular or dome-like configuration. Longitudinally oriented SP fibres (double arrowheads) are observed to associate with longitudinally oriented dendrites or sympathetic preganglionic neurons. SP fibres are also distributed around the central canal (CC) (triple arrows). Bars, 100 μm.

Fig. 24. Horizontal section at T2 showing SP fibres in the central canal of the spinal cord of SHR (triple arrowheads). Bar, 100 μm.

Fig. 25. Horizontal section at T2 showing SP fibres associated with sympathetic preganglionic neurons of WKY rats. Also shown are ladder-like medially oriented SP fibres (single arrows), longitudinally oriented SP fibres (double arrowheads), laterally oriented SP fibres (single arrowheads), and some descending SP fibres (double arrows). Bar, 100 μm.

distribution patterns were noted, the significance of such in relation to the regulation of blood pressure in SHR and WKY rats remains to be investigated.

Sympathetic preganglionic neurons are the final common pathway from central cardiovascular control centres to peripheral targets. Since they receive afferent inputs directly or indirectly from supraspinal regions (Loizou, 1969; Saper et al. 1976; Amendt et al. 1979; Swanson & McKellar, 1979; Saper & Loewy, 1980), any change in these centres may cause changes in sympathetic preganglionic neurons and vice versa. The shape of the dendritic tree is an expression of the receptive field of a neuron (Peters et al. 1991); hence a cell with widespread dendrites would be able to receive information from fibres of diverse origins and function. The observation of a reduction in the dendritic distribution patterns of sympathetic preganglionic neurons in the ILf and ILp of SHR in the present study suggests that these neurons had a more restricted receptive field than those of normal (i.e. WKY) rats.

Jänig & McLachlan (1986) proposed that sympathetic preganglionic neurons were composed of functionally different groups of neurons, the vasoconstrictor neurons being located laterally in the ILf and ILp, whereas neurons projecting to the viscera were located more medially and spread towards the midline. In the present study, the morphological differences between hypertensive and normotensive rats were observed only in neurons in the ILf and ILp. This suggests that these neurons might be related to the regulation of blood pressure.

Substance P is one of the putative neurotransmitters around sympathetic preganglionic neurons (Helke et al. 1982; Holets & Elde, 1982; Krukoff et al. 1985*a*; Oldfield et al. 1985). It is possible that some of these

SP fibres may originate from sensory neurons in the dorsal root ganglion, local spinal interneurons, from supraspinal regions (Holets & Elde, 1982; Oldfield et al. 1985) or sympathetic preganglionic neurons (Krukoff et al. 1985*b*). There is, however, evidence that the majority of SP fibres in the intermediolateral column arise from supraspinal sources (Helke et al. 1982; Holets & Elde, 1982). Our study suggests that some SP fibres may also arise from sympathetic preganglionic neurons. First, the SP fibres are arranged longitudinally and mediolaterally in the same manner as those of sympathetic preganglionic neurons labelled after injection of CB-HRP into the scg. Secondly, if these SP fibres arise from supraspinal regions, one would expect to find many longitudinally-oriented descending SP fibres, but only a few longitudinally oriented SP fibres have been observed. Gilbey et al. (1983) have shown that when SP was applied iontophoretically, it could cause excitation of identified sympathetic preganglionic neurons of rats. Coexistence of SP with thyrotropin-releasing hormone (TRH) in fibres and terminals apposing identified sympathetic preganglionic neurons (Appel et al. 1987) and coexistence of SP with both TRH and serotonin (5-HT) in medullary neurons projecting to the spinal cord have also been demonstrated (Johansson et al. 1981). Furthermore SP has also been shown to cause a concentration-dependent inhibition of TRH receptor binding in the spinal cord (Sharif & Burt, 1983). All these studies suggest that the mechanism or role of SP on sympathetic preganglionic neurons may be complex. Very interestingly, our study showed the presence of SP fibres in the central canal of the spinal cord in SHR. Such fibres around and in the central canal may possibly serve a neurosecretory function (Barber et al. 1979). Cerebrospinal fluid (CSF)-contacting neurons of the central canal have been reported in spinal cord of the ray, opossum, mouse, guinea pig and other species (Vigh et al. 1983). Cytochemical studies have revealed that some CSF-contacting neurons contained monoamines, some were acetylcholinesterase-positive (Vigh et al. 1983), some contained serotonin and other gamma aminobutyric acid (GABA) (Vigh & Vigh-Teichmann, 1992). Vasoactive intestinal polypeptide (VIP) neurons contacting the cerebrospinal fluid have also been reported in monkey and cat spinal cord (LaMotte, 1987). In these species the dendrites of these neurons penetrated the ependyma to enter the central canal. The results of our study suggest that SP-positive CSF-contacting neurons might also be present in the spinal cord of hypertensive rats.

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