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Gap junctions between perineuronal satellite cells increase in number with age in rabbit spinal ganglia

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SUMMARY - The gap junctions between perineuronal satellite cells were studied in the spinal ganglia of 12, 42, and 79-month-old rabbits. The mean number of gap junctions per 100 μm² of surface of the section occupied by satellite cells was significantly greater in old rabbits than young adults. Since the mean length of individual gap junctions did not change with age, the increase in number of gap junctions cannot be due to fragmentation of pre-existing gap junctions but is very likely due to the formation of new gap junctions. The increase in number of gap junctions cannot be related to an increase in number of perineuronal satellite cells since the mean number of these cells is significandy smaller in aged rabbits than in young adults. It is suggested that the increase in number of gap junctions with age may enhance the suggested neuroprotective role of satellite cells towards ganglionic neurons. The present findings, together with previous observations, suggest that the gap junctions between perineuronal satellite cells are dynamic structures, able to adapt to varying neuronal demands and varying environmental conditions.

Key Words: *dorsal root ganglia - peripheral neuroglia - intercellular junctions - ageing - sensory ganglia - Oryctolagus cuniculus*

INTRODUCTION

In the spinal ganglia of adult animals, gap junctions occur between the satellite cells within a single perineuronal sheath (in thin sections: Pannese, 1974; Lieberman, 1976; in freeze-fracture preparations: Pannese *et al.*, 1978; Pannese, 1981). Gap junctions between perineuronal satellite cells also occur in mammalian sympathetic ganglia (Elfvin and Forsman, 1978).

In recent years a number of age-related structural changes in the perineuronal satellite cells of spinal ganglia have been reported (Ledda *et al.*, 1999, 2003; Martinelli *et al.*, 2003). However, as far as we are aware, age-related changes of the gap junctions of these cells have not been studied. In view of the important role played by satellite cells in neuronal support (for reviews, see Pannese, 1981, 1994) and the fact that gap junctions are actively involved in the diffusion of substances and propagation of signals between cells (*e.g.*, see Sheridan and Atkinson, 1985; Dermietzel *et al.*, 1990; Wolburg and Rohlmann, 1995; Bruzzone *et al.*, 1996; Bruzzone and Ressot, 1997) we decided to investigate changes in satellite cell gap junctions with age.

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MATERIALS AND METHODS

Rabbits (*Oryctolagus cuniculus*) aged 12 months (three animals, 3.4-3.5 kg body weight), 42 months (three animals, 3.6-3.8 kg body weight), and 79 months (three animals, 4.0-4.2 kg body weight) were used. The rabbits were treated according to the European Community Council Directive (86/609/EEC) for the care and use of laboratory animals. The dates of birth of these animals were documented; all had been raised by a specialist rabbit breeder with particular attention to hygiene and regular veterinary inspections and had been fed an unrestricted diet. Because the life span of the normal healthy *Oryctolagus* is 60-72 months (Harkness and Wagner, 1983) or 84-96 months (Weisbroth *et al.*, 1974) the 12-month-old rabbits we studied were young adults, the 42-month-old rabbits were intermediate-aged animals, and the 79-month-old rabbits were aged animals. Furthermore, the end of fertility is usually considered to mark the onset of senescence and female rabbits are not normally fertile after 60 months, so that the 79-month-old animals are to be considered aged also from this point of view.

The animals were perfused transcardially with a solution containing 2% formaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) under deep anaesthesia with Nembutal i.p. (80 mg/kg). After fixation for about 3 h, the thoracic spinal ganglia were removed, washed in cacodylate buffer (0.2 M, pH 7.3) for 2 h and then post-fixed on ice for 1.5 h in 2% OsO_4, buffered with 0.1 M sodium cacodylate. The specimens were washed in distilled water, stained with 2% aqueous uranyl acetate, dehydrated in alcohol and embedded in Epon-Araldite resin. Several semithin sections were prepared from each ganglion and stained with 0.5% toluidine blue in 1% sodium borate. They were then examined in the light microscope to check the quality of fixation. Only the best preserved ganglia were used and neither the nerve cell bodies nor the perineuronal satellite cell sheaths in these ganglia showed signs of swelling or shrinkage. Overall, 72 ganglia (8 for each animal) were used for this study.

Isotropic uniform random (IUR) sections were obtained following the orientator procedure (Mattfeldt *et al.*, 1990). For each ganglion, a single IUR thin section (about 0.15 × 0.10 mm) was photographed under the electron microscope. Each section was photographed in its entirety at a magnification of ×8,000 and the negatives printed to a final magnification of ×32,000. A montage of 60-70 prints was necessary to reconstruct each section. The sectional area of the satellite cell sheath enveloping each nerve cell body was measured with the aid of a digitising tablet connected to a computer. Successively, the following were determined in each ganglion: 1) the mean number of gap junctions per 100 μm^2 of surface of the section occupied by satellite cells, and 2) the mean length of individual gap junctions.

The values obtained for the three rabbits in each age group were compared by one-way ANOVA to establish whether they differed significantly. Subsequently, the values obtained for each age group were compared. The latter statistical comparison employed the two-tailed Student's *t*-test (differences with $p < 0.01$ were considered significant) and for each value the 99% confidence limits were calculated. All data analyses were carried out using a statistical graphics program (Statgraphics software STSC).

TABLE I

Mean number and mean length of gap junctions between satellite cells in young adult, intermediate-aged, and old rabbits

Rabbit	Age (months)	Number of ganglia examined	Total surface of the section occupied by satellite cells (μm^2)	Total number of gap junctions	Mean number (± SE) of gap junctions per 100 μm^2 of surface of the section occupied by satellite cells	Mean length (± SE) of individual gap junctions (μm)
Values for each rabbit						
1	12	8	11045.31	20	0.143 ± 0.036	0.420 ± 0.116
2	12	8	9976.78	19	0.174 ± 0.039	0.452 ± 0.135
3	12	8	10515.82	21	0.156 ± 0.038	0.449 ± 0.135
4	42	8	10234.49	20	0.256 ± 0.048	0.514 ± 0.127
5	42	8	10447.92	23	0.227 ± 0.044	0.444 ± 0.108
6	42	8	9864.25	21	0.203 ± 0.043	0.524 ± 0.148
7	79	8	9412.37	37	0.311 ± 0.053	0.413 ± 0.116
8	79	8	9236.53	36	0.329 ± 0.073	0.482 ± 0.159
9	79	8	8657.32	33	0.321 ± 0.075	0.500 ± 0.164
Values for each age group						
Young adult		24	31537.91	60	0.158 ± 0.022[a]	0.445 ± 0.075[d]
Intermediate-aged		24	30546.66	64	0.224 ± 0.027[b]	0.491 ± 0.076[e]
Old		24	27306.22	106	0.320 ± 0.038[c]	0.460 ± 0.082[f]

There are no significant differences between [a] and [b] and between [b] and [c], whereas the difference between [a] and [c] is significant ($p < 0.01$).
There are no significant differences between [d] and [e], between [e] and [f], and between [d] and [f] ($p < 0.01$).

RESULTS

In all three age groups each nerve cell body was usually enveloped by a single satellite cell sheath, which was in turn surrounded by connective tissue (Fig. 1). Gap junctions were usually present between satellite cells making up a single perineuronal sheath (Fig. 2). Their structure conformed to literature descriptions of these junctions. The junctional area was sometimes curved, sometimes smoothly undulating, and sometimes straight. No correlation between the linearity of the junctional area and the age of the animals was found. In all ages, most gap junctions occurred singly; only in a few cases were they arranged in junctional complexes together with adhering junctions. Sometimes mitochondria were associated with gap junctions. The frequency of this association did not change with age. It was not possible to determine whether this association was due to chance or had some functional significance.

The mean number of gap junctions per 100 μm^2 of surface of the section occupied by perineuronal satellite cells increased with age (Table 1). The mean number of gap junctions present in old rabbits differed significantly ($p < 0.01$) from the number present in young adult animals. By contrast, the mean length of individual gap junctions did not differ in the three age groups (Table 1). Gap junctions between the nerve cell body and its attendant satellite cells were never observed.

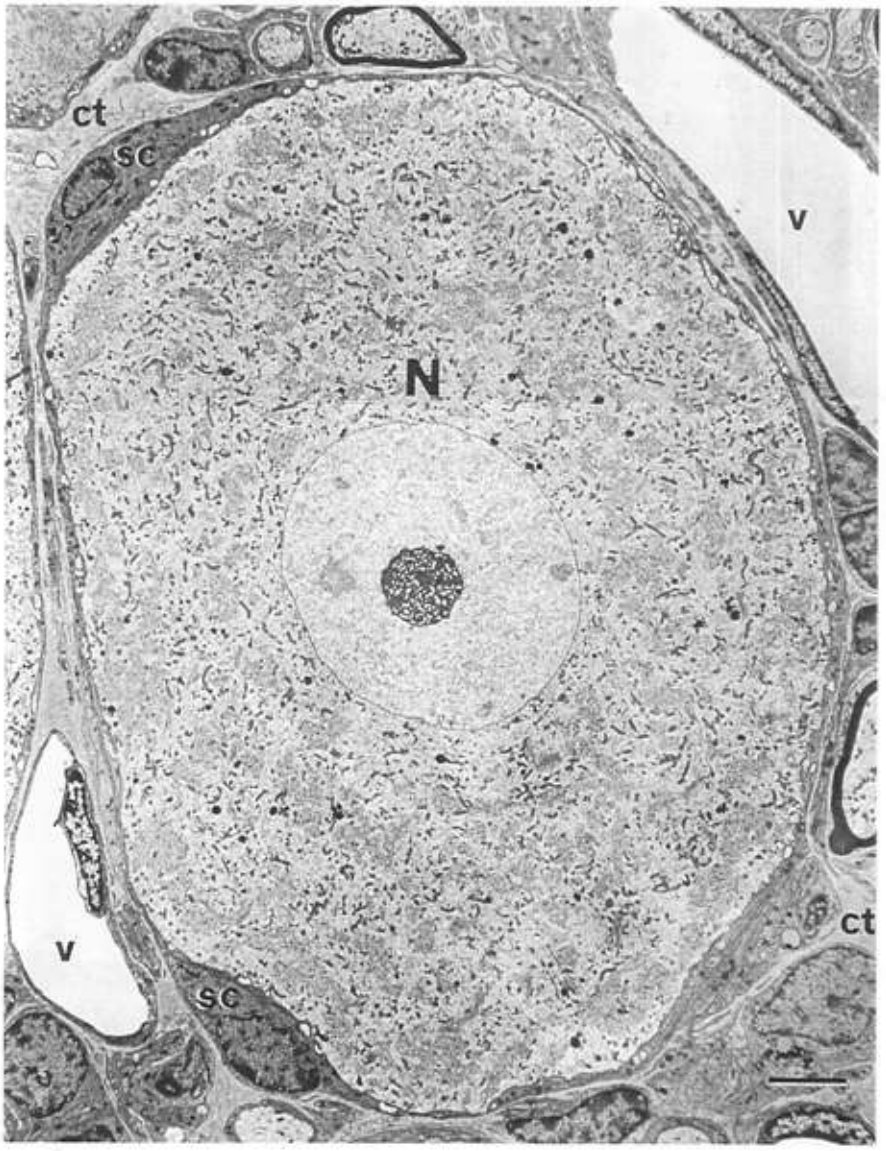

FIGURE 1 Nerve cell body (N) of a sensory neuron enveloped by its satellite cell sheath (sc). The latter is surrounded by connective tissue (ct). v: blood vessel. Electron micrograph. Spinal ganglion from a 79-month-old rabbit. Bar = 2 μm.

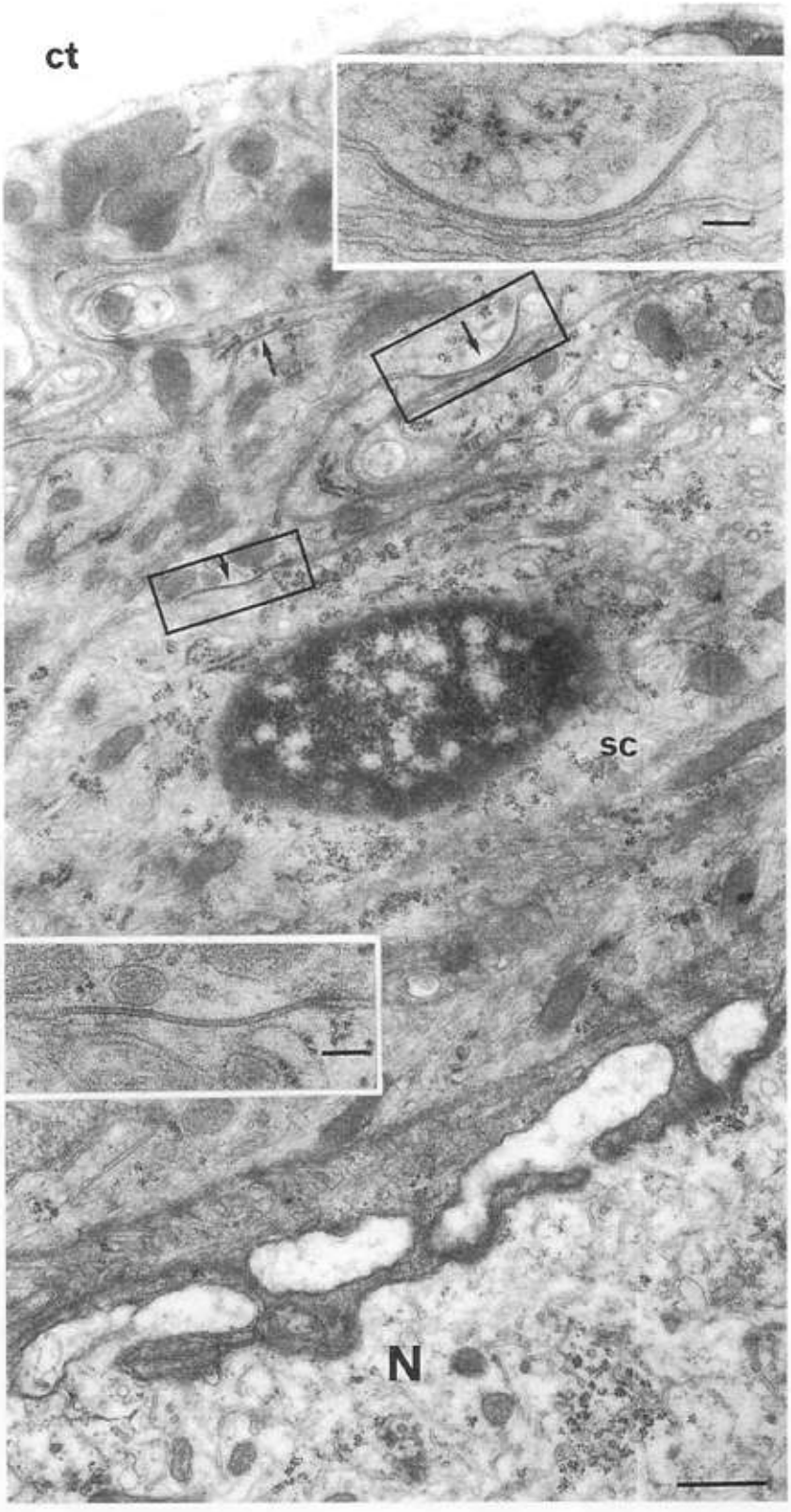

FIGURE 2 Portion of a satellite cell sheath (sc) enveloping a nerve cell body (N). Arrows indicate gap junctions between satellite cells. The insets show adjacent sections of the outlined areas at greater enlargement. ct: connective tissue. Electron micrographs. Spinal ganglion from a 79-month-old rabbit. Bar = 0.5 μm; insets: bars = 0.1 μm.

DISCUSSION

In the present study we have found that the mean number of gap junctions between perineuronal satellite cells was significantly greater in old rabbits than young adults. Since the mean length of individual gap junctions did not change with age, this increase in gap junction number with age cannot be due to fragmentation of pre-existing gap junctions but is very likely due to the formation of new gap junctions. The formation of new gap junctions cannot be due to an increase in the number of perineuronal satellite cells since the mean number of these cells has been found to be significantly lower in aged rabbits than in young adults (Pannese *et al.*, 1997). The present study provides, we believe, the first demonstration that new gap junctions form in the perineuronal satellite cell sheaths of rabbit spinal ganglia with advancing age.

To our knowledge, no other studies have been published concerning relationships between ageing and number of gap junctions securely identified using the electron microscope, in the nervous system. It is not possible, therefore, to compare our findings in spinal ganglia with the situation in other regions of the nervous system. We note, however, the findings of Cotrina *et al.* (2001). These authors showed that in the mouse hippocampus the permeability of astrocyte gap junctions did not differ significantly between old and young animals and that, although there was an overall tendency to decreased coupling with age, functional coupling between astrocytes remained high in old mice.

The following roles have been proposed for perineuronal satellite cell sheaths in sensory ganglia (for reviews, see Pannese, 1981, 1994): 1) control of the traffic of materials to and from the ganglionic neuron, 2) control of ion concentrations and of levels of neuroactive amino acids in the microenvironment of each ganglionic neuron, and 3) 'trophic' support of the neuron. The increase in number of gap junctions with age may be related to an enhancement of one or more of these functions.

However, it is also possible to advance a different hypothesis. There is evidence that astrocytes play a neuroprotective role. For example, cultured neurons are less vulnerable to various neurotoxic treatments if they are associated with astrocytes (Mattson and Rychlik, 1990). Astrocytic gap junctions probably participate in this neuroprotective function, as shown by the finding that following exposure to oxidative insult, the blockade of gap junctional communication in astrocytes results in a markedly enhanced generation of intraneuronal peroxides and an increase in neuronal death (Blanc *et al.*, 1998). It is possible that satellite cells exert a similar neuroprotective function. If this is the case, the increase in the number of gap junctions between satellite cells with age could be interpreted as a mechanism to more effectively protect old neurons from oxidative injury or other insults.

It has been reported recently that the number of gap junctions between perineuronal satellite cells increases significantly following axon injury of spinal ganglion neurons (Hanani *et al.*, 2002; Pannese *et al.*, 2003). The present study shows that the number of these junctions significantly increases in senescence. Considered together, these findings suggest that the gap junctions between perineuronal satellite cells are dynamic structures, able to adapt to varying neuronal demands and varying environmental conditions.

Gap junctions are composed of a family of proteins called connexins. Numerous studies have identified the connexins expressed by the various cell types within the central nervous system (for reviews, see Dermietzel and Spray, 1993; Wolburg and Rohlmann, 1995; Bruzzone *et al.*, 1996; Giaume and McCarthy, 1996; Spray *et al.*, 1999; Nagy and Rash, 2000). However, we know very little about the connexins expressed by satellite cells in sensory ganglia. The only study we are aware of in this area reported that connexin43 is present in the satellite cells enveloping large neurons in the rat petrosal ganglion, while immunostaining for this connexin was absent from the satellite cells enveloping the other neurons of the ganglion (Chen *et al.*, 2002). Further investigations are necessary to determine what types of connexin are present within perineuronal satellite cell sheaths of spinal ganglia and whether their quantities change in parallel with the age-related increase in the number of gap junctions demonstrated in the present study.

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