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The permeability of capillaries among the small granule-containing cells in rat superior cervical ganglia: an ultrastructural lanthanum tracer study

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Summary. The permeability of blood capillaries associated with small granule-containing (SGC) cells in rat superior cervical ganglia was investigated at ultrastructural level by employing ionic lanthanum as an electron dense tracer. In rat superior cervical ganglia, the majority of blood capillaries were nonfenestrated. Both fenestrated and nonfenestrated capillaries were observed in the area associated with SGC cells. Lanthanum tracer was observed in the luminal surface, the interendothelial cleft and the subendothelial perivascular spaces of both fenestrated and nonfenestrated capillaries associated with SGC cells. The external lamina of the Schwann cell which surrounded the neurons, nerve fibres and SGC cells were clearly delineated by the lanthanum tracer. Furthermore, the perineuronal space, the periaxonal space, and the pericellular space of the SGC cells were readily accessible to the lanthanum ion. The results demonstrated an absence of blood-nerve barrier, blood-ganglionic and blood-SGC cell barrier to the lanthanum ion in the parenchymal area of the SGC cells in rat superior cervical ganglia. It is proposed that lanthanum may pass through the endothelial cells via 1) the fenestrae of fenestrated capillaries, 2) the intercellular junctions of both fenestrated and nonfenestrated capillaries, i.e., a paracellular pathway; and 3) the process of endocytosis/exocytosis, i.e., a transcellular pathway, to reach the subendothelial space and be distributed in the parenchyma of SGC cells in rat superior cervical ganglia.

Key words: Lanthanum Tracer, Capillaries, Permeability, Small granule-containing (SGC) cells, Rat superior cervical ganglia

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Introduction

Ultrastructural observations have revealed two types of capillaries: continuous and fenestrated, in the rat superior cervical ganglia. The majority of capillaries within rat superior cervical ganglia were of a continuous type, equipped with tight junctions and mainly located among nerve fibres and principal ganglionic neurons. Fenestrated capillaries are primarily adjoined to SGC cells (Siegrist et al., 1968; Matthews and Raisman, 1969; Lu et al., 1976; Matthews, 1989). SGC cells may act as a neuroendocrine element and release their catecholamine-containing granules via exocytosis into microcirculation (Benítez et al., 1974; Heym and Williams, 1979; Matthews, 1989) and thus the permeability of the capillaries among SGC cells may play an important role in the neuroendocrine function within the ganglia (Lu et al., 1976; Hsiao and Lu, 1982; Matthews, 1989).

It is generally accepted that fenestrated capillaries are recognized as permeable vessels and can be penetrated by some tracers such as horseradish peroxidase (HRP), ferritin, etc. (Jacobs, 1971; Shimamura and Morrison, 1973). However, Pino and Essnar (1980) and Chen et al. (1989) reported that some fenestrated capillaries are non-permeable in rat choriocapillaries and hamster pineal complex by HRP. Therefore, the permeability of capillaries determined by the presence of fenestrae is inadequate. Moreover, Costran and Karnovsky (1967) reported that a dose of HRP greater than 0.2 mg/g body weight was capable of producing pronounced vascular leakage in several species, the effect being most pronounced in the rat. In contrast, lanthanum ion has been suggested as an ideal tracer to detect the permeability of the capillaries because of its smaller size and nontoxicity (MacKenzie et al., 1984, 1987).

Since the permeability of capillaries associated with SGC cells in the sympathetic ganglia has not yet been determined, the ultrastructure and permeability of these

capillaries in the rat superior cervical ganglia will be examined. In order to avoid the toxicity of the HRP, the lanthanum ion was used as a tracer in the present study.

Materials and methods

Sixteen adult rats (Long-Evans strain) of both sexes, weighing between 200-300 gm body weight were used for the present study. All animals were divided into two groups, ten animals were used in lanthanum tracer experiments and the others were used for conventional electron microscopy and served as controls. All animals were kept 3-4/cage at room temperature (22° C) and 14/10 light/dark photoperiod, and food and water were supplied *ad libitum*. They were anaesthetized by an intraperitoneal injection of chloral hydrate (300 mg/kg b.w.) before sacrifice.

For lanthanum tracer studies, animals were perfused through the left ventricle with 15 ml of 1% sodium nitrite dissolved in 0.85% sodium chloride solution and immediately followed by 200-250 ml lanthanum solution for 15 minutes. After the perfusion of lanthanum

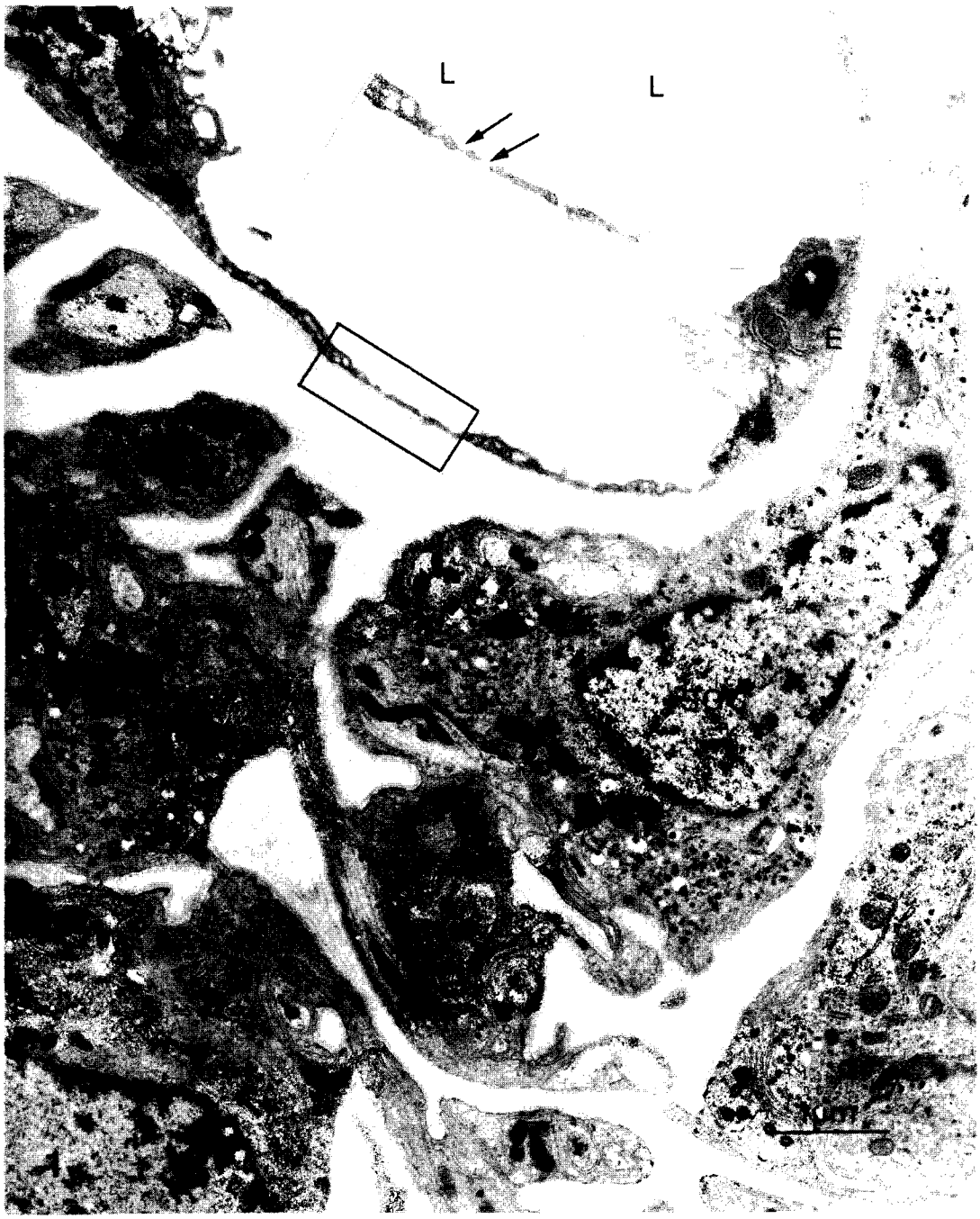


Fig. 1. Electron micrograph of a fenestrated capillary close to the SGC cells (SGC) in the rat superior cervical ganglion.
Inset: The fenestrae of the endothelial cell were provided with diaphragms (arrows).
E: endothelial cell;
L: lumen of the capillary.

solution, 200-250 ml 2% paraformaldehyde and 2% glutaraldehyde in sulfate balanced salt solution (pH 7.4) was administered for another 15 minutes (De Pace, 1984). For conventional electron microscopy, the animals were perfused with a fixative containing 2% glutaraldehyde and 2% paraformaldehyde in 0.067M cacodylate buffer (pH 7.4) for 15 minutes.

After perfusion, superior cervical ganglia of all animals were fixed *in situ* overnight to induce the fluorescence of the small intensely fluorescent (SIF) cells. Superior cervical ganglia were then dissected out and cut into 50 μm sections in agar with an Oxford vibratome. Areas containing SIF cells in thick sections were identified by fluorescence microscopy and dissected under a stereomicroscope. After several rinses in buffer, thick sections were postfixed in 1% osmium tetroxide, dehydrated through a graded series of alcohol, and then embedded in Epon-Araldite mixture. Thin sections were either unstained or doubly stained with uranyl acetate and lead citrate before being examined in a Hitachi-HU12A electron microscope at 100 Kv.

Results

Conventional electron microscopy revealed that the majority of blood capillaries associated with SGC cells were of a fenestrated type (Fig. 1). Fenestrae of the endothelial cell were equipped with a thin layer of diaphragm (Fig. 1 and inset). Occasionally, a few profiles of nonfenestrated capillaries were also observed near SGC cells and their processes.

After perfusion of lanthanum tracer, the electron-dense deposits of lanthanum sulfate were observed on the luminal surface of endothelial cells of all the blood vessels. In the regions of rat superior cervical ganglia, where the principal neurons and nerve fibres were highly populated, the tracer substance was found on the luminal surface but not in the extravascular space of the nonfenestrated capillaries (Fig. 2). In the SGC cell area, lanthanum was deposited in both luminal and abluminal sides of nonfenestrated (Fig. 3) and fenestrated (Fig. 4) capillaries as well as in the subendothelial tissue. Lanthanum tracer was observed

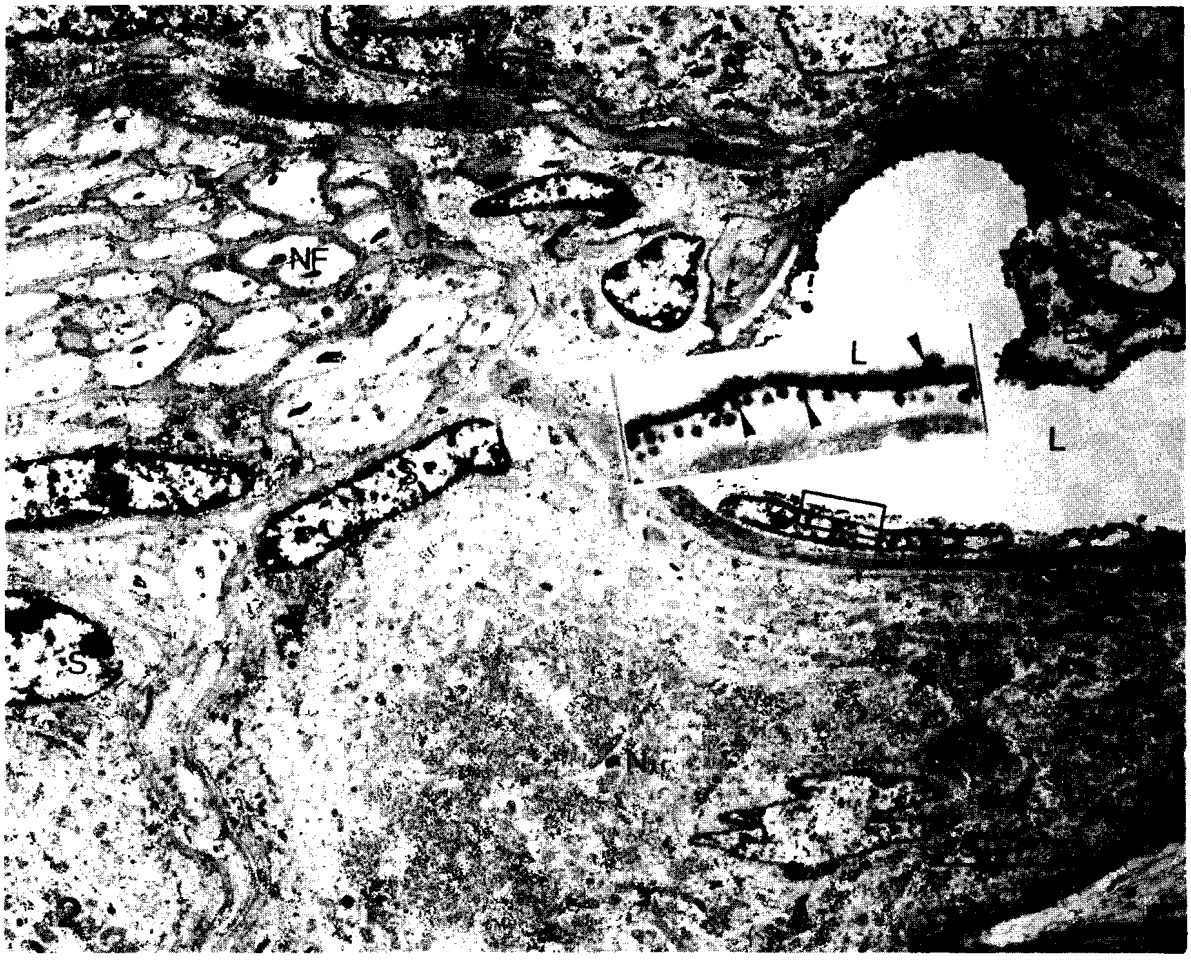


Fig. 2. A low power electron micrograph from a lanthanum-treated rat showing a nonfenestrated capillary in the parenchyma of the superior cervical ganglion devoid of SGC cells. Note the luminal surface is clearly depicted by the lanthanum tracer, but not in the subendothelial space. cf: connective tissue fibres; E: endothelial cell; L: lumen of capillary; N: postganglionic neurons; NF: nerve fibres; S: Schwann cells. Inset: An area of the endothelial cell similar to the square of Fig. 3 showing an abundance of lanthanum-filled small vesicles attached to the luminal membrane (arrowheads).

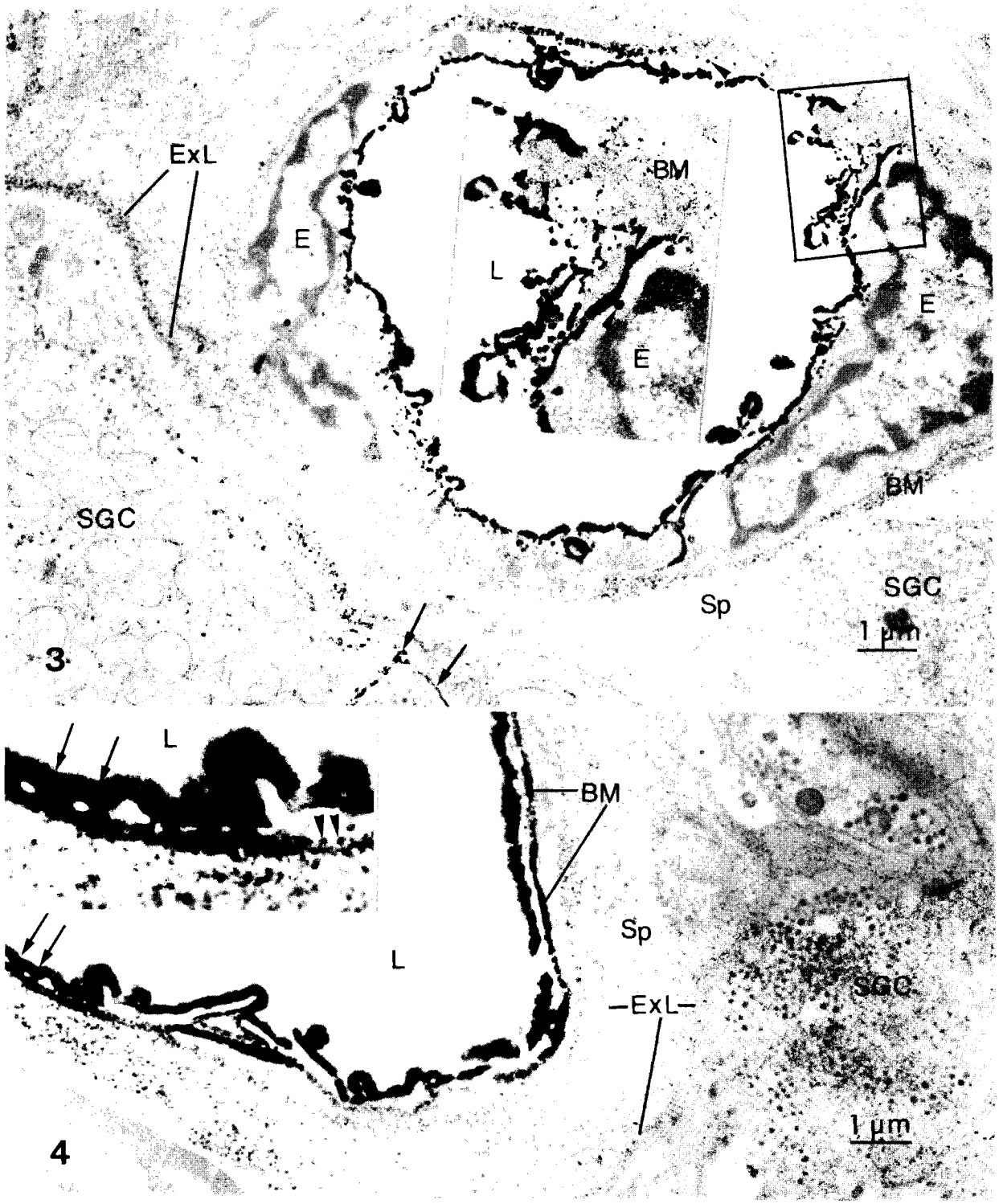
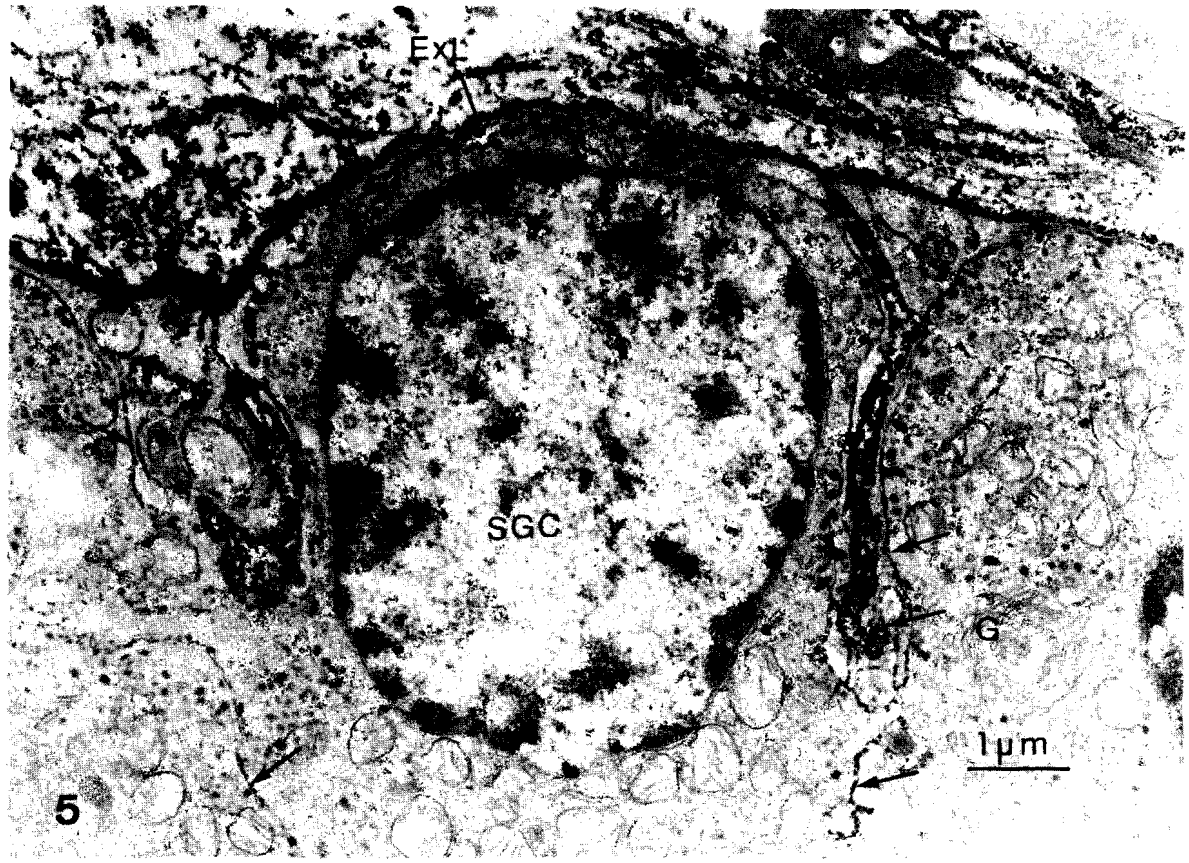


Fig. 3. A nonfenestrated capillary close to SGC cells (SGC) in lanthanum-treated rat superior cervical ganglion. Luminal surface of the endothelial cell is delineated by lanthanum tracer. Precipitates are also observed on the basement membrane (BM) of the endothelial cells (E) and the surrounding interstitial space (Sp). Lanthanum is also located on the external lamina (ExL) of Schwann cells which encircle the SGC cells. Arrows indicate the deposition of lanthanum in the elaborate intercellular space of the SGC cells. Inset: High power magnification of the square area showing that lanthanum fills almost the entire length of the junctional cleft. Arrowheads indicate the lanthanum-filled vesicles attached to the tracer substance in the intercellular space between two adjacent endothelial cells.

Fig. 4. Lanthanum ion extends across the fenestrated portion of endothelial cell and accumulates within the extracellular space. Inset: The possible location of intervening diaphragms (arrows) in the fenestrated diaphragm are obscured by dark tracer product. Arrowheads indicate the possible exocytotic vesicles in the endothelial cell. BM: basement membrane; ExL: external lamina; SGC: SGC cells; Sp: interstitial space.



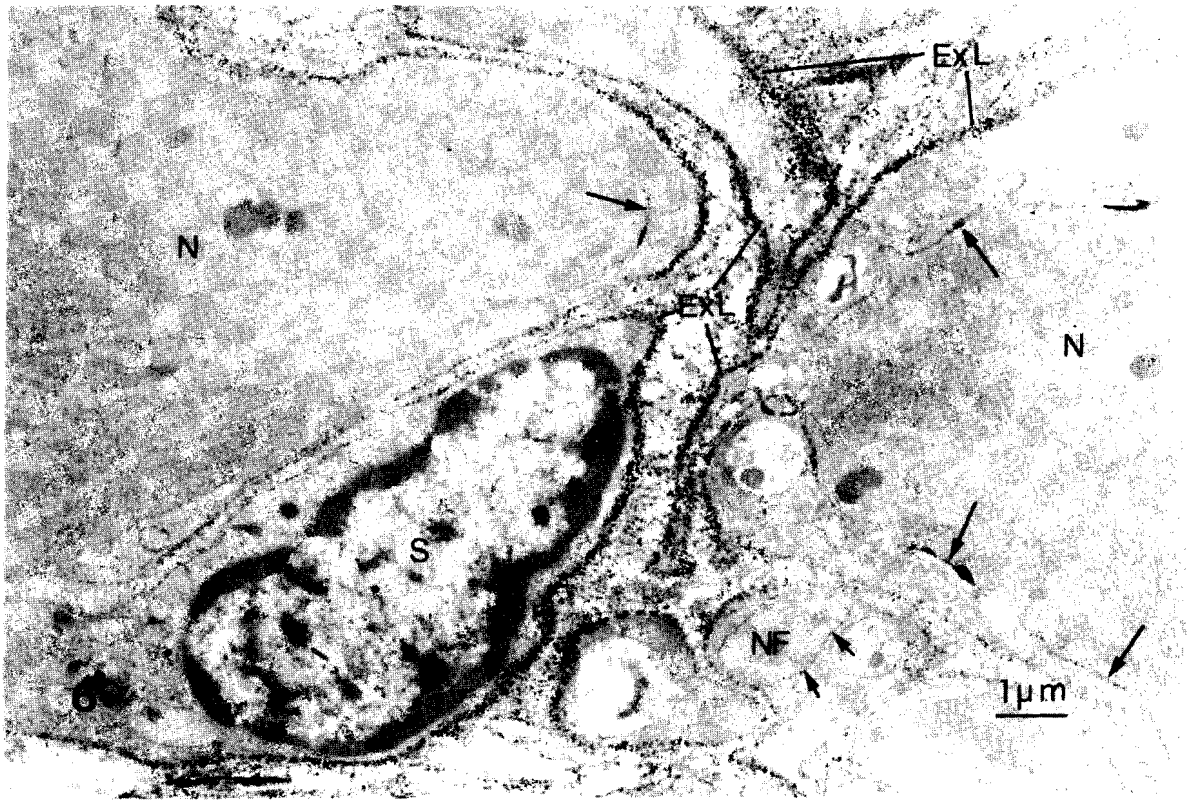


Fig. 5. Electron micrograph showing tracer deposits on the external lamina (ExL) of Schwann cells and their processes covering the SGC cells (SGC). The tracer invades into the pericellular space of SGC cells (arrows). G: Golgi complex of SGC cell.

Fig. 6. Electron micrograph demonstrating that heavy accumulation of lanthanum is deposited on the external lamina (ExL) of the Schwann cells (S). The tracer penetrates deeper into the perineuronal (long arrows) and periaxonal space (short arrows). N: postganglionic neurons; NF: nerve fibres.

to fill almost the entire length of the intercellular junction between the adjacent nonfenestrated endothelial cells and to penetrate into the subendothelial perivascular space (Fig. 3 and inset). In fenestrated capillaries, an accumulation of lanthanum sulfate precipitates appeared to extend across the fenestrae of the endothelium and thus the intervening diaphragm was obscured by the dark product, as shown in the inset of Fig. 4.

In both fenestrated and nonfenestrated capillaries, many intra-endothelial vesicles containing tracer product were present at the luminal and abluminal sides of the endothelium. Some tracer-filled and omega-shaped vesicles facing the interendothelial cleft, the luminal and abluminal surfaces, suggesting an uptake from the luminal side and a release into extracellular space of the tracer products via endocytosis/exocytosis, were observed (Insets of Figs. 2-4).

Lanthanum tracer deposited in the perivascular space of the subendothelial tissue frequently penetrated further into the adjacent parenchyma of SGC cells (Figs. 3-5). Heavy accumulation of lanthanum ions was frequently encountered on the external lamina of Schwann cells which surrounded the nerve fibres, neurons and SGC cell clusters (Figs. 5, 6). Intricate intercellular spaces of contiguous SGC cells were depicted by the lanthanum ions (Fig. 5). The external surface of neurons and axons (i.e., perineuronal and periaxonal spaces) was also delineated by the tracer products (Fig. 6).

Discussion

Two types of capillaries exist in the rat superior cervical ganglion: the fenestrated and nonfenestrated type. The endothelial cell of blood vessels and capillaries among postganglionic neurons and nerve fibres is nonfenestrated. Capillaries associated with the SGC cells are mainly fenestrated (Matthews and Raisman, 1969; Lu et al., 1976; Hsiao and Lu, 1982; Matthews, 1989). In the present study, we confirm the presence of these two types of capillaries and the preferential distribution of the fenestrated capillaries in the SGC cell region of rat superior cervical ganglia.

Lanthanum was chosen as a tracer because of its exquisite permeability, small size (atomic size = 0.114 nm) and non-toxic property to detect the inter-endothelial junctions of blood vessels. Machen et al. (1972) and Whittembury and Rawlins (1971) reported that lanthanum passes through «leaky» or «macular» tight junctions but that it is restrained by «continuous» or «tight» tight junctions. Used as an intravascular perfusate, lanthanum would therefore be restricted to the luminal side of endothelial cells joined by continuous tight junctions but could penetrate the walls of other types of vessel (Shaklai and Tavassoli, 1982; MacKenzie et al., 1984; Bishop, 1985). Bouldin and Krigman (1975) demonstrated that the blood vessels in cerebral cortex possess a hematic barrier and restrict the passage of the lanthanum ions. DePace (1984) reported that the majority of the capillaries in sympathetic ganglia have continuous, nonfenestrated endothelial cells with tight junctions that prevent the escape of the lanthanum ion from intravascular lumen to intercellular space. The results that lanthanum precipitated in the luminal side of the nonfenestrated capillaries in the area devoid of SGC cells indicate the presence of a blood-ganglion barrier and the continuous tight junctions in these capillaries of rat superior cervical ganglia. The permeability of ganglionic vessels has been investigated by Jacobs (1977) and Arvidson (1979) using protein tracers of varying molecular size. The permeability of ganglionic vessels to HRP and ferritin lead these investigators to suggest that ganglionic vessels do not offer the same protection to principal neurons as blood brain barrier does for neurons in the central nervous system. However, DePace (1981, 1982) described a blood ganglion barrier in the superior ganglion of the rat by intravenous injection of HRP. Using the lanthanum tracer, DePace (1984) demonstrated that rat sympathetic ganglia possess anatomic features that provide a hematic barrier, i.e., a blood ganglionic barrier. Our results confirm the existence of a blood-ganglion barrier in the rat superior cervical ganglion.

The present results show that the lanthanum tracer precipitated not only in the lumina of the blood capillaries but also in the extravascular space and the extracellular spaces between the SGC parenchymal cells of rat superior cervical ganglia. The information obtained from these results may be summarized and interpreted as follows. The results that lanthanum deposited on both luminal side and abluminal side (i.e., basal lamina and perivascular space of blood capillaries) of the fenestrated capillaries associated with SGC cells suggest that these fenestrated capillaries were readily penetrated by the lanthanum ion. We speculate that the tracer, in a large part, may enter the subendothelial space via fenestrae of fenestrated endothelia. Moreover, the results that some of lanthanum-loaded small vesicles in the cytoplasm of endothelial cell attached to the endothelial membranes (luminal, abluminal, and junctional) imply that a transendothelial transportation of the lanthanum tracer through the endocytosis/exocytosis is another possible route for the tracer to penetrate into the fenestrated and nonfenestrated capillaries. In addition, a paracellular pathway through the interendothelial junction is also responsible because the interendothelial clefts were filled with lanthanum tracer. Therefore, we suggest that an exchange of substance between the SGC cells and their associated microcirculatin can occur in the regions of SGC cells in the rat superior cervical ganglion and there are three possible routes responsible for the penetration of lanthanum in the SGC cell area: 1) through fenestrae, 2) by a transcellular pathway via the process of endocytosis/exocytosis and 3) via a paracellular interendothelial extravasation.

In a study on the ultrastructure and permeability of the interendothelial junction of cardiac capillaries in the rat, Ward et al. (1988) reported that after tilting the specimen the zonular region of the two adjacent endothelial cells which appeared to fuse to each other

under conventional electron microscopy turned out to have a gap of mean 5.4 nm between the adjacent membranes. They further concluded that these narrow regions sieve macromolecules based on the size of the molecule, although other factors may determine permeability properties. By employing the freeze-fracture techniques, Connell and Mercer (1974) and Castel et al. (1974) recognized that the tight junction can be divided into «very tight» and «leaky» tight junction according to the number of the junctional ridges in the zonular region. Fig. 3 reveals an apparently fused junctional region of the adjacent nonfenestrated endothelial cells near the SGC cell area, through which the lanthanum ions penetrated, we do not know yet whether this is because of the leaky tight junction or the presence of a narrow «gap» between the nonfenestrated capillaries which has not been revealed by the conventional electron microscopy as mentioned by Ward et al. (1988). However, because the intercellular junction of the nonfenestrated capillaries is not completely filled with the lanthanum tracer, the possibility that interendothelial junctions of nonfenestrated capillaries are not leaky and that the lanthanum can only pass through the fenestrated ones cannot be ruled out. Further studies by freeze-fracture or tilting the specimen are needed to clarify this problem.

In the present study we have demonstrated the precipitation of lanthanum ions on the external lamina of Schwann cells, in the perineuronal and periaxonal spaces and the pericellular space of SGC cells. The lanthanum precipitation on these structures may be derived from the ions that penetrated through the capillaries (both fenestrated and non-fenestrated types) and then were diffussed through the intercellular and perivascular space to reach the principal neurons, nerve fibres and SGC cells. Therefore, we suggest that, in the SGC cell-associated area of rat superior cervical ganglia, there is no barrier between these structures and blood vessels (i.e., blood-neuron barrier, blood-nerve barrier and blood-SGC cell barrier).

Finally, we would like to report that the longer the specimen was left in the lanthanum solution, the deeper the lanthanum penetrated into the tissue. This may indicate that lanthanum tracer could reach the subendothelial connective tissue by simple diffusion because of the concentration gradient. That other factors, such as pH and temperature, may facilitate or inhibit the penetration of tracer substance cannot be excluded.

In conclusion, we emphasize that: 1) fenestrated or nonfenestrated capillaries associated with SGC cells were permeable to lanthanum; 2) lanthanum tracer could pass through the fenestrae, trans-endothelial pathway via pinocytotic vesicles and the interendothelial junctions and reach the subendothelial connective tissue; and 3) blood-SGC cell barrier, blood-neuron barrier and blood-nerve barrier were lacking in the region of the SGC cells in the rat superior cervical ganglion.

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