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Macrophages in Pacinian Corpuscles

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Key Words. Pacinian corpuscle · Outer bulb lamellae · Endoneurial space · Macrophage · Horseradish peroxidase

Abstract. The presence of macrophages in the outer bulb region of mouse, monkey and human Pacinian corpuscles was demonstrated by light and electron microscopy. In the normal, nontreated, Pacinian corpuscles, a few particular cells were located in the spaces between lamellae of the outer bulb. These cells contained numerous vesicles and vacuoles, and various cytoplasmic processes. When horseradish peroxidase (HRP) was injected locally or systemically, many HRP-positive cells, which were considered to be similar to the particular cells described above, were found in the outer bulb region of the corpuscles. Electron microscopy revealed that these cells contained HRP in vesicles and vacuoles, suggesting that they were macrophages vigorously taking up exogenous HRP. Macrophages in the Pacinian corpuscles are considered to work as scavengers to keep the inner environment of the corpuscles clear and constant with regard to its macromolecular content.

Introduction

Pacinian corpuscles are found in the subcutaneous regions of the volar side of mammalian fingers. Morphologically as well as physiologically they are well-defined sensory corpuscles [*Pease and Quilliam,* 1957; *Hunt,* 1961; *Nishi* et al., 1969; *Loewenstein,* 1971; *Munger,* 1971; *Spencer* et al., 1973; *Ilinsky* et al., 1976; *Ide and Saito,* 1980], consisting of the inner and outer bulbs. The axon terminal is in the center of the inner bulb. The outer bulb is a continuation of the perineurial sheath [*Shanta and Bourne,* 1968] and consists of multilayered lamellae. There are wide spaces between lamellae of the outer bulb, in which no structural substance is found except for some fibrillary material near the lamellae. No other type of cell has been reported thus far in the Pacinian corpuscle.

During the study on the mechanism of horseradish peroxidase (HRP) uptake by the axon terminal of the Pacinian corpuscle, it was found that there were some peculiar cells vigorously taking up HRP, located in the outer bulb. When normal, nontreated Pacinian corpuscles were examined by light and electron microscopy, it became obvious that these peculiar cells contained numerous vesicles and vacuoles, being attached with long cytoplasmic processes to the outer bulb lamellae.

The present study was carried out to examine these particular cells, both in nontreated and in HRP-treated Pacinian corpuscles. It was demonstrated that they were macrophages migrating in the spaces between the outer bulb lamellae. The possible significance of macrophages in the Pacinian corpuscle is discussed.

Materials and Methods

Pacinian corpuscles of human, monkey and mouse fingers were used in the present study.

Human Pacinian Corpuscles

Human Pacinian corpuscles were obtained by biopsy from the volar subcutaneous tissue of a volunteer's fourth finger distal phalanx. The tissue flaps were immediately fixed in modified Karnovsky's fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.4). After fixation for 3 h, the tissue flaps containing Pacinian corpuscles were selected and processed for conventional electron microscopic observation. Serial Epon sections were cut and observed by light microscopy after toluidine blue staining. In the course of such serial sectioning, some ultra-thin sections were cut at the appropriate levels for electron microscopic observation.

Monkey Pacinian Corpuscles

A rhesus monkey, which was to be sacrificed for other purposes in the laboratory, was used for the present experiment. The monkey was anesthetized by injecting Nembutal (sodium pentobarbital, 50 mg/kg body weight) and about 0.05 ml of a 20% HRP (Sigma, type II) saline solution was injected into the volar side of the distal phalanx of the left third finger. After 2.5 h, the monkey was fixed by perfusion through the heart with the fixative described above. The skin flap together with the subcutaneous tissue was excised, divided into small blocks and further fixed in the same fixative for 3 h at 4°C. The tissue blocks were stored overnight in 0.1 *M* cacodylate buffer containing 10% sucrose. About 40-µm thick frozen sections were cut from these tissue blocks on a freezing microtome. Sections were stored in 0.1 *M* Tris-HCl buffer (pH 7.4) containing 10% sucrose at 4°C.

Mouse Pacinian Corpuscles

About 30 mice were used for the experiments. Mice were anesthetized by intraperitoneal injection of Nembutal (30 mg/kg body weight). About 0.1 ml of a 20% HRP saline solution was injected through a tail vein. The animals were fixed by perfusion through the heart with the same fixative as above 50 s, 5 min, 1 h, 2 h, 6 h, 9 h and 18 h after the HRP injection. Pacinian corpuscles, which were clustered on the fibular periosteum as in the case of rat [*Zelená,* 1978], were identified with a dissecting microscope and excised together with the periosteum. These specimens were fixed in the same fixative for a further 5 h at 4°C, and washed overnight in 0.1 *M* cacodylate buffer (pH 7.4) containing 10% sucrose. Frozen sections about 40-µm thick were prepared and were stored in 0.1 *M* Tris-HCl buffer (pH 7.4) containing 10% sucrose at 4°C.

Incubation

The frozen sections of monkey and mouse origin kept in the Tris-HCl buffer as described above, were first immersed for 1 h in 3,3'-diaminobenzidine solution (5 mg/10 ml Tris-HCl buffer, pH 7.4) at 4°C. The sections were then transferred and stored in an incubation medium containing both 3,3'-diaminobenzidine at the same concentration as above and 0.015% H_2O_2 for 1.5 h with intermittent agitation at 4°C. After replacing the incubation medium with fresh medium, the incubation of sections was carried out with continuous agitation for 3 h at 37°C [*Graham and Karnovsky,* 1966]. During this incubation the medium was replaced twice. After incubation the sections were washed for 1 h in 0.1 *M* Tris-HCl buffer (pH 7.4) containing 10% sucrose. For light microscopic observation, several sections were picked up and mounted on glass slides with gelatin jelly. The remaining sections were processed for electron microscopic observation. They were post-fixed for 1 h in 1% osmium tetroxide solution at 4°C, washed briefly, dehydrated through a series of ethanol concentrations and embedded in Epon 812. Thin sections were prepared using an LKB Ultrotome and observed with a Hitachi H-700 electron microscope after staining with both uranyl acetate and lead citrate solutions.

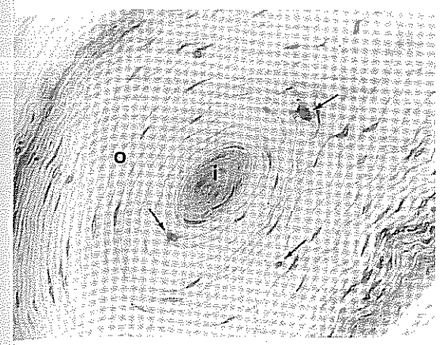

Fig. 1. A cross section of a Pacinian corpuscle obtained by biopsy from the volunteer's finger pad. A few peculiar cells (arrows), apparently different from lamellar cells, are situated in the spaces between lamellae of the outer bulb (o). i = Inner bulb. 1 µm thick Epon section. ×260.

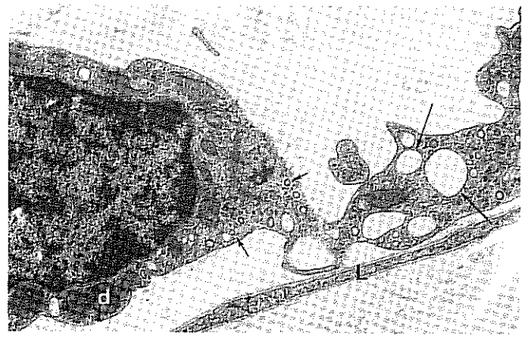

Fig. 2. An electron micrograph of the particular cell such as seen in figure 1. This cell extends cytoplasmic processes, and contains numerous vesicles (short arrows) and vacuoles (long arrows) as well as dense bodies (d). L = outer bulb lamella. ×9,800.

Results

Normal, Nontreated Corpuscles

Light microscopy of Epon sections stained with toluidine blue showed a few cell profiles attached to the lamellae of the outer bulb in human Pacinian corpuscles obtained from the finger pad (fig. 1). Electron microscopy showed that the cells were in close contact with the lamellar cell basement membrane and possessed long cytoplasmic processes (fig. 2). These cells contained numerous vesicles and vacuoles of various sizes including coated vesicles, and a well-developed Golgi apparatus. No basement membrane was seen on these cells. These characteristic features suggested that these

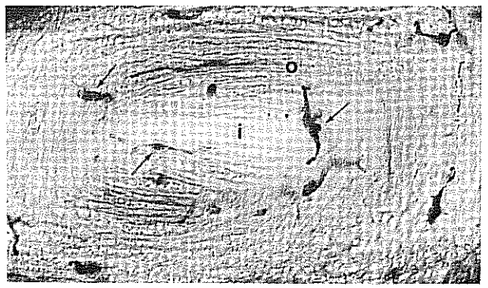

Fig. 3. A longitudinal section of a Pacinian corpuscle from the monkey finger pad, to which HRP was locally applied 2.5 h prior to fixation. Many HRP-positive cells (arrows), extending long cytoplasmic processes, are seen in the outer bulb region (o). I = inner bulb. ×260.

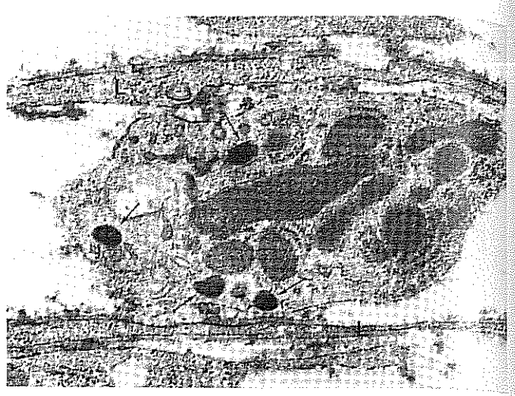

Fig. 5. A part of a mouse Pacinian corpuscle 50 s after the systemic injection of HRP. This particular cell located between outer bulb lamellae already takes up HRP into vesicles (arrows). L = outer bulb lamellae. ×23,400.

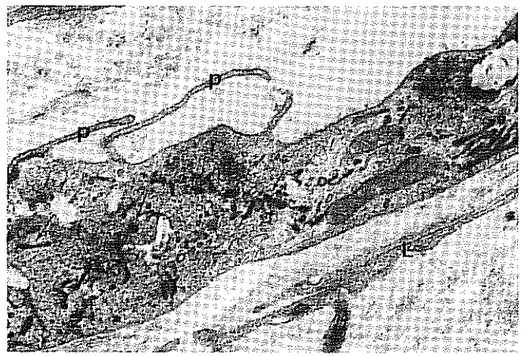

Fig. 4. An electron micrograph showing one of HRP-positive cells as seen in figure 3. The vesicles of various sizes and shapes contain HRP (arrows), indicating that this cell vigrously takes up the exogenous HRP. Long slender cytoplasmic processes (p) are extended from the cell. L = outer bulb lamellae. ×14,300.

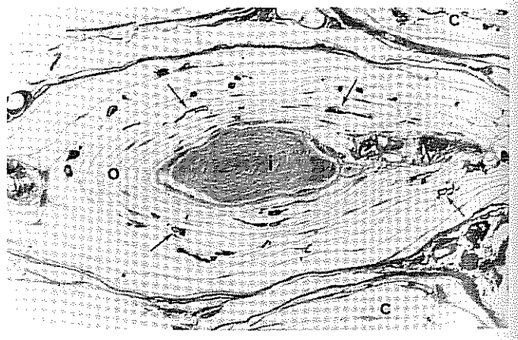

Fig. 6. An almost transversely-cut section of a mouse Pacinian corpuscle fixed 6 h after the systemic injection of HRP. Many HRP-positive cell bodies (arrows) are seen in the outer bulb. The cell bodies can be identified as such when the cell profiles contain nuclei which appear clear due to devoid of HRP-reaction. Parts of two other neighbouring Pacinian corpuscles (c) are seen. i = inner bulb; o = outer bulb. ×260.

cells might be macrophages migrating in the outer bulb compartment.

Local Application of HRP

When HRP was locally applied to the connective tissue around the Pacinian corpuscles in the finger of a monkey, several peculiar cells with long cytoplasmic processes appeared in the outer bulb space. These cells were darkly stained due to the uptake of HRP (fig. 3). By electron microscopy it was shown that these darkly-stained cells were similar in morphology to the cells described in the preceding paragraph, i.e. they were located in the outer bulb extending long cytoplasmic processes to the lamellae. They contained vesicles and vacuoles of various sizes. Almost all the vesicles and vacuoles contained HRP, indicating that these cells had the characteristics of vigorously taking up the HRP (fig. 4). No basement membrane was seen on these cells.

These findings indicated that these particular cells were macrophages.

Systemic Application of HRP

Cells vigorously taking up the HRP as described above and identified as macrophages were observed at successive time intervals following intravenous injection of HRP. Electron microscopy showed that as early as 50 s after injection the cells had taken up the HRP into vesicles of approximately 100 nm in diameter (fig. 5). From about 5 min after injection, the cells taking up the HRP became identifiable by light microscopy as faintly-stained dark cells in the outer bulb of corpuscles of thick frozen sections. Such HRP-positive cells gradually became darker and appeared spread over much wider regions of the outer bulb of the corpuscle. About 1 h after injection, electron microscopy showed that the cells were filled with larger vacuoles containing HRP. 6–18 h after the injection, the cells exhibited an intense HRP reaction, giving rise to the black cell-contour. The nuclei of such cells appeared clear as reaction-free regions in 1-μm thick Epon sections stained with toluidine blue (fig. 6). The number of the cells judged from the number of nuclei seemed to be much larger in the HRP-treated corpuscles than in normal, nontreated corpuscles, suggesting that macrophages increased in number in response to the HRP injection.

Discussion

The presence of macrophages within the outer bulb of corpuscles was demonstrated by performing the HRP-uptake experiment. A few macrophages have always been thought to exist within normal, nontreated corpuscles. Macrophages seemed to increase in number in response to exogenously-applied HRP. Capillaries are found in the outer bulb region [*Pallie* et al., 1970], the endothelial cells of which are partly fenestrated, suggesting that some macromolecules, e.g. HRP, might easily filtrate into the interior of the corpuscle [*Jacobs* et al., 1976]. The endoneurial space of the peripheral nerves is considered to be separated functionally from the surrounding connective tissues. The locally-applied HRP can not penetrate the perineurial sheath of the axon innervating the corpuscle. Cells forming the perineurial sheath have tight junctions segregating the inner endoneurial space from the outer connective tissue compartment [*Reale* et al., 1975; *Jacobs*, 1980: *Chi and Carlson*, 1981]. Macrophages have been demonstrated to be present in the endoneurial space in normal mouse sciatic nerve by HRP-treatment [*Arvidson*, 1977]. The outer bulb of the Pacinian corpuscles, which is the continuation of the perineurial sheath, might work as barrier like the perineurial sheath. In this sense the macrophages in the outer bulb of Pacinian corpuscle can be considered to have the same functions as those present in the endoneurial space.

It is interesting to compare Pacinian corpuscles with Meissner corpuscles in terms of whether they have barriers of perineurial sheath. Meissner corpuscles are not surrounded by the definite perineurial sheath nor by its derivatives, thus having no effective barrier between the corpuscle and the surrounding connective tissues [*Cauna and Ross*, 1960; *Ide*, 1976; *Castano and Ventura*, 1979]. In fact, exogenous HRP can easily gain access to the lamellar cells and axon terminals [*Chouchkov*, 1974; *Jirmanová and Zelená*, 1980]. No macrophage is observed in Meissner corpuscles.

The attention should be paid to the fact that the axon which enters the Pacinian corpuscle and the associated inner bulb lamellar cells are always stituated in the endoneurial compartment, never being exposed all the way along its course to the surrounding connective tissues. This fact shows that the interior of the Pacinian corpuscle is completely segregated from the outer connective tissues, indicating that the axon terminal and the associated lamellar cells of the inner bulb are situated in the highly conditioned environment guaranteed by the outer bulb. Macrophages are considered to move, in response to the invasion of foreign materials, into the corpuscle and work as scavengers to keep the inner environment of the corpuscle clear and constant with regard to its macromolecular content.

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Arterielle Gefässversorgung der Cauda pancreatis unter besonderer Berücksichtigung der cauda-corporealen Gefässbeziehungen

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Key Words. Cauda pancreatis · Blood supply · Cauda-corporeal relations

Abstract. The cauda pancreatis has a characteristic pattern of vascularization. Among the big arterial stems surrounding it, up to 4 arteries, 'caudal arteries', nourish the arterial system of the cauda. These stems originate especially in the Arteria gastroepiploica sinistra and in the lower main splenic branch of the Arteria lienalis. The vascular relations between corpus and cauda can be of different kinds. Five basic types of relations can be identified: type I: the cauda is supplied exclusively by caudal arteries; type II: at least one caudal artery anastomoses with the vessels of the corpus; type III: the cauda is supplied both by the caudal arteries and by vessels of the corpus (non-anastomosing); type IV: combined forms of blood supply by caudal arteries and corpus arteries by way of anastomoses and non-anastomosing vessels are found; type V: the cauda is supplied exclusively be vessels stemming from the corpus. In each of these five types, individual vessels supplying the cauda can assume the function of a terminal vessel.

Einleitung

Eine fast unüberschaubare Fülle von Arbeiten beschäftigen sich mit der Gefässversorgung des Pankreas, wobei der Caudagefässversorgung im allgemeinen weniger Beachtung geschenkt worden ist. Auf die Bedeutsamkeit der genauen Kenntnis der Caudaversorgung weisen klinische Arbeiten hin, die die Möglichkeit und Gefahr von postoperativen Caudanekrosen mit vaskulärer Komponente aufzeigen [*Baronofsky* et al., 1951; *Chérigiée* et al., 1962; *Bourde* et al., 1972].

In der Literatur finden sich sehr unterschiedliche Angaben über Anzahl und Ursprung der die Cauda versorgenden Arterien, zudem wird die Caudagefässversorgung zumeist in die Betrachtung des Corpusgefässsystems miteinbezogen. Die zweifellos vorliegenden Unterschiede der Gefässsysteme und deren dennoch bestehende vielfältige Verknüpfungsweisen werden dabei ausser acht gelassen.

Die vorliegende Untersuchung versucht zu einer Systematisierung dieser Aspekte beizutragen.

Material und Methode

An 63 Leichen beiderlei Geschlechts im Alter von 49–82 Jahren wurde das arterielle Gefässsystem der Cauda pancreatis im Korrosionsverfahren und in der Aufhellung untersucht. Zu diesem Zweck wurden der Zwölffingerdarm, die Bauchspeicheldrüse und die Milz, zusammen mit dem dahintergelegenen Aortenabschnitt im Block entnommen. Nach Ligatur der Arteria gastrica sinistra wurde eine Kanüle vom Aortenstumpf ausgehend in den Truncus coeliacus eingebunden und die Injektion vorgenommen: Für die Herstellung der Aufhellungspräparate wurde eine dünnflüssige Kleistermasse nach Spanner verwendet. Die anschliessende Aufhellung erfolgte nach Spalteholz in der Modifikation nach Drahn. Die Korrosionspräparate wurden mit dem Kunstharz Swebond hergestellt. Anhand der Aufhellungen wurde in der Lupenbetrachtung die gesamte Problemstellung bearbeitet. Die an Zahl stark zurücktretenden Korrosionspräparate dienten der Bestätigung der Ergebnisse im gröberen Bereich.

5 Präparate wurden wegen starker Füllungsdefekte ausgeschieden, so dass insgesamt 58 Präparate zur Auswertung gelangten.

Befunde

Im untersuchten Material zeigt sich, dass in den meisten Fällen das Gefässgebiet der Cauda von eigenen