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AUTONOMIC NERVES TERMINATING ON SMOOTH MUSCLE CELLS OF VESSELS IN THE PINEAL ORGAN OF VARIOUS MAMMALS

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The significance of autonomic nerves reaching the pineal organ was already investigated in connection to the innervation of pinealocytes and mediating light information from the retina for periodic melatonin secretion. In earlier works we found that some autonomic nerve fibers are not secretomotor but terminate on arteriolar smooth muscle cells in the pineal organ of the mink *(Mustela vison)*.

Studying in serial sections the pineal organ of the mink and 15 other mammalian species in the present work, we investigated whether similar axons of vasomotor-type are generally present in the wall of pineal vessels, further, whether they reach the organ via the conarian nerves or via periarterial plexuses.

In all species investigated, axons of perivasal nerve bundles were found to form terminal enlargements on the smooth muscle layer of pineal arterioles. The neuromuscular endings contain several synaptic and some granular vesicles. Axon terminals are also present around pineal veins. In serial sections, we found that the so-called conarian autonomic nerves reach the pineal organ alongside pineal veins draining into the great internal cerebral vein. Similar nerves present near arteries of the arachnoid enter the pineal meningeal capsule and septa by arterioles, both perivenous and periarterial nerves form terminals of vasomotor-type. The arteriomotor and venomotor regulation of the tone of the vessels of the pineal organ may serve the vascular support for circadian and circannual periodic changes in metabolic activity of the pineal tissue.

Keywords: Pineal organ – vasomotor nerves – conarian nerve – periarterial nerve plexus – fine structure – various mammals

INTRODUCTION

Autonomic nerves of the pineal organ were studied by several authors. A part of these nerve fibers originate from the superior cervical ganglion and reach the organ via the so-called conarian nerves [2, 3, 5, 7, 9, 10, 20, 24, 28]. In mammals, the role of peripheral afferentation was predominantly investigated in connection to the pineal melatonin-secretion and the mediation of environmental light periodicity to the pineal organ from the retina [1, 7, 8, 10, 12, 19]. Synaptic contacts were described between sympathetic axon terminals and pinealocytes in the rat [6] and monkey [11].

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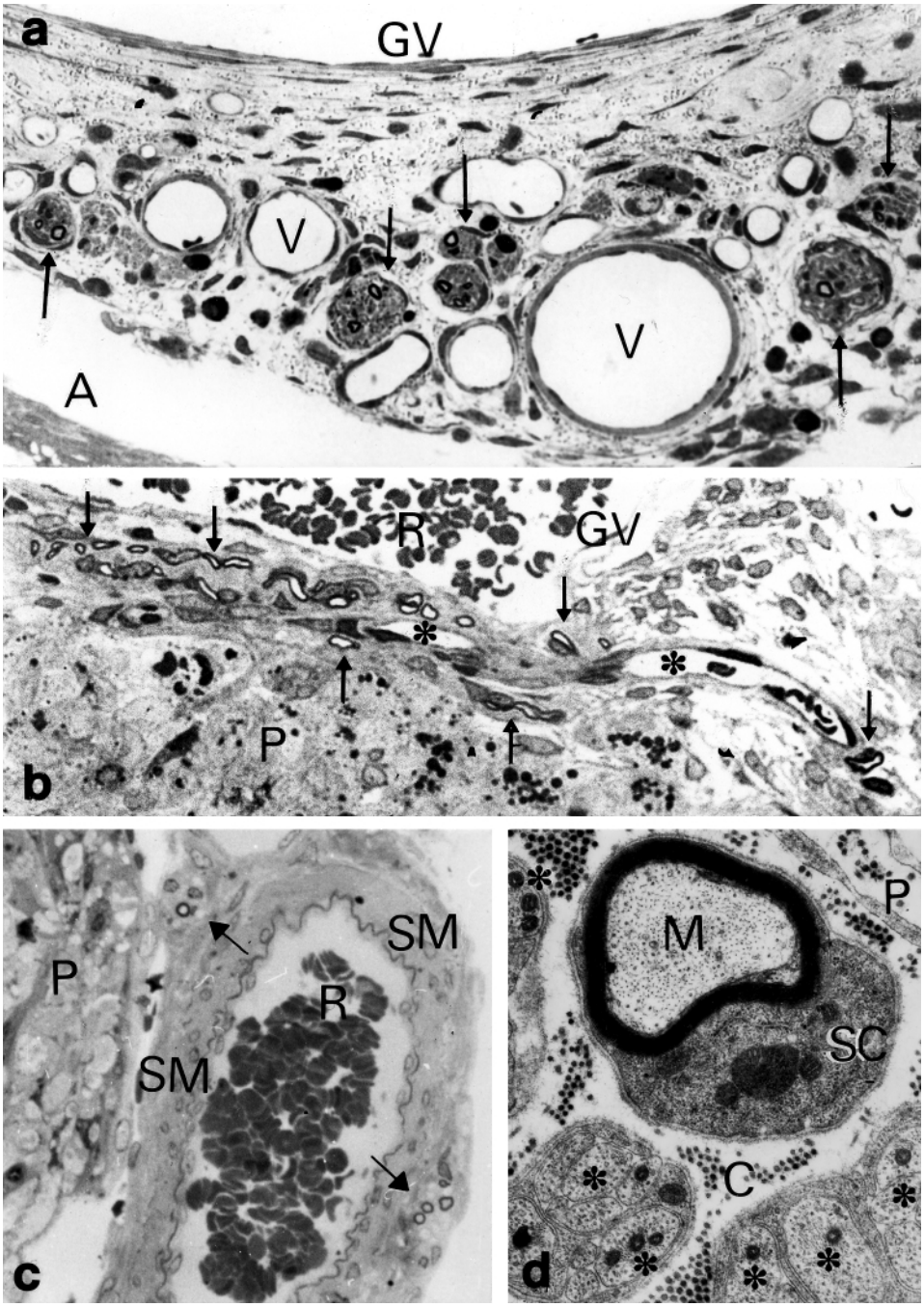
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There are no detailed fine-structural investigations on the relation of autonomic nerves to smooth muscle cells of pineal vessels.

In our earlier studies, we found some unmyelinated autonomic fibers of the mink *(Mustela vison)* innervating smooth muscle cells of pineal arterioles [25, 26, 27]. The question arises whether autonomic axons terminating on smooth muscle cells are generally present in the pineal organ of the mink and of various mammals. We also wanted to study whether these peripheral nerves reach the pineal organ via the conarian nerves and/or via periarterial plexuses. Therefore, in the present work, we studied the fine structure of the vascular innervation of the pineal organ in the mink and in 15 other species belonging to various mammalian groups and followed in serial sections the conarian and perivasal nerves supplying pineal vessels. We investigated species predominantly used in the pineal research, further some carnivorous animals similar to mink. We also studied some insectivorous mammals as structures of these undifferentiated species usually permit easier understanding of basic principles of organisation than that of more differentiated ones.

MATERIALS AND METHODS

The pineal organ in both sexes of various mammals – 3 animals from each species – were investigated from the following mammalian groups: conventional laboratory animals generally used in pineal research were laboratory white rat and mouse, Guinea-pig *(Cavia cobaya),* hamsters *(Meriones unguiculatus, Mesocricetus auratus)* and rabbit *(Orictolagus cuniculus)*. Cat and carnivorous mustelids as the mink *(Mustela vison),* ferret *(Putorius furo)* and marten *(Martes foina)* were also investigated, further, some insectivorous mammals as shrew *(Sorex araneus),* mole *(Talpa europea),* hedgehog *(Erinaceus roumanicus),* microchiropteran bats *(Myotis myotis, Nyctalus noctula)* and the megachiropteran fruit eating bat *(Cynopterus sphinx)*. Carnivorous mammals were bought from Hungarian dealers, insectivorous mammals were collected partly in Palmela, Portugal, partly (microchiroptreans) in caves near Budapest with the permission of the Hungarian authorities protecting environment and wild life, *Cynopterus* were collected in India and fixed in the Pineal Research Laboratory of the Banaras Hindu University, Varanasi.

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Fig. 1. Perivenous and periarterial pineal nerves of mammals. a: Conarian nerves (arrows) containing unmyelinated and myelinated fibers around the great cerebral vein (GV) follow pineal veins (V) of Martes foina. A: pineal arachnoid. Semithin section, toluidine blue stain, ×520. b: Conarian nerves (arrows) alongside pineal vessels (asterisks) of the rat. GV: great cerebral vein, P: pineal tissue, R: erythrocytes. Semithin section, toluidine blue stain, ×480. c: Myelinated and non-myelinated nerve fibers (arrows) in the wall of a pineal arteriole of Mustela vison. P: pineal tissue, R: erythrocytes, SM: smooth muscle cell layer, ×600. d: Ultrastructure of perivasal nerve fibers of *Putorius furo.* C: collagenous fibers of endoneurium, M: myelinated nerve fiber, P: perineurium, SC: Schwann's cell, asterisks: unmyelinated fibers, ×13 000

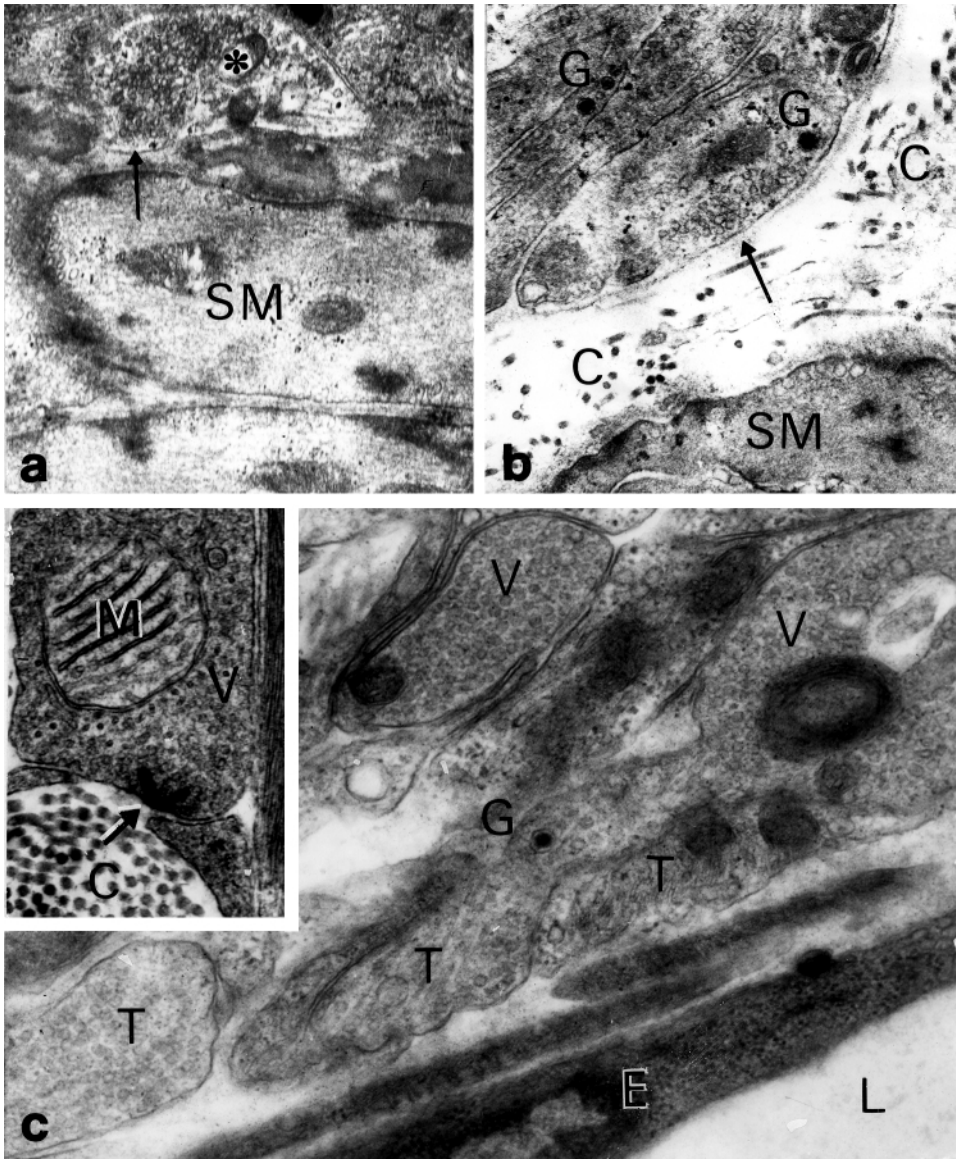

Fig. 2. Fine structure of nerve terminals on pineal vessels of mammals. a: Accumulation of synaptic vesicles (arrow) in an axon terminal (asterisk) on arteriolar smooth muscle cell (SM) in the pineal organ of Mustela vison. ×14 000. b: Nerve terminal (arrow) at arteriolar smooth muscle cells (SM) in the pineal organ of *Cynopterus sphinx.* C: collagenous fibers, G: granular vesicles, ×18 000. c: Nerve terminals (T) in the wall of a pineal vein of Myotis myotis. E: endothelial cell, G: granular vesicle, L: lumen of the vein, V: synaptic vesicles, ×28 000. Inset: ribbon- (arrow) containing terminal of a pinealocyte of the cat on the vascular surface of the pineal tissue. C: collagenous fibrils, M: large pinealocytic mitochondrium, V: synaptic vesicles, ×30 000

The animals were kept in special cages under normal laboratory conditions and anesthetised with phenobarbital prior to fixation. All procedures were made according the international resolutions of animal care. The perfusion through the aorta with 1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) was preceded by a saline flush. Some pineals were fixed by immersion. The pineal organs were further fixed in 1% OsO_4 for 1.5 h, and then dehydrated and embedded in Poly Bed (Polysciences, St. Goar, Germany). Semithin serial-sections and ultrathin sections were cut on a Reichert Ultracut S ultramicrotome. The semithin sections were stained by toluidine blue-azur II and the ultrathin section with uranyl acetate and led citrate.

RESULTS

In all species investigated, autonomic nerves reach the pineal organ alongside vessels. Conarian nerves follow the wall of the great cerebral vein and enter the organ around pineal veins emptying into the great cerebral vein (Figs 1a, b). Autonomic nerves also reach the pineal tissue by periarterial plexuses of vessels supplying the organ (Fig. 1c). Both run in the pineal capsule and intralobular septa formed by the arachnoid and pia mater. Like in other parts of the brain, even the smallest vessels are separated from the pineal tissue itself by basal lamina. The perivasal autonomic nerves contain some myelinated and numerous unmyelinated fibers encapsulated by Schwann-cells and thin endo- and perineural connective tissue (Fig. 1d).

Several axons of the perivasal nerve bundles form terminal enlargements on or near smooth muscle cells of pineal arterioles (Figs 2a, b). Attached by half-desmosomes to the basal lamina of the nerves, the terminal part of the axons are free from covering Schwann-cell processes. The terminals contain several synaptic and some granular vesicles (Figs 2a, b). Axon terminals are present not only in the wall of arterioles but also around pineal veins (Fig. 2c). Also pinealocytic processes form synaptic ribbon-containing neurohormonal terminals on the vascular surface of the pineal tissue (Fig. 2c inset). In serial sections, nerves in the wall of arterioles are the continuation of periarterial plexuses supplying the organ, perivenous nerves are the continuation of conarian nerves.

The number of the perivasal terminals slightly differs in the species studied. The highest number of perivasal nerves was found in carnivorous animals, in the cat and in mustelids. In the rabbit, arterioles follow the margin of the pineal organ and also have autonomic nerve terminals in their vascular adventitia. In the insectivorous bats investigated, the paired internal cerebral veins run both sides in close contact of the pineal organ. There are several autonomic nerves around these veins. In bats, numerous ribbon-containing axons of pinealocytes terminate on the inner surface of the basal lamina of pineal tissue in front of the internal cerebral veins. Axons entering the interlobular pia mater septa from the pineal tissue by crossing its basal lamina also were found in all species investigated.

DISCUSSION

Several types of peripheral fibers were already identified in the pineal organ of mammals [4, 13, 14, 15, 16, 17, 18, 21, 22, 23, 24]. As already mentioned in the Introduction, the significance of autonomic nerves was generally studied in connection to the innervation of pinealocytes, the relation between autonomic nerves and smooth muscle cells of pineal vessels was not yet investigated. Most of the experimental works demonstrated the importance of sympathetic fibers in the melatonin secretion of the organ by mediating light information from the retina to the pinealocytes in mammals [1, 6, 7, 8, 11, 19, 20].

Unmyelinated autonomic fibers found by us to terminate on smooth muscle cells of pineal arterioles of the mink indicated that some of these fibers are not secreto-

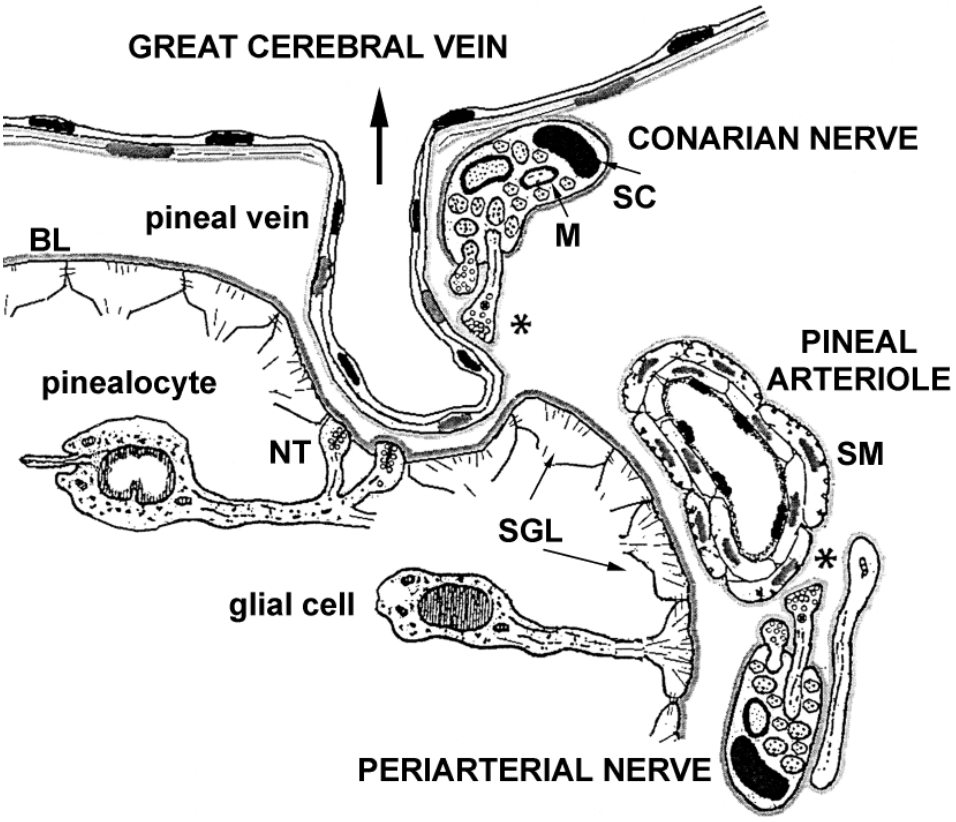

Fig. 3. Schematic drawing on the pineal perivasal nerves of mammals. BL: basal lamina of the pineal nervous tissue, M: myelinated nerve fiber, NT: synaptic ribbon-containing neurohormonal terminals of pinealocytes, SC: Schwann's cell of the peripheral nerv, SGL: superficial glial limiting membrane of the pineal nervous tissue, asterisks: autonomic nerve terminals on vessels, SM: smooth muscle cell-layer of the arteriole

motoric but vasomotoric in nature [25, 26, 27]. Comparing pineal vessels of rodents, carnivorous and insectivirous mammals in the present work, similar nerve terminals were found in the wall of pineal arterioles in all species studied. Therefore, we suppose that a general task of the pineal autonomic fibers is to regulate the pineal blood supply. Terminals found around veins also may represent venomotor fibers (Fig. 3).

We have also studied the relation between pineal perivascular nerves and the conarian nerves running alongside the great cerebral vein. Autonomic fibers were found to reach the pineal organ from both, by periarterial and perivenous nerve plexuses. Conarian nerves run to the pineal tissue alongside pineal veins emptying to the great cerebral vein or directly to the confluens sinuum. Nerves forming terminals around pineal arterioles and veins may have different origin: arteriolar nerves seem to be the continuation of the periarterial nerve plexus of the arteries supplying the brain and perivenous nerves from the conarian nerves following veins. The arteriomotor and venomotor regulation of the tone of the vessels of the pineal organ by peripheral nerves may serve the vascular support of the periodic circadian and circannual changes in metabolic activity of the pineal tissue.

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