Synthetic smooth muscle in the outer blood plexus of the rhinarium skin of *Lemur catta* L.

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Submitted December 19, 2016; accepted in final form February 7, 2017

**Abstract**

The skin of the lemur nose tip (rhinarium) has arterioles in the outer vascular plexus that are endowed with an unusual coat of smooth muscle cells. Comparison with the arterioles of the same area in a number of unrelated mammalians shows that the lemur pattern is unique. The vascular smooth muscle cells belong to the synthetic type. The function of synthetic smooth muscles around the terminal vessels in the lemur rhinarium is unclear but may have additional functions beyond regulation of vessel diameter.

**Key words:** Vascular smooth muscles, rhinarium, *Lemur catta*

**Introduction**

The ring-tailed lemur (*Lemur catta* L.) has a very thin epidermis apart from a few glabrous portions (1). The nose skin was not included in the investigation but it belongs to the areas with a thick epidermis. We observed an unusual pattern of vascular smooth muscle cells in the lemur nose.

The morphology of smooth muscle cells comprises of a continuum between two extremes, the contractile and the synthetic types. The capacity to contract depends on cell shape and is most developed in the contractile type (2). This type is characterized by morphologically elongated, spindle-shaped cells, which are rich in contractile filaments. Cuboidal cells with many organelles participating in protein synthesis are typical for the synthetic type. Mechanical strain tends to enhance the contractile type whereas over- or under-stressing facilitates the synthetic type, which is also involved in repair after injury (3).

Smooth muscle cells form a sheet of varying thickness around arteries, which, in mammalian skin, are termed arterioles because of their small sizes. Those with a diameter of less than 50 µm are termed terminal arterioles. The term terminal arterioles apply specifically to those arterioles with only one layer of smooth muscles (4). The arterioles in the skin are arranged in two plexa, one being subpapillary and the other subepidermal (5, 6). Additional plexa may be found around adnexal structures (7).
terioles of the subepidermal plexus. This holds true for abdominal skin as well as the rhinaria skin of many mammals. However, the arterioles of the outer plexus in the skin of the lemur rhinaria deviate somewhat from the appearance of other studied rhinaria. They are invested with smooth muscle cells identifiable as the synthetic type.

### Materials and Methods

The following mammalian species were used in our investigation: Order Primates, *Lemur catta*, ring-tailed lemur (Linne 1758, protocolnr LuBi LC 2014-01, -02, Tropikariet Helsingborg local ID 34, 36, 37), Order Artiodactyla, *Sus scrofa*, pig (Linne 1758, protocolnr LuBi SS 2012-01, -02), *Ovies aries*, sheep (Linne 1758, protocolnr LuBi OA 2012-01), *Bos taurus*, cattle (Linne 1758, protocolnr LuBi BT 2012-01); Order Carnivora *Felis catus*, cat (Linne 1758, protocolnr LuBi FC 2012-01), *Canis familiaris*, dog (Linne 1758, protocolnr LuBi CF 2012-01, -02, -03), *Mustela putorius*, ferret (Linne 1758, protocolnr LuBi MP 2013-01), *Ursus arctos*, bear cub (Linne, 1758, protocolnr LuBi UA 2015-03); Order Rodentia *Rattus norvegicus*, brown rat (Berkenhout, 1769, protocolnr LuBi RN 2013-01, -03). The artiodactylan species were obtained via sacrifice on a farm, the rat was euthanized for biomedical research and the cat, dog, ferret, bear and lemur were euthanized for terminal illness or serious injury in local zoos. Five adult lemurs were investigated and between one and three animals in each species.

Skin biopsies, 3 mm in diameter, from the nasolabial area and the abdomen (belly) were obtained from the same animal. The biopsies were immersed in 3% glutaraldehyde (Agar Scientific, Stansted, UK) in sodium cacodylate buffer 0.15 M (pH 7.2–7.3) (Agar Scientific) and the preparations were left in fixative overnight at 4 °C. After fixation the preparations were rinsed repeatedly in buffer before further processing.

The preparations for transmission electron microscopy were post-fixated 1% OsO₄ (Agar Scientific) in 0.15 M sodium cacodylate buffer for 1 h at room temperature (about 20 °C). After rinsing in buffer, the preparations were carefully dehydrated in a graded ethanol series, transferred to acetone, and Epon (Agar Scientific). The Epon was polymerized at 60 °C for 48 h. Ultra-thin sections (50 nm) were cut with a Leica Ultracut UCT microtome (Leica Microsystems GmbH, Wetzlar, Germany). Staining on copper grids was performed with 2% uranyl acetate (Ted Pella Inc., Redding, CA, USA) and 1% lead citrate (Merck KGaA, Darmstadt, Germany) for 30 min. Sections were studied in a Jeol 1230 electron microscope (Jeol Ltd., Tokyo, Japan) coupled with a Gatan camera 791 (Gatan Inc., Pleasanton, CA, USA) and a Jeol 1240 Plus.

Some preparations were sectioned for light microscopy and the sections were stained in a 1% mixture of methylene and Azur II blue.

### Results

The external surface of the lemur rhinarium has a rhinoglyphic pattern (8) that is mirrored by the internal structure of the skin. There is an undulating border between the epidermis and dermis. Epidermal papillae project down into the dermis (rete ridges) reaching a depth of 300 µm. Dermal papilla rise towards the surface between the ridges and the epidermis becomes thin, approximately 50 µm. Many nerves and blood vessels, both forming plexa, traverse the collagen bundles of the dermis. By contrast, blood vessels and nerves are sparse in the dermis of abdominal skin.

The outer plexus of blood vessels in lemur nose skin is situated approximately 100 µm below the epidermal ridges (Fig. 1A). Single-unit smooth muscle cells invest the terminal arterioles. These are densely packed forming a continuous layer around the vessel (Figs. 1B–E). The cells are cuboidal and abut one another forming a palisade around the vessel (Fig. 1D). In some places they consist of more than one layer (Fig. 1E). The smooth muscle cells
have a slightly elongated central nucleus (Fig. 1D). The cell membrane is usually irregular and forms protrusions of various sizes. The cell border has rows of caveolae and dark patches (Fig. 2A). The latter continue into the cytoplasm as strands of fibers. The fibrillar cytoplasm contains dark bodies and mitochondria. Ribosomes are found both free in the cytoplasm and attached to the endoplasmic reticulum. A pair of centrioles next to the nucleus is often encountered in the cells (Fig. 1F). The frequent contact points between the cells have dark cytoplasm plaques and they can be referred to as adherens junctions (11).

The smooth muscle cells are separated by a fairly regular intercellular space of approximately 0.3 µm in width (Fig. 2A). An amorphous substance, sometimes granular and filamentous, fills the space. Connective tissue bundles neighbor smooth muscle cells outside the basal lamina of the vessels.
Capillaries branch from the arterioles and enter the dermal papillae (Fig. 1A). They consist of endothelial cells surrounded by a basal lamina and are devoid of smooth muscles. The diameter of the capillaries is that of one blood cell whereas that of the arterioles is around 50 µm and increases further down in the dermis.

Arterioles connecting the outer and inner plexa differ from those of the outer plexus in having smooth muscle cells of the contracting type arranged in one layer (Figs. 2B and C). The veins of the circulatory system in the dermis have endothelial cells and a basal lamina but no coat of smooth muscle cells.

We can confirm the results obtained by Montagna and Yun (1) regarding the abdominal skin of the lemur. The epidermis is 10 to 20 µm thick. It has a straight border between the epidermis and dermis. Few nerves and blood vessels traverse the dermis. The blood vessels of the outer plexus have smooth muscles of the contracting type, which form a thin envelope around the vessels (Figs. 2D and E).

The rhinaria of a number of other mammalians were examined for comparison of the structure of the vascular smooth muscle. The artiodactyl species pig, sheep, and cattle; the carnivoran species cat, dog, ferret,
Synthetic smooth muscle in the Lemur nose

and brown bear (cub); and the rodent species brown rat (see material and methods) were examined for reference in this study. The nose discs of pigs are not ‘true’ rhinaria, because they are dry (9), but are certainly homologous structures and were therefore also studied. In none of these cases was a synthetic type of smooth vascular muscle found. In all cases a thin coat of contractile smooth muscles surrounds the arterioles (Fig. 2F).

Discussion

The organization and structure of cutaneous blood vessels is well known (5, 6). Vascular smooth muscles invest deeper arteries as a thick tissue and they diminish in thickness as the vessels divide and become thinner toward the skin surface. Skin arterioles are organized in two horizontal plexa. The peripheral vessels, terminal arterioles, in the outer plexus have smooth muscles arranged as a one-cell layer belonging to the contractile type. This established general structure was true for the arterioles of the outer plexa of abdominal and nose skin in most species investigated. However, the smooth muscle cells surrounding the arterioles of the outer plexus in the rhinaria skin of the lemur deviated from that of the other species. They belong to the synthetic type.

The ultrastructure of smooth muscle is well established (10–12). Vascular smooth muscle cells can vary between contractile and synthetic phenotypes (13–15) and display intermediates between the two extremes. The smooth muscle phenotypes can vary within one species, within one vessel and within the same site. Genetic, epigenetic and local factors are thought to influence the type of muscle (15 and references therein).

The lemur vascular smooth muscles in the outer blood plexus of the arterioles of the rhinaria skin show all the characteristics of synthetic smooth muscle cells. They display an epithelial morphology and the frequent appearance of centrioles in the cells indicates an increased proliferation as ascribed to the synthetic cells. The synthetic activity of matrix proteins within the intercellular space is manifested by the wide intercellular spaces between the smooth muscle cells, the contents of which are unstructured. At the same time the smooth cells are not devoid of contractile elements and can be presumed to retain some contractile capacity.

The skin of the lemur nose is a highly active organ. It has many nerves and is well vascularized. It contains the Eimer’s organ-like mechanoreceptor compounds (16). Eimer’s organ in short-nosed bandicoot and opossum exists together with terminal arterioles in the outer blood plexus with one layer of smooth muscle of the contractile type. See Fig. 12 in (17). Hence, any connection between Eimer’s organ and terminal blood vessels with synthetic smooth muscles does not seem to exist.

The synthetic vascular smooth muscles can be associated with injuries of blood vessels and are used in human medicine as grafts to restore contractile smooth muscles (18). The synthetic type of smooth muscle of the lemur nose was found in five adult specimens with no visible injuries to the rhinaria, and can therefore be assumed to be permanent. It may therefore represent a local innate specialization with a function yet to be disclosed.

Descriptions of observed lemur behavior suggest a function of the rhinaria in collecting long-chained pheromones (19). In this behavior, the rhinaria is frequently rubbed against substrates of all kinds and this may put so much mechanical strain on the outer layers of the skin that constant repair is necessary. However, we found smooth muscles of the contractile type in the pig nose disc, which undoubtedly also experiences substantial mechanical strain during foraging. Our morphological finding motivates a specific investigation into lemur behavior with regard to the use of the rhinaria.

Conflict of Interest

The authors declare that they have no conflict of interest.
Acknowledgments

The animals were supplied by Bo Holmqvist, Kovads Gård, Karl-Johan Nordfeldt, Skånes and Ölands Djurpark, Tropikariet in Helsingborg and Monica P. Bauden, Dept of Surgery BMC, Lund. We are very grateful for their efforts and support. The valuable technical assistance with microscopical preparations by Rita Wallén, Carina Rasmusson and Eva Landgren is gratefully acknowledged. Dr James J Foster provided linguistic corrections, which are greatly appreciated. The Royal Physiographic Society and Crafoord Foundation funded the study.

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