Original article

Pattern of adrenergic innervation of aorta in goat (Capra Hircus)

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Abstract

Adrenergic innervation of aorta influences its physicomechanical properties and functioning. The pattern may vary between species. The goat is a suitable model for studying cardiovascular disease, but the pattern of its innervation is largely unreported. The purpose of this study was therefore to describe the pattern of adrenergic innervation of its aorta. Aortae for this study were obtained from four adult male goats (capra hircus). Specimens were obtained immediately after euthanasia from ascending aorta, aortic arch, proximal (T6), middle (T9), distal (T12) thoracic and abdominal aorta. Fresh 2 mm long transverse specimens were embedded using OCT compound (Tissue-Tek II) in cryostat chamber at –30 °C. Cut sections were prepared for demonstration of tissue monoamines by the sucrose-potassium phosphate-glyoxylic acid (SPG) method. Examination under Leitz-Ortholux© fluorescent microscope revealed that adrenergic innervation is dense in the proximal segments where nerve terminals penetrate into the tunica media and colocalise with muscle islands present in the adventitial half. Density of innervation declines caudally such that in the abdominal aorta, there are only sparse terminals confined to the media-adventitial border. This dense adrenergic innervation of goat aorta and its regional variation may underpin regional differences in physicomechanical properties, functions and disease distribution in this vessel.

Keywords: adrenergic innervation, aorta, smooth muscle, goat.

1 Introduction

Adrenergic innervation of the aorta influences its mechanical properties, and alters its diastolic pressure diameter relationship reducing the diameter for any given pressure (NICOLOSI and PIEPER, 1975; PANGANI, SCHWARTZ, BISHOP et al., 1975). These features may affect the pattern of distribution of disease and aging along the aorta. Nonetheless, adrenergic innervation of the aorta has been reported to be generally sparse (KUCHII, SHIBATA and MORI, 1973; KIENECKER and KNOCHE, 1978; LUFF and MCLACHLAN, 1989). Recent studies suggest that in the goat, for example, adrenergic terminals co-exist with muscle cells (OGENG'O, MALEK and KIAMA, 2009) suggesting species variation in adrenergic innervation of mammalian aorta. Since goat is a suitable model for studying vascular disease (LEMSON, DAEMON, KITSHAAR et al., 1999), this study aimed at detailing the pattern of adrenergic innervation of its aorta.

2 Materials and methods

Aortae for this study were obtained from four healthy adult domestic male goats purchased from private livestock farmers in Nairobi. Ethical approval for the study was granted by the Kenya Physiological Society Animal Ethics and Research Committee. The animals were euthanized with overdose of sodium pentabarbitone 20 $mg.mL^{-1}$ injected intravenously. After opening the thorax and abdomen, and retracting away the lungs and abdominal viscera, 2 mm long specimens were taken from ascending, arch, proximal (T6), middle (T9), distal (T12) thoracic and abdominal aortae. The specimens were wrapped in aluminium foil and stored in dry ice, then embedded in OCT compound (Tissue-Tek II) in a cryostat chamber at –30 °C. Sixteen micrometer (16 µm) sections were prepared for demonstration of tissue monoamine by the sucrose-potassium phosphate – glyoxilic acid (SPG) method.The sections were dipped 3 times in the SPG solution and excess solution drained off. Slides were dried at 40 °C then placed in an oven maintained at 100 °C under liquid paraffin for 5 minutes. They were cover-slipped using fresh liquid paraffin, and examined under a Leitz ortholux© fluorescent microscope using 250/4 ultra high pressure mercury lamp with a Leitz Bp 546/filter block.

3 Results

In the ascending aorta, aortic arch and proximal thoracic aorta, adrenergic nerve terminals are present in the tunica media (Figure 1a-d). These terminals are confined to the outer half of the tunica media where they co-localise with muscle islands (Figure 1b, c). Nerve terminals appear as discrete varicosities and are distinguishable from the fluorescence of the elastic lamellae and fibres by their greater intensity and nodular appearance. Whereas in the proximal parts of the aorta, the fluorophores are numerous and penetrate into the outer half of the tunica media (Figure 1a), in the distal thoracic aorta, they are fewer, and confined to the outer one quarter of the tunica media (Figure 1d). In the abdominal aorta, the fluorophores are very few if any, and are confined to the media-adventitial border (Figure 1e).

4 Discussion

The proximal segments of the goat aorta are densely innervated. This is at variance with reports from laboratory

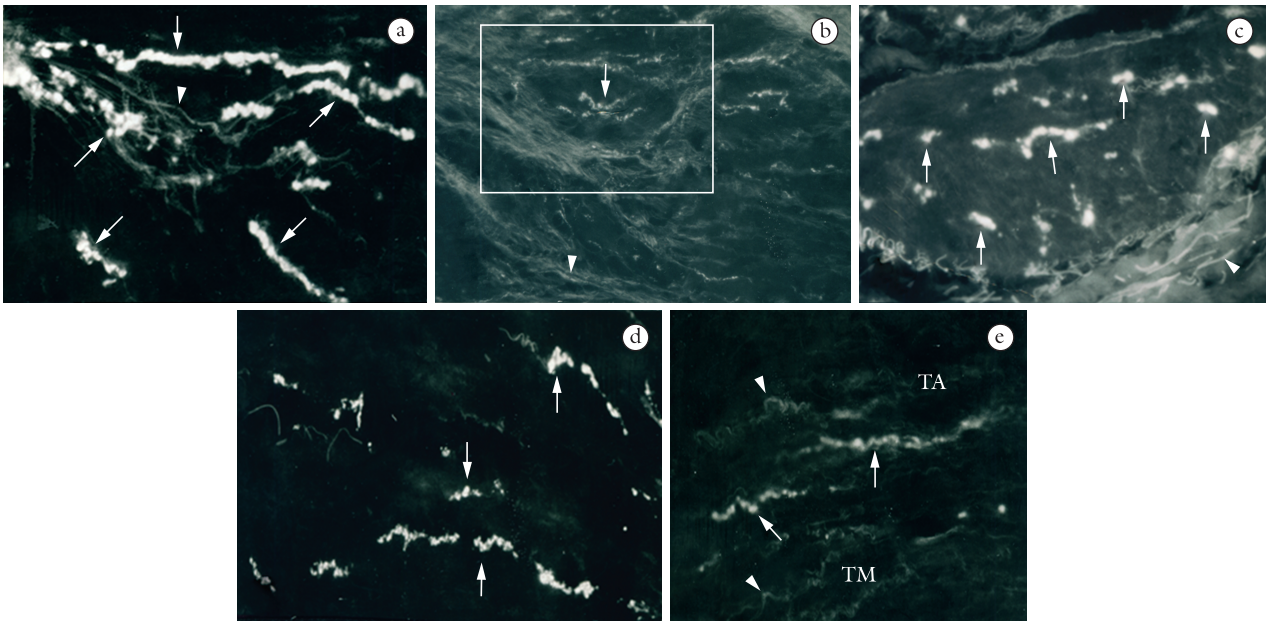

Figure 1. a-e) Fluorescent photomicrographs of goat aorta showing Adrenergic innervation. a) Ascending aorta: Note the fluorophores (arrow) distinguishable from elastic fibres (arrowhead) by their greater fluorescence and nodular appearance. ×1,100; b) Proximal thoracic aorta showing the adrenergic fluorophores (arrow)co-localised with the muscle islands and distinguishable from elastic fibres (arrowhead) by greater fluorescence. ×440; c) Proximal thoracic aorta showing adrenergic fluorophores (arrow) co-localised with a muscle island. The elastic fibres (arrowhead) are shown outside the island. ×1,100; d) Distal thoracic aorta, showing diminution of fluorophores (arrow) as compared with Figure 1a above. ×1,100; and e) Abdominal aorta, showing fewer fluorophores (arrows), as compared with figures a and d, and their relative confinement between tunica media (TM) and tunica adventitia (TA). Note the elastic fibres (arrowheads) in the tunica media and adventitia. ×1,100.

animals that the adrenergic innervation of the aorta is sparse (KUCHII, SHIBATA and MORI, 1973; BURNSTOCK, 1975; OSBORN-PELLIGRIN, 1978,). This sparsity of aortic innervation may be due to abundance of elastic fibres with only a few smooth muscle cells. These workers suggested that the stretch-recoil properties of elastic fibres suffice in passively maintaining blood flow, and that adrenergic fibres would produce too great latency in functional adaptation (KIENECKER and KNOCHE, 1978). Observations of the present study suggest that whereas the luminal zone of the tunica media and the elastic bundles between the muscle islands function in this passive manner, the areas with the muscle islands, by neurogenic muscular response, operate in a different manner that enables the aorta to act as an auxillary pump, and also participate in regulating blood flow(OGENG'O, MALEK and KIAMA, 2009).

Adrenergic nerves penetrate into the tunica media of the goat aorta, as in the aorta of fish and amphibians (KIRBY and BURNSTOCK, 1969), cat, fox and badger (ABRAHAM, 1969) and dog (ABRAHAM 1969; DOLEZEL, 1972). These findings are, however, at variance with reports from laboratory animals that adrenergic nerves in the aorta are confined to the medio adventitial border (BURNSTOCK, 1975; LUFF and MCLACHLAN, 1989). Penetration of nerves into the tunica media is usually retained in those parts of the arterial tree where a predominantly sympathetic control would be of some physiological value (FURNESS, 1971; BEVAN and PURDY, 1973), as in the case of the proximal arteries of mammals which undergo reflex vasoconstriction during diving (WHITE, IKEDA and ELSNER, 1973). A notable difference is that whereas in these studies a uniform pattern of distribution is described, observations of the present study reveal a circumferential asymmetry in which the nerve fibres occur in patches co-localized with smooth muscle islands. This implies that a close functional relationship exists between the sympathetic nerves and the smooth muscle cells. The sympathetic nerves in the smooth muscle islands may serve to suppress the proliferation, differentiation or, retro-differentiation of the smooth muscle cells into morphogenic cells, thus maintaining the contractile phenotype. Observations of *in vitro* studies show that sympathetic nerve fibres inhibit phenotypic modulation of smooth muscle cells, and their proliferation (HARTLEY and CAMPBELL, 1987) and also suppress their tendency to de-differentiate (KACEM, SEYLAZ, ISSERTIAL et al., 1995).

A second function of the adrenergic nerves in the tunica media of the goat aorta may be to modulate its mechanical properties. The importance of neurohumoral influences on the mechanical properties of the aorta has been suggested in experimental animals (GEROVA, GERO, DOLEZEL et al., 1973; NICOLOSI and PIEPER, 1975). Increased sympathetic activity alters the aortic diastolic pressure diameter relationship, reducing the diameter for any given pressure in the dog (GEROVA, GERO, DOLEZEL et al., 1973) and cat (PANGANI, SCHWARTZ, BISHOP et al., 1975). Neurogenic contraction of smooth muscle cells in the islands may contribute to aortic stiffness, thus constituting part of the mechanism for preventing aortic rupture in the wake of a diminished tunica adventitia in the thoracic aorta. A third function of the nerves co-localized with the smooth muscle cells may be to modulate the phenotypic characteristics of the cells by exerting their trophic effect

as has been shown in other arteries (BEVAN and BEVAN, 1981; DIMITRIADOU, AUBINEAU, TAXI et al., 1988; MUELLER and RUSTERHOLZ, 1983; TSURU, TANIMUTSU and HURAI, 2001).

The density of adrenergic innervation decreases caudally such that the distal descending thoracic and abdominal aortae are only sparsely innervated with nerves confined to the medio-adventitial border. Similar paucity of adrenergic innervation of the abdominal aorta has been reported in dogs (GEROVA, GERO, DOLEZEL et al., 1973), rats (OSBORNE-PELLIGRIN, 1978) and rabbits (COWEN and BURNSTOCK, 1980). In cases where nerves are confined to the medio-adventitial border, there is dual control of the vessel by the nerves, and circulating catecholamines which diffuse in an intimo-medial direction (AVAKIAN and GILLEPSIE, 1968; BURNSTOCK and IWAYANA, 1971). Activation of nerves at the medio-adventitial border, indirectly activates the other muscles which are electronically coupled. Since such coupling is precluded by elastic lamellae in the aorta, it is possible that after the action of neurogenically and myogenically driven smooth muscle nests has augmented the elastic recoil in the thoracic region, the abdominal aorta may not need any special modifications, and continues to function mechanically.

In conclusion, the adrenergic innervation of goat aorta is dense and displays regional variation. This pattern may underpin regional differences in physicomechanical properties, function and disease distribution in the aorta.

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Received September 16, 2010
Accepted March 22, 2011