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RESEARCH ARTICLE

Enhanced constriction of supplying arteries — A mechanism of femoral head Necrosis in Wistar rats? ☆

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ABSTRACT

Femoral head necrosis occurs in 8–15% of Wistar Kyoto (WKY) rats, and in up to 46% of spontaneous hypertensive (SHR) rats. For SHR rats, the etiologic factors have been described while the pathomechanism of femoral head necrosis in Wistar rats remains unclear.

The aim of this study has been to compare the vasoconstrictive effect of noradrenaline on femoral arteries in common Wistar and SHR rats.

Four male SHR rats 180–209 days of age, and four male Wistar rats 179–185 days of age, were used. Seven femoral artery segments were harvested from the SHR rats while 6 artery segments were harvested from the WKY rats. The arterial segments were mounted as ring preparations on a small vessel myograph for isometric force development. The arteries were stimulated cumulatively by adding noradrenaline (0.3–30 μM at 2-min intervals) to obtain the dose–response curve of isometric wall tension.

Noradrenaline elicited a concentration-dependent vasocontraction in all arteries. The lumen diameter at standard passive tension L_{100} was not different. The dose–response curve showed a stronger constriction of the femoral artery segments in WKY than in SHR rats. The maximal active tension of noradrenaline was 6.4 ± 3.4 mN in the WKY, and 3.0 ± 1.8 mN (mean ± S.D), significantly lower, in the SHR group ($p < 0.05$).

This study showed that vasoconstriction of the femoral artery in WKY rats was stronger than that of SHR rats. This may be a pathomechanical factor in femoral head necrosis of WKY rats.

1. Introduction

Osteonecrosis of the femoral head is a severely disabling disease affecting young people. Femoral head ischemia is widely accepted to be the pathomechanism of this disease (Mont and Hungerford, 1995). Blood flow to bone is regulated by the degree of wall tension of the supplying arteries (Brinker et al.,1990). It has been shown that vasoconstriction increases perfusion pressure and reduces blood flow in bone arteries (Brinker et al., 1990). The role of enhanced vasoconstriction has been postulated earlier in the pathogenesis of nontraumatic femoral head necrosis (Drescher et al., 2006).

The blood supply in rats is comparable to that in humans and derives from branches of the medial and lateral circumflex femoral arteries (Boss and Misselevich, 2003).

Osteonecrosis of the femoral head resembling that of human Perthes' disease occurs in 8–15% of Wistar-Kyoto (WKY), and in up to 46% of spontaneous hypertensive (SHR) rats. For SHR rats, the pathomechanism has been thoroughly described (Tomita et al., 1999; Suehiro et al., 2000). In the SHR rats, abnormalities such as stenosis and obstruction of the lateral epiphyseal arteries, the main supplying vessels to the femoral head, have been described (Hirano et al., 1989, 1996). Also, vascular occlusion at the site, where the lateral epiphyseal arteries penetrate the cartilage to enter the femoral head, has been found in SHR rats (Hirano et al., 1989). It has not yet been fully clarified by which mechanism the femoral head blood supply is disturbed in WKY rats. Nothing is known about wall tension of the femoral artery (Spencer and Brookes, 1988) in either animal. Enhanced constriction of the femoral artery may result in a lower arterial inflow to the femoral head (Shim, 1968), and be a relevant cofactor in the early

☆ The experiment was approved by the local ethics committee of the University Hospital Schleswig-Holstein, Kiel, Germany.

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pathogenesis of femoral head necrosis. Noradrenaline is the most important vasoconstrictor agent in most parts of the arterial bed, especially in the bone (Lundgaard et al., 1996), and in the femoral head (Drescher et al., 2004).

The aim of this study has been to investigate whether the vasoconstrictive effect of noradrenaline on femoral arteries is stronger in common WKY than in SHR rats.

2. Materials and methods

2.1. Study design

Four male SHR rats 180–209 days of age, and four male Wistar–Kyoto (WKY) rats 179–185 days of age, were used. Femoral artery segments were harvested from both groups of rats.

2.2. Myographic method

The animals were sacrificed by ether anesthesia. Immediately after, the femoral artery was approached by a medial skin incision, a segment was cut from the femoral artery, and placed in a Petri dish in physiological saline solution at 4 °C (Fig. 1).

Arterial segments 2 mm in length were threaded onto two stainless steel wires 40 μm in diameter, and mounted as ring preparations on a Mulvany–Halpern small vessel myograph (Fig. 2; model 500; JP Trading, Aarhus, Denmark) for isometric force development (Mulvany and Halpern, 1976). The test chamber contained 10 mL bicarbonate-buffered physiological saline solution (PSS), which was heated to 37 °C and bubbled continuously with 95% O_2 and 5% CO_2 to give a pH of 7.4–7.5. After an initial equilibration time of 30 min, the arteries were stretched stepwise to characterize the relationship between the vessel circumference and the passive wall tension. The vessel size was normalized by defining L_{100} as the internal circumference the vessel would have if relaxed and exposed to a transmural pressure of 100 mm Hg (Mulvany and Halpern, 1977). The vessels were kept at $0.9 \times L_{100}$, since this degree of stretch allows maximal active force development (Lundgaard et al., 1996). Experiments were carried out by adding the vasoactive agents directly to the chamber and recording the isometric force development in the vessel wall. The vasoactive agent used was noradrenaline (Sigma, St. Louis, MO, USA).

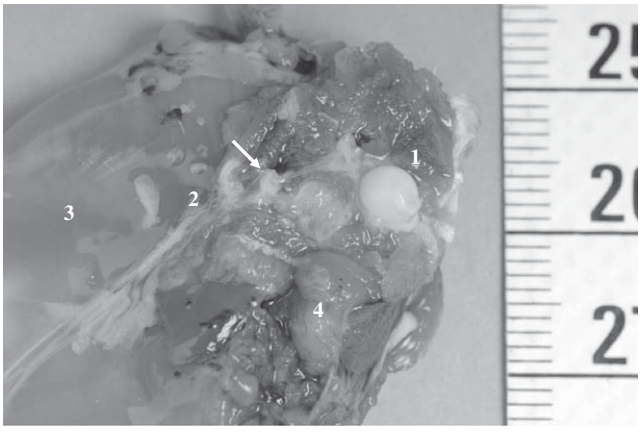

Fig. 1. A view from medially onto the lower extremity after removal of the skin and exarticulation of the hip joint. The cartilage of the femoral head is visible (1). The dissected femoral artery is marked with an arrow (→), and runs within the neurovascular bundle together with the femoral nerve (2). The quadriceps femoris (3) and ischiocrural muscles (4) are also marked.

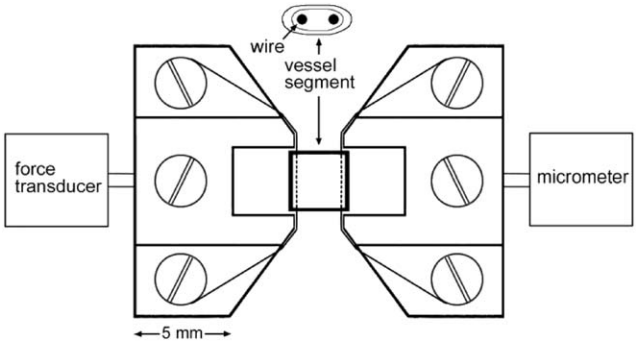

Fig. 2. The principle of the Mulvany–Halpern small vessel myograph (Mulvany and Halpern, 1976). 2-mm arterial segments are threaded onto 40-μm steel wires, which are fixed on the myograph clamps in a physiologic saline bath at 37 °C temperature. Connected to these are the force transducer and micrometer, which register changes in isometric wall tension.

All arteries were stimulated 3 times with 10 μmol noradrenaline for 2 min at 5-min intervals and then once for 5 min. This standard start carried out prior to all protocols mobilizes the vessel and is used to evaluate smooth muscle function (Lundgaard et al., 1997). Vessels with an active force development corresponding to contraction against a pressure of at least 100 mm Hg were included in the protocol (Mulvany and Halpern, 1977). Thus, seven femoral artery segments could be included from the SHR rats while 6 artery segments were included from the Wistar rats. The arteries were stimulated cumulatively by adding noradrenaline (0.3–30 μM at 2-min intervals) to the chamber to obtain the dose–response curve of isometric wall tension.

2.3. Calculations and statistics

Isometric force (F) exerted by the arterial segment on the force transducer was registered. Data are presented as mean ± SD. Agonist sensitivity was expressed as EC50, defined as the agonist concentration (in mol/L=M) resulting in half maximal effect. Normal distribution was documented by q–q-plotting. The homogeneity of variances was checked by Levene's test. Comparison of the two experimental groups of rats was done by means of the independent samples t-test. P values of less than 0.05 (two-tailed) were considered significant.

3. Results

Noradrenaline elicited a concentration-dependent vasoconstriction in all arteries (Fig. 3). The vessel diameter at normalized passive wall tension L_{100} was 457 ± 49 μm in the WKY, and 443 ± 80 μm (mean ± S.D.) in the SHR rats was not significantly different ($p > 0.05$).

The dose–response curve showed a greater constriction of the femoral artery segments of WKY than in those of SHR rats (Fig. 3). The maximal active tension of noradrenaline was 6.4 ± 3.4 mN in the Wistar, and 3.0 ± 1.8 mN (mean ± S.D), significantly lower, in the SHR group ($p < 0.05$).

4. Discussion

This *in vitro* study showed that noradrenaline elicited greater constriction of the femoral artery in WKY 26–28-week-old male rats. Stronger constriction of the femoral artery leads to diminished arterial inflow to the femoral head. This may represent