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RAPID COMMUNICATION

Sex-Dependent Differences in Growth of Vascular Smooth Muscles Cells From Spontaneously Hypertensive Rats

L. BAČÁKOVÁ, J. KUNEŠ

Institute of Physiology, Academy of Sciences of the Czech republic, Prague

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Summary

The growth capacity of cultured vascular smooth muscle cells (VSMC) obtained from the thoracic aorta of 8-week-old male and female spontaneously hypertensive rats (SHR) was compared. Explants from the intima-media complex were cultured in Dulbecco minimum essential medium supplemented with fetal calf serum (10 %). The migration of VSMC out of the explants started on day 2 in both sexes but on day 18 the number of explants with VSMC migration was 100±32 explants/flask in male VSMC and only 24±5 explants/flask in female ones. The doubling time at the early exponential phase of growth was shorter (13.5±0.5 h) and the [^{3}H]-thymidine Labelling Index was higher (34.0±2.3 %) in male VSMC than in those from females (19.9±0.6 h and 23.9±1.9 %, $p<0.01$, respectively). The difference in the doubling time became even more apparent in the late exponential phase of growth (male VSMC: 51.8±2.0 h, female VSMC: 91.5±5.8 h, $p<0.001$). Moreover, at the end of the exponential growth phase, the male VSMC reached significantly higher ($p<0.001$) maximum population density than VSMC from females. Our data provide evidence of different growth characteristics of cultured VSMC isolated from male and female SHR aortas.

Key words

Sex – Spontaneously hypertensive rat – Smooth muscle cells – Cell proliferation – Thymidine incorporation

Proliferation of vascular smooth muscle cells (VSMC) has been implicated in the pathogenesis of atherosclerosis (Ross and Glomset 1976, Schwartz *et al.* 1990) and may also be involved in the vascular complications that often accompany hypertension development (Folkow 1978, Mulvany 1991). There is a growing body of evidence that men are more sensitive to atherosclerosis than women (Goldbourt and Neufeld 1986) and sex-dependent differences also exist in the prevalence of hypertension (Ostrander and Lamphiear 1974). It was shown that aortic VSMC isolated from normotensive male rats proliferate more rapidly than those from female rats (Travo *et al.* 1980, Bačáková and Baudyšová 1990). To our knowledge, there is no information about the proliferation of VSMC isolated from male and female rats with genetic hypertension. Therefore, we have attempted to elucidate the sex-dependent differences in growth of VSMC isolated from both male and female spontaneously hypertensive rats (SHR).

Male and female SHR were bred in our institute. Blood pressure of 8-week-old SHR of both sexes was measured directly in the carotid artery under light ether anaesthesia. The rats were decapitated and their thoracic aortas were removed under sterile conditions. Cultured VSMC were obtained by an explant method as described previously (Bačáková and Baudyšová 1990). Briefly, the media-intima complex was cut into small fragments (1 mm^2) and treated with 0.1 % collagenase solution in the Dulbecco minimum essential medium (MEM) for 1 h at 37 °C. After washing, the fragments were placed in 25-cm^2 tissue culture flasks (NUNC). Each flask contained 200 fragments and 2 ml Dulbecco MEM supplemented by 40 μg/ml gentamycin and 10 % foetal calf serum. The flasks were gassed with 95 % air and 5 % CO_2 and placed in a 37 °C incubator.

VSMC began to grow from the explants within 2 days. After reaching confluence the cells exhibited hill-and-valley pattern typical of smooth muscle cells in culture. They were then passaged by trypsinization with proteases for tissue cultures P-TC (TK Media, Bratislava, Slovak Republic). Proliferation of VSMC was determined for 10 days by counting the cells in a Bürker chamber and the resultant values were used for computing the doubling time of cells (Bačáková and Baudyšová 1990). The [^{3}H]-thymidine Labelling Index was measured in the early exponential phase of growth after the incubation of the cells with [^{3}H]-thymidine (1 μCi/ml) for 2 and 26 h. The maximum VSMC population density per culture area unit was likewise determined. The results are given as mean ± S.E.M. Statistical analysis was performed by Student's t-test. $P<0.05$ value was considered statistically significant.

Body weight of 8-week-old SHR males was significantly higher in comparison with the age-matched SHR females (216±9 vs 147±1 g, $p<0.001$). The same was also true for their blood pressure. Systolic blood pressure was significantly higher in the SHR males than SHR females (201±6 vs 180±2 mm Hg, $p<0.01$). The migration of VSMC out of the explants started on day 2 in both sexes but on day 18 the number of explants with VSMC migration was 100±32 explants/flask in male VSMC and only 24±5 explants/flask in female ones.

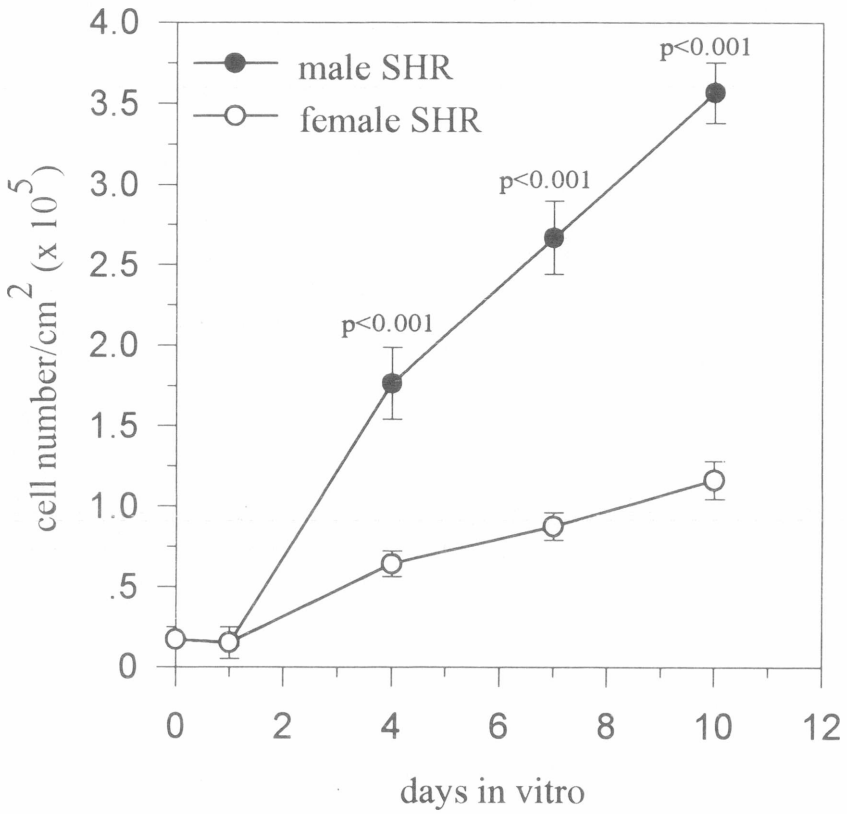

Fig. 1
Growth curves of vascular smooth muscle cells isolated either from male or female spontaneously hypertensive rats.

Growth curves of VSMC from both sexes were evaluated after the second passage (Fig. 1). In spite of the same initial plating density, the VSMC from SHR males grew significantly faster than those from SHR females. The doubling time at the early exponential phase of growth was shorter in male VSMC than in those from females. This difference in the doubling time became even more apparent in the late exponential phase of growth (Table 1). Moreover, at the end of the exponential growth phase, VSMC from SHR males reached a significantly higher maximum population density when compared with those from SHR females (3.57±0.18 vs 1.16±0.12 cells x 10^5/cm^2, $p<0.01$). Growth of VSMC in 10 % foetal calf serum as determined by [^{3}H]-thymidine incorporation into newly synthesized DNA was also sex-dependent. After 2 h incubation with [^{3}H]-thymidine, the percentage of labelled cells was higher in male VSMC in comparison with female VSMC (34 vs 24 %, $p<0.01$) and this difference was even more pronounced after 26 h (86 vs 68 %, $p<0.001$).

Table 1
Doubling time (in hours) of vascular smooth muscle cells isolated either from male or female spontaneously hypertensive rats

Phase of growth	MALE	FEMALE
Early exponential (Day 1–3)	13.5±0.5	19.9±0.6*
Late exponential (Day 3–7)	51.8±2.0	91.5±5.8**

*Data are means ± S.E.M. from three experiments performed in triplicate. * $p<0.01$, ** $p<0.001$ from male values.*

The proliferation of VSMC has been considered a consequence of vascular injury induced by hypertension (Folkow 1982). However, the pathophysiological role of vascular smooth muscle cell hyperplasia in hypertension still remains controversial. Although this hyperplasia may be, in part, secondary to high blood pressure (Owens 1987), it has been widely demonstrated that cultured VSMC from male SHR proliferate more than VSMC from normotensive Wistar-Kyoto rats (Yamori *et al.* 1981, Clegg *et al.* 1986, Hadrava *et al.* 1989). In addition, VSMC from SHR are more sensitive to growth factors (Hadrava *et al.* 1989, Agrotis *et al.* 1993) suggesting an abnormal balance between stimulatory and inhibitory control mechanisms in SHR.

We have recently demonstrated sex-dependent differences in growth of VSMC isolated from normotensive Wistar rats (Bačáková and Baudyšová 1990). The results of the present study have shown that these sex-dependent differences in VSMC growth exist even in the cells isolated from SHR. The aortic VSMC from male SHR proliferate more rapidly than those from SHR females. This was seen not only by counting the number of cells but also by measuring the incorporation of [^{3}H]-thymidine into newly synthesized DNA. It could be speculated what is the cause of this sex-dependent difference in growth of VSMC in vitro. Travo *et al.* (1980) ascribed it to the different conditions to which VSMC are exposed during in vivo conditions, such as the action of different hormones in males and females. Testosterone can stimulate the growth of VSMC by the accumulation of cholesterol esters inside the cells, providing them with essential material for construction of the cell membrane (Libby *et al.* 1985, Naseem and Heald 1987). On the other hand, oestrogens inhibit VSMC proliferation (Adams *et al.* 1987). The role of sex hormones is not clear because sex-dependent differences in VSMC growth exist even in cells isolated from neonatal Wistar rats (Bačáková, unpublished results). Nevertheless, several authors (Yamori *et al.* 1981, Clegg *et al.* 1986, Hadrava *et al.* 1989) suggested that enhanced proliferation of cultured VSMC from SHR is a genetic abnormality in hypertension since the cells deprived of high blood pressure continue to grow faster even *in vitro*. The results of our study might be in good agreement with this statement. Another possible explanation of the faster growth of male VSMC might be related to the presence of the Y chromosome which is considered to contribute to the higher blood pressure level in male SHR (Ely *et al.* 1994).

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Reprint Requests

Dr. J. Kuneš, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic