Mechanisms of structural vascular changes in genetic hypertension: analyses on cultured vascular smooth muscle cells from spontaneously hypertensive rats

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Summary

1. The basic mechanism underlying the structural vascular changes occurring in hypertension was studied in cultured aortic smooth muscle cells (SMC) obtained by an explant method from spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) and compared with that in normotensive Wistar-Kyoto (WKY) rats.

2. The growth rate of SMC from SHR and SHRSP at the age of 2-5-11 months was greater than that of SMC from WKY rats even after repeated passages.

3. [3H]Thymidine and [14C]leucine incorporation, and ornithine decarboxylase (ODC) activity of SMC were increased in SHR and SHRSP in comparison with WKY rats.

4. The application of isoprenaline but not noradrenaline to the culture media increased ODC activity acutely in SMC from WKY rats and this increase was blocked by propranolol.

5. These results indicate that SMC from SHR and SHRSP are more prone to proliferate than those from WKY rats and that a β-adrenergic neurohumoral mechanism accelerates SMC growth independently of blood pressure.

Key words: cultured smooth muscle cells, isoprenaline, leucine, noradrenaline, ornithine decarboxylase, propranolol, spontaneously hypertensive rats, thymidine.

Abbreviations: ODC, ornithine decarboxylase; SMC, smooth muscle cells; SHR-SMC, SHRSP-SMC and WKY-SMC, smooth muscle cells from spontaneously hypertensive rats, stroke-prone SHR and Wistar–Kyoto rats respectively.

Introduction

Structural vascular changes are important in the development and maintenance of various forms of hypertension [1]. Biochemical mechanisms underlying such structural changes, namely, the acceleration of vascular non-collagen and collagen protein synthesis secondary to hypertension, have been studied in spontaneously hypertensive rats (SHR) [2, 3] and in other experimental models of hypertension [4]. However, since this acceleration of vascular protein synthesis is detected even at the very early stages in SHR, not only blood pressure itself but other factors such as genetic predisposition and neural activation of protein synthesis in vascular smooth muscle cells (SMC) also appear to contribute to the structural changes occurring in hypertension [5–7]. In order to eliminate the effect of blood pressure on the vascular wall, SMC were obtained by an explant method from the aortas of SHR, stroke-prone SHR (SHRSP) [8] and Wistar–Kyoto (WKY) rats. Growth rate, the incorporation of labelled thymidine or leucine, and ornithine decarboxylase (ODC) activity, which was the rate-limiting step for polyamine biosynthesis and thus regarded as an indicator of cellular hypertrophy or hyperplasia [9, 10], were compared.

Furthermore, because accumulating findings in vivo indicate the important role of neural activation in vascular protein synthesis [5–7], the neural influence on vascular protein synthesis was analysed by observing the acute effects of α- and β-agonists or blockers on the ODC activity of cultured SMC.

Methods

Cultured SMC were obtained by an explant method from the thoracic aortas of age-matched
2.5–11 month old SHR, SHRSP and WKY rats. The media was neatly removed, dissected out as a 1 mm × 1 mm tissue strip and explanted in plastic culture flasks containing medium 199 supplemented with 10% foetal calf serum [11]. Migrated cells from these explants after 30 days of incubation were trypsinized and subcultured by repeated passages thereafter to observe growth curves. Skin fibroblasts were also cultured from neonatal rats of the three strains as a control experiment. Confluent cultured SMC of three to seven passages were used for the following experiments.

DNA and protein syntheses of these cultured SMC were observed by counting the radioactivity of SMC or protein after 12-h incubation in a medium containing [3H]thymidine and [14C]leucine at a concentration of 0.5 μCi/ml respectively. The number of SMC was counted by a Coulter counter and protein was assayed by Lowry’s method.

For measuring ODC activity [9], 10% foetal calf serum or catecholamines (2 μg/ml) with or without DL-propranolol (5 μg/ml) or 1-methyl-3-isobutylxanthine (0.3 mmol/l) was added to the cultured SMC, which had been placed in medium 199 free of foetal calf serum for 16–18 h. At selected time intervals SMC were washed with phosphate-buffered saline and suspended in a solution containing dithiothreitol (5 mmol/l), pyridoxal phosphate (0.2 mmol/l) EDTA (4 mmol/l) and Tris/HCl (pH 7.4; 50 mmol/l). After sonication and centrifugation the supernatant fraction was assayed for the generation of 14CO₂ from L-[1-14C]ornithine, which was trapped with Hyamine hydroxide and counted by a liquid scintillation spectrometer. Enzyme activity was calculated as pmol of 14CO₂ produced h⁻¹ of incubation mg⁻¹ of protein at 37°C.

Results

Growth curve of cultured aortic SMC from SHR, SHRSP and WKY rats

Cultured SMC from the aortas of SHR and SHRSP at the age of 2.5–11 months migrated faster from the explants than those from the age-matched WKY rats. Such a difference in growth rate was checked by counting the cell population for 8 days and confirmed by repeated passages as previously observed [12]. However, no difference in growth rate was observed among cultured skin fibroblasts from neonatal rats of these three strains.

DNA and protein synthesis in cultured aortic SMC from SHR, SHRSP and WKY rats

Both [3H]thymidine and [14C]leucine incorporations into cultured SMC were significantly greater in the cultured SMC from SHR or SHRSP (SHR-SMC or SHRSP-SMC) than in those from WKY rats (WKY-SMC) and the rates of these incorporations in SHR and SHRSP were similar. The incorporation rates (mean ± SE, d.p.m. 12 h⁻¹ 10⁴ cells) of [3H]thymidine and [14C]leucine in the SHR-SMC at the seventh passage were 1294 ±68 and 523 ± 21, and those in WKY-SMC were 193 ± 19 and 397 ± 40 respectively. Such differences in the incorporation of [3H]thymidine between SHR-SMC and WKY-SMC were still observed at the tenth and 24th passages, although the incorporation rates were increased by repeated passages; 3612 ± 167 vs 1881 ± 146 at the tenth passage and 4514 ± 226 vs 2306 ± 94 at the 24th passage.

Foetal calf serum-stimulated ODC activity in cultured aortic SMC from SHR, SHRSP and WKY rats

ODC activity became activated 30–40 times above the basal level 5 h after the addition of foetal calf serum. ODC activity assayed under this condition was 1.7 and 1.5 times greater in SHRSP-SMC and SHR-SMC respectively than in WKY-SMC.
Effects of α- or β-agonists with or without a β-adrenoceptor blocker or phosphodiesterase inhibitor on the ODC activities of cultured aortic SMC from WKY rats

The ODC activity of cultured SMC was clearly increased after exposure to isoprenaline and reached the maximum increase (threefold) 3 h after exposure, whereas it was only slightly elevated after exposure to noradrenaline. As shown in Fig. 1, isoprenaline activation of ODC activity 3 h after exposure was completely blocked by the addition of the β-adrenoceptor blocker, propranolol, although propranolol itself at this dose did not affect the basal level of ODC activity. Similarly, the phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine, did not affect basal ODC activity of cultured SMC but clearly augmented isoprenaline activation of ODC activity.

Discussion

Differences in growth rate, labelled thymidine and leucine incorporation and ODC activity observed between cultured aortic SMC from SHR or SHRSP and from WKY rats suggest that vascular SMC from the rats with genetic hypertension are intrinsically (or genetically) different independently of blood pressure. However, it is still possible that aortic SMC from SHR or SHRSP even as young as 2-5 months old are already transformed into the active form due to the exposure to mild hypertension for 1 month or so, since growth rates of the cultured cells from atherosclerotic aortas were recently reported to be greater than those of control cells in rabbits fed with cholesterol for 6 months [13].

Since ODC activity was regarded as a sensitive indicator of protein synthesis [9, 10], the effect of catecholamines with or without a β-adrenoceptor blocker was observed in the present experiment, which clearly demonstrated that β-adrenoceptor stimulation activated protein synthesis of cultured vascular SMC similarly to its activation of ODC activity in myocardial cells in vivo [14]. Thus we conclude that β-adrenoceptor stimulation activates vascular protein synthesis independently of blood pressure, as suggested by previous observations in vivo [15]. The augmentation of isoprenaline-induced ODC activation by a phosphodiesterase inhibitor further indicates that cyclic AMP is involved in the activation of protein synthesis by β-adrenoceptor stimulation.

These data suggest that sympathetic tone itself accelerates the structural vascular changes through the β-agonistic effects of released catecholamines from vasomotor nerve endings or from the adrenal medulla on vascular protein synthesis. Therefore, chronic treatment of hypertension by β-blockers is theoretically beneficial, by reducing and preventing cardiovascular structural changes [15] which may finally result in cardiovascular complications in hypertension.

References