6) Genetic and Environmental Influence on Cultured Vascular Smooth Muscle Cells from Spontaneously Hypertensive Rats (SHR). Yukio Yamori, Toshimi Kanbe, Yasuo Nara, Masahiro Kihara, and Ryoichi Horie. Department of Pathology, Shimane Medical University, Izumo 693, Japan.

INTRODUCTION

It is now well established that structural and functional vascular changes are important in the pathogenesis of genetic hypertension. Medial hypertrophy of arterial walls in hypertension is generally regarded as being secondary to blood pressure rise but involved in the further development and maintenance of hypertension (Folkow, B. et al.: CIRC RES 32/33 S1: 2, 1973). However, studies from our laboratory have indicated that the thickening of arterial walls is noted even in the prehypertensive SHR, suggesting that vascular smooth muscle may be altered genetically in SHR and have a tendency to more active proliferation even under normotensive state (Yamori, Y. et al.: CLIN SCI 61, 1981 in press). In order to eliminate secondary hemodynamic effects or the influence of humoral factors on smooth muscle cells (SMC), we have obtained SMC from the aortas of SHR, SHRSP and WKY under a tissue culture condition and studied whether or not genetic predisposition and neural activation contributed to the active protein synthesis leading to the structural vascular changes in hypertension.

MATERIALS AND 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. The media was neatly removed and the explants were cut off approximately into 1mm² sections. Then the explants were transferred into tissue culture flasks and 5ml of medium 199 supplemented with 10% fetal calf serum (FCS) was added carefully to avoid the detachment of explants from flasks. Cells migrating from these explants after 30 days of incubation were trypsinized and subcultured to observe growth curves. Skin fibroblasts from neonatal rats of the three strains were also cultured to observe growth curves as a control experiment.

DNA and protein synthesis of these cultured SMC were observed by counting the radioactivity of SMC or protein after 12 hrs incubation in a medium containing ³H-thymidine and ¹⁴C-leucine, respectively.

For measuring ornithine decarboxylase (ODC) activity which is regarded as an early biochemical indicator of cellular hypertrophy or hyperplasia, 10% FCS or catecholamines (2 µg/ml) with or without propranolol (5 µg/ml) or 1-methyl-3-isobutylamines (3x10⁻⁴M) were added to the cultured SMC which had been placed in FCS-free medium for 16-18 hrs. At selected time intervals the cells were washed with phosphate buffered saline and suspended in a solution containing 5mM dithiothreitol, 0.2mM pyridoxal phosphate, 4mM EDTA and 50mM Tris-HCl (pH7.4). After sonication and centrifugation the supernatant fraction was assayed for ODC activity by determining the generated ¹⁴CO₂ from L(1-¹⁴C)-ornithine. The ¹⁴CO₂ evolved was trapped using hyamine hydroxide and counted by a liquid scintillation spectrometer. Enzyme activity was calculated as pmol of ¹⁴CO₂ produced/mg protein·h⁻¹ incubation at 30°C.

To determine membrane abnormality in cultured SMC from SHR and SHRSP, intracellular Na⁺ and K⁺ contents were measured in the presence of 1mM ouabain. At selected time intervals after the addition of 1mM ouabain, SMC were washed rapidly six times with 0.1M MgCl₂ at 4°C and then 5% trichloroacetic acid was added to extract Na⁺ and K⁺. Total cell Na⁺ and K⁺ were measured with a flame photometer and expressed in µmol/mg of cell protein.

²²Na⁺ uptake was assayed in the medium containing 140mM choline chloride, 2.7mM KCl, 10mM Tris-HCl (pH7.5) and 1mM ouabain. After one hour incubation at 37°C 1µCi/ml of ²²Na⁺ was added to SMC. The reaction was stopped 5 min later by placing the dish on ice and washing six times with 0.1M MgCl₂. ²²Na⁺ in SMC was extracted into 5% TCA and counted in a gamma counter.

RESULTS

Cultured SMC from the aortas of SHR and SHRSP at the age of 2.5-11 months grew faster than those from age-matched WKY. However, no difference in growth

rate was observed among cultured skin fibroblasts from neonatal rats of these three strain. Both ^3H -thymidine and ^{14}C -leucine incorporation into cultured SMC were significantly greater in the SMC from SHR or SHRSP than in those from WKY at the seventh, tenth and 24th passages. Moreover, ODC activity in cultured SMC was 1.7 and 1.5 times greater in SMC from SHRSP and SHR respectively than in those from WKY after the addition of 10% FCS.

The ODC activity of cultured SMC exposed to isoproterenol (2 $\mu\text{g/ml}$) was clearly increased (three fold) after the incubation from 2 hrs, whereas it was slightly increased after exposure to norepinephrine (2 $\mu\text{g/ml}$).

The activation of ODC activity induced by isoproterenol was completely blocked by the addition of the β -adrenoceptor blocker, propranolol, although propranolol itself at this dose did not affect the ODC activity. Moreover, the phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine, clearly augmented isoproterenol-induced activation of ODC activity in cultured SMC.

In order to study the membrane abnormality in cultured SMC from SHR and SHRSP, changes in intracellular Na^+ and K^+ contents of SMC from these three models were measured. When Na^+ - K^+ pump was inhibited by 1mM ouabain, the Na^+ contents of cultured SMC increased as the K^+ contents decreased, and cultured SMC from SHR and SHRSP showed a greater Na^+ influx and K^+ efflux than those from WKY. K^+ turnover in cultured SMC from SHRSP was significantly different from that in WKY. ^{22}Na influx assayed in a Na^+ -depleted medium was also significantly greater in SMC from SHRSP than in SMC from WKY.

SUMMARY WITH DISCUSSION

Differences in growth rate, labelled thymidine and leucine incorporation and ODC activity observed between cultured SMC from SHR or SHRSP and those from WKY suggest that aortic cultured SMC from SHR and SHRSP would be intrinsically (or genetically) more liable to proliferate.

The effect of catecholamines with or without a β -adrenoceptor blocker on ODC activity suggest that the sympathetic tone itself accelerates the thickening of arterial walls through β -agonistic effect of released catecholamines from vaso-motor nerve endings or adrenal medulla on the protein synthesis in vascular SMC. The augmentation of isoproterenol-induced ODC activation by a phosphodiesterase inhibitor further indicates that cyclic AMP is involved in the activation of protein synthesis by β -adrenoceptor stimulation.

Increased ouabain-resistant Na^+ and K^+ permeability was noted in cultured SMC from SHR and SHRSP, suggesting that such membrane abnormalities might activate the proliferation of vascular SMC in genetic hypertension.

We conclude that not only blood pressure itself but also other factors such as neural activation and intrinsic membrane abnormalities may induce the hypertrophy or hyperplasia of vascular SMC, resulting in the increment of vascular resistance.