The effect of antihypertensive agents on cell proliferation in cultured vascular smooth muscle cells from different strains of genetically hypertensive rats. Masanori Hamada, Takashi Ueyama, Takuzo Hano, Yuuji Ueno and Ichiro Nishio. Division of Cardiology, Department of Medicine, Wakayama Medical College, Wakayama city, 640.

It has been reported that enhanced cell proliferation in cultured vascular smooth muscle cells (CVSMCs) from spontaneously hypertensive rat (SHR) compared to Wistar-Kyoto rat (WKY). The genetically enhanced cell proliferation in hypertensive strains of rats and "slow presser mechanism" by angiotensin II or other neuro-humoral mediators, such as vasopressin and catecholamines, make it easy to understand both vascular hypertrophy and development of hypertension. We have reported that SHR CVSMCs is hard to respond to competence factors because of easy pass from GO phase to G1 phase in cell-cycle, which suggested a shortening in GO/G1 phase in SHR CVSMCs.

In this study, we used flowcytometry with pulse-label method by bromodeoxyuridine (BrdU) to reveal which phase of cell-cycle is shortened in SHR CVSMCs compared to WKY CVSMCs. We also compared cell-proliferation curves and effects of antihypertensive agents in three different strains of genetically hypertensive rats, namely, SHR/WKY, New Zealand genetically hypertensive rat (GH) / its normotensive control rat (N) and Dahl salt sensitive rat (DS) / Dahl salt resistant rat (DR), to try to find a possible difference in cell proliferative character and to find the effect of antihypertensive agents on them.

CVSMCs were prepared by enzyme digestion method from twelve week-old SHR, WKY, GH, N, DS and DR. Systolic blood pressures were 175±10 for SHR, 108±20 for WKY, 195±8 for GH, 118±19 for N, 168±8 for DS and 148±10 for DR. Five to seventh passages of CVSMCs were used in these studies. Cell-doubling time (Dt) was significantly shorter in SHR CVSMCs compared to WKY CVSMCs (30 hours for SHR and 43 hours for WKY). There were no obvious differences both between GH and N and between DS and DR. Flowcytometric data demonstrated that S1 phase cells in SHR CVSMCs were significantly more than in WKY CVSMCs. No significant difference in a proportion of each cell-cycle phase was observed in between GH and N CVSMCs. Durations of various phase of cell-cycle in SHR and WKY CVSMCs were calculated by the formula of Watanabe. The result clearly demonstrated the three hours shortening in GO/G1 phase duration in SHR CVSMCs compared to WKY CVSMCs.

We tested nicardipine, diltiazem, atenolol, propranolol, amiloride and enalaprilat on cell proliferation in the three different genetically hypertensive strains of rats and their control rats. The inhibitory effects of these agents were measured by dye-staining method by neutral red. The data were expressed by percentage of cell number compared to agent-free Dulbecco's modified Eagle medium (DMEM) containing 10% FCS condition. Nicardipine decreased cell proliferation at the concentration of 10^{-5} M in SHR and WKY CVSMCs, and at 10^{-4} M in GH, N, DS and DR. Diltiazem showed inhibitory effect on cell proliferation at the concentration of 10^{-4} M. No significant difference was observed among the six strains tested. Propranolol, a fat-soluble beta
adrenoceptor blockade, decreased cell proliferation at the concentration of 10^{-5} in SHR and WKY CVSMCs but at 10^{-4} in the other four strains. On the other hand, atenolol, a water-soluble beta adrenoceptor blockade, influenced cell proliferation only at 10^{-4}M in SHR and WKY cells but did not in GH, N, DS and DR CVSMCs. Amiloride, a potent blocker of Na^{+}-H^{+} antiporter, inhibited cell proliferation at the concentrations of 10^{-5} in SHR CVSMCs and at 10^{-4} in the others. Enalapril has been reported to prevent vascular hypertrophy in vivo SHR and cell proidity in vitro culture study. Our data showed that enalaprilat decreased cell proliferation at the concentration of 10^{-4}M. It did not influenced cell proliferation in other four strains of rats even at the concentration of 10^{-3}M.

In conclusion, these data suggests that the enhanced cell proliferation observed in SHR CVSMCs compared to WKY CVSMCs is not an essential factor to develop the genetical hypertension in rats. The antihypertensive agents affected SHR and WKY cells more sensitively than other four strains. No obvious difference in response to antihypertensive agents was demonstrated between genetically hypertensive and normotensive pairs.