ENDOTHELIAL DYSFUNCTION UNDERLYING THE INCREASED CONTRACTILITY IN AORTA FROM OLDER RATS

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Background: Aging is associated with marked changes in the cardiovascular system and is one of the most important independent risk factors for cardiovascular diseases. The purpose of this study was to elucidate whether smaller increases in age influences the vascular contractile effect of various stimuli in rat thoracic aorta. An additional aim was evaluate the contribution of vascular smooth muscle and endothelium in case there is an altered behavior of the contractility of the aorta in various age groups. **Methods:** Open rings of male Wistar rats of 7, 11 and 15 weeks were used in this study. The experiments were done in both endothelium-denuded as well as endothelium-intact preparations. Isometric contraction was measured by a force-displacement transducer. **Results:** KCl-induced contraction was not different in endothelium-denuded preparations from the three groups of animals, however, phenylephrine (PE), $PGF_{2\alpha}$ and endothelin-1 (ET-1) produced an exaggerated contractile response in aorta from 15 week animals. Carbachol relaxed the contraction produced by PE in a concentration-dependent manner; however the magnitude of relaxation was more marked in 7 week animals compared to that in 11 or 15 week rats. **Conclusion:** Our study shows for the first time that even a modest increase in age from 7 to 15 weeks produces changes in VSM as well endothelial cells resulting in exaggerated contractile response to agonists.

Key words: aging, vascular smooth muscle, endothelium, rat, aorta

INTRODUCTION

Aging is associated with marked changes in the cardiovascular system and is one of the most important independent risk factors for cardiovascular diseases. In the vessels, these changes may be at the level of vascular smooth muscle (VSM) cells, endothelial cells as well as extracellular matrix.[1] Arteries show an increase in the thickness of media, increase in the number of VSM cells and increase in the content of collagen. All these factors lead to the stiffening of vessels.[2-4] Guyton et al (1983) have also reported endothelial dysfunction in aged animals.[2] Endothelial dysfunction is generally defined as a decrease in the capacity of the endothelium to dilate blood vessels in response to physical and chemical stimuli. At functional level, aged vessels have been shown to exhibit enhanced contractility towards agonists.[5,6] This enhanced contractility may be due to hyper-responsiveness of the VSM cells as well as the blunted endothelial function, which has been documented in animals[7], as well as in humans.[8] In the literature, in order to study the effects of aging on cardiovascular status, the experiments have been conducted on the markedly older animals[9], however to the best of our knowledge, no study has compared the effects of age on the contractile response to stimuli in at relatively younger animals. The purpose of this study was to elucidate whether smaller increases in age influences the vascular contractile effect of various stimuli in rat thoracic aorta. An additional aim was evaluate the contribution of VSM and endothelium in case there is an altered behavior of the contractility of the aorta in various age groups.

MATERIALS AND METHODS

The experiments of this study were carried out mainly at the Tohoku University, Sendai, Japan and partly at the Aga Khan University, Karachi, Pakistan. All procedures were designed in accordance with the Institutional Guidelines for the care and use of laboratory animals.

Tissue preparation

Male Wistar rats of 7, 11 and 15 weeks were used in this study. The animals were stunned and killed. Open rings of approximately 3 mm width were made from the aorta. The experiments were done in both endothelium-denuded as well as endothelium-intact preparations. Endothelium was removed by gently rubbing the endothelial surface with moistened finger tip. The lack of endothelium was confirmed by failure of carbachol (1 μM) to cause relaxation of phenylephrine (1 μM)-induced contraction.

Measurement of isometric contraction

The aortic strips were suspended vertically, in a 6-ml organ bath (aerated with 95% O_2 and 5% CO_2), containing HEPES-buffered physiological salt solution (PSS). All experiments were carried out at 37°C. The tissues were adjusted to a preloaded resting tension of 10 mN and equilibrated for at least 1 h. Isometric contraction was measured by a force-displacement transducer (Nihon Kohden, Tokyo, Japan). The aorta was contracted 3 times by 64.8 mM

KCl. The last contraction to KCl was employed as a standard in certain experiments (appropriately mentioned in the results section) and thereafter, other contractions were normalized with this contractile response. For the determination of absolute contraction induced by various stimuli, wet weight and length of the aortic strips were measured. Absolute contraction was calculated from the formula: contraction in mN x length of the tissue in mm / wet weight of the tissue in mg.

Solutions

The composition of PSS (in mM) was: NaCl 120, KCl 4.8, $MgSO_4$ 1.3, $CaCl_2$ 1.2, $NaHCO_3$ 25.2, glucose 5.8, KH_2PO_4 1.2 and HEPES 20. 64.8 mM KCl was made by replacing 60 mM of NaCl in PSS with eqimolar KCl.

Materials

Wistar rats were purchased from Kumagai (Sendai, Japan). Endothelin 1 (ET-1), phenylephrine and prostaglandin F2 alpha ($PGF_{2\alpha}$?were obtained from Sigma-Aldrich Chemical Co (St Louis, USA).

Statistics

All values are expressed as mean ± SEM. n represents the number of experiments performed. Data were analyzed by Student's t test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of various stimuli on the contractility of endothelium-denuded aortic strips

After equilibration, the endothelium-denuded aortic strips from three groups of animals viz 7, 11 and 15 week old rats were subjected to KCl and various contractile agonists. Absolute contraction was determined as explained in METHODS section. As shown in Fig. 1, KCl-induced contraction was not different in the three groups, however the contractile response to 10 µM phenylephrine (PE), 3 µM $PGF_{2\alpha}$ and 100 nM endothelin-1 (ET-1) was statistically different in different groups. PE and $PGF_{2\alpha}$ caused higher contraction in aortic strips from 15 week rats compared to 7 and 11 weeks animals. Whereas, ET-1-inuced contraction was significantly more in tissues from 11 and 15 week rats compared to those from 7 week old animals.

Effect of PE on contractility of endothelium-intact aortic strips

In order to explore the status of endothelial function during the active contraction, the endothelium-intact aorta was challenged with 10 µM PE. As shown in Fig. 2, PE-induced contractile response was markedly higher in aortic tissue from 15 week rats compared to those from 7 weeks.

Concentration-response relationship of carbachol in PE-induced contraction

In order to elucidate the functional status of endothelium, a dose-response curve of carbachol was obtained in 1 µM PE-induced contraction in endothelium-intact aortic strips. The strips were initially contracted with 1 µM PE. When the contraction reached a maximum and steady level, carbachol was added to the bathing solution in a cumulative manner. As shown in Fig. 3, carbachol at a concentration of 0.3 µM markedly relaxed the vessel; the degree of that relaxation was significantly higher in 7 week old rats compared to 11 and 15 week old animals. However, further increasing the dose of carbachol up to a concentration of 1 µM abolished the difference in the relaxant response between 7 and 11 weeks animals. Nevertheless, the difference in the magnitude of relaxation persisted between 7 and 15 weeks rats at this concentration of carbachol.

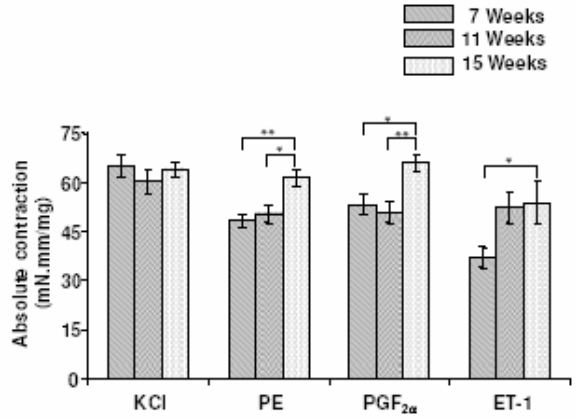

Figure-1: Effect of KCl, phenylephrine (PE), $PGF_{2\alpha}$ and endothelin-1 (ET-1) on isolated endothelium-denuded aorta from Wistar rats of 7, 11 and 15 weeks. After equilibration, the aorta was contracted with 10 mM PE, 3 mM PG or 100 nM ET-1. The absolute contraction was determined as explained in material and methods A. The data represent the means ± SEM of 6-8 independent experiments. *p < 0.05; **p < 0.01 between the groups as indicated by the lines.

DISCUSSION

In the present study, we studied the effects of age on the contractile response to KCl and various agonists in the aorta from Wistar rats. We have demonstrated an age-dependent increase in the contractility of vessels caused by PE, $PGF_{2\alpha}$ and ET-1 but not that caused by KCl.

Previous studies have shown an age-,[10] BP,[11] and strain-dependent[12] differences in the contractile response of vessels. However, those studies have used much older animals, where an exaggeration of vasoconstrictor response due to apparent reasons like atherosclerosis can be well anticipated.[13] In the present study, we performed experiments on three

groups of rats but with relatively small differences in age viz; 7, 11 and 15 weeks of age. Thus the primary aim of our study was to see whether small increases in age has any influence on the contractile response.

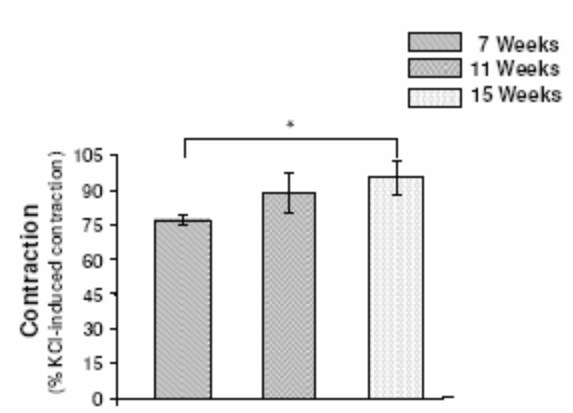

Figure-2: Effect of phenylephrine (PE) on isolated endothelium-intact aorta from Wistar rats of 7, 11 and 15 weeks. After equilibration, the aorta was contracted with 10 mM PE. The data represent the means ± SEM of 6-8 independent experiments. *p < 0.05 between the groups as indicated by the lines.

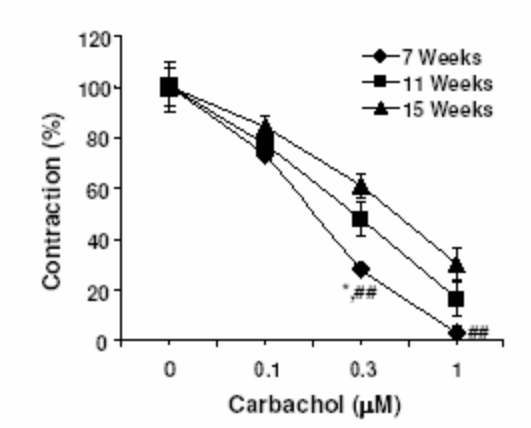

Figure-3: Concentration-relaxation curves of carbachol on isolated endothelium-intact aorta from Wistar rats of 7, 11 and 15 weeks. After the equilibration phase, the aorta was contracted with 1 mM phenylephrine (PE) and when the response to PE attained a maximum and steady level, carbachol was added to the bathing solution in a cumulative manner. The figure shows the comparative analysis of the degree of relaxation caused by various concentrations of carbachol in isolated aorta from all three groups of rats. n = 6-7 in each group. *p < 0.05 between 7 and 11 weeks; ##p < 0.01 between 7 and 15 weeks.

It was observed that all agonists used in this study; PE, $PGF_{2\alpha}$ and ET-1 induced greater contraction in aorta from relatively older animals. This shows that a smaller increase in age has a definite effect on the response of the vascular tissue towards contractile agonists. This finding is in consistence with the previous ones, who have shown an exaggerated response in aged animals.[14,15] However, it was interesting to note that unlike agonists, KCl did not show any differential effect on the contractility. This finding was unexpected. It is difficult to explain this phenomenon from these preliminary experiments. However one can deduce that the likely reason for this difference lies in the mechanisms of contraction utilized by the agonists and the KCl. PE, $PGF_{2\alpha}$ and ET-1 cause contraction acting through G-protein coupled receptors, followed by the receptor-operated Ca^{2+} influx and intracellular Ca^{2+} release.[16,17] On the other hand, KCl does not exploit these mechanisms; instead it produces constriction of the vessels by depolarizing the cell membrane of the VSM cells with consequent Ca^{2+} entry through voltage-dependent Ca^{2+} channels.[18] Nevertheless, further experiments are required to elucidate this discrepancy in the contractile response to KCl and agonists.

Another conclusion can easily be drawn from the above findings that the exaggeration in contractile response observed in older rats was due to hyper-responsiveness of the VSM cells. This conclusion has been drawn from the fact that endothelium was removed from the strips in these experiments, and functionally checked by carbachol. From all these observations, it is reasonable to deduce that age causes changes in the VSM properties resulting in the enhanced contraction caused by receptor agonists.

Endothelial cells exert their relaxant action during active contraction as well as during resting level. These cells tend to produce vascular relaxation through a number of mechanisms notably via nitric oxide,[19] and endothelium-derived hyperpolarizing factor.[20] Previous studies have shown that aging is associated with endothelial dysfunction.[21,22] Having evaluated the role of VSM in the exaggerated contraction, we later tried to investigate the impact of age on the endothelial function and hence the contraction. In order to evaluate the role of endothelium, endothelium-intact aortic strips were prepared and were contracted with PE. It was interesting to note that the PE-induced contraction in 15 week old rat aorta was significantly higher than that in 7 week rats. Since in these preparations, the apparent contraction is the sum of the contractile function of VSM cells as well as the relaxant effect of endothelial cells, one may argue that the higher contraction observed in endothelium-intact preparations was indeed due to hyper-responsiveness of the VSM cells as concluded earlier: hence no decreased role of endothelium. In order to elucidate further the functional status of endothelium, experiments were conducted on the similar endothelium-intact strips and concentration-relaxation response of carbachol was determined. These experiments showed that carbachol at a

concentration of 1 μM though relaxing the PE-induced contraction almost completely in tissues form 7 week animals, could not do so in preparations from 15 week rats. A residual contraction (approximately 35% of the maximum) was still there in the presence of carbachol. From these findings, it is not unreasonable to conclude that in addition to hyper-reactivity of VSM cells, older age is also associated with changes in endothelial function that results in the blunted relaxant effect of the endothelium.

Concluding, our study shows for the first time that even a modest increase in age from 7 to 15 weeks produces changes in VSM as well endothelial cells resulting in exaggerated contractile response to agonists.

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