Editorial

Endothelial nitric oxide synthase and estrogen

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Received 3 April 2000; accepted 5 April 2000


Andersen and Stender [1] have reported regional variations in endothelial nitric oxide synthase activity in the endothelial cells of the rabbit aorta. This paper adds an interesting new twist to the link between atherosclerosis and e-NOS activity. The rabbit has been used frequently as a preferred model for studying the effects of cholesterol feeding on endothelial function for nearly fifteen years. In the process, much has been learned about the evolution of the lesions and the specific histological changes observed both during the phase of feeding and following cessation of feeding diets supplemented with cholesterol. There is consensus in three areas: loss of endothelial dependent relaxation (EDR) associated with cholesterol feeding [2,3], the protective effect of L-arginine on EAR [4] and the type and location of lesions formed [5]. The early lesions can be described best as fatty streaks and are located principally in the arch and upper thoracic aorta with relatively few lesions in the abdominal aorta [6]. Andersen and Sender have attempted to provide an explanation for this characteristic distribution. They have suggested that this effect is due to a regional variation in e-NOS activity in the endothelial cells of the aorta, the activity being lowest in the arch and highest in the abdominal aorta.

The authors have taken the first step towards testing this hypothesis by developing a sensitive assay for e-NOS in rabbit aortic endothelial cells. Instead of using cells derived from culture, they adapted a method previously described in the rat to harvest endothelial cells from specific locations in the aorta and examine basal e-NOS activity. The cells were harvested by scraping the endothelial cells from the internal surface of the aorta and suspended in 70 l. of buffer. A small aliquot of this suspension was removed for counting the cells in a hemocytometer. The assay was conducted in the remaining cells. The assay was based upon the conversion of L-arginine to L-citrulline and nitric oxide (NO). Since NO and citrulline are formed in equi-molar quantities, NO formation was expressed in terms of citrulline production per million endothelial cells. Thus it was possible to standardize the NO production and e-NOS activity and thereby make comparisons between different regions of the aorta. The data presented clearly indicates that in male rabbits there is indeed a gradient in e-NOS activity in cells harvested from the aorta. The activity was least in the arch and highest in the abdominal aorta. In female rabbits, the gradient in activity is not as marked as in males, possibly due to the fact that the thoracic aorta was examined as a single entity while in males, the thoracic aorta was examined in two portions. In addition, the values for e-NOS activity appeared to be greater in females as compared to males. The authors have cautioned against drawing any inferences from this difference because the studies in the two genders were undertaken 6 months apart.

This study provides a means of investigating e-NOS activity in cells maintained in-situ in contrast to tissue culture. In the majority of e-NOS assays of blood vessels reported so far, the entire tissue was homogenized and the activity was expressed in terms of mg of protein. The methods described by Andersen and Sender is a much simpler procedure which also carries several other advantages. One is that the endothelial cells could be exposed to agonists while they were in situ. Another is that it will be possible to examine age related changes in the rabbit aorta. It has been suggested that EAR is altered with age. Finally, it will provide a means of evaluating e-NOS systematically by linking the activity of the enzyme to conventional markers of gene expression (DNA, mRNA) thus providing a means of testing hypotheses relating to mechanisms which regulate e-NOS.

The second part of the report is concerned with the potential role of estrogen in EAR. This is an important issue for two reasons, the attenuation of EAR in the post menopausal state and the current debate on the role of estrogen in the prevention of atherosclerosis. The data presented in Fig. 2 of the paper by Andersen and Sender

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merit scrutiny. The experiment was undertaken in oophorectomized rabbits given either a vehicle or estrogen. In the former, the gradient in e-NOS activity described previously was observed. The group given estrogen showed an increase in e-NOS activity in the upper thoracic aorta. Of even greater interest is that there was no significant increase in the lower thoracic aorta. These findings, if confirmed by other investigators, indicates the existence of a unique mechanism linking oestrogen and endothelial cells in this region. This mechanism appears to be absent in the lower thoracic aorta. This paper has opened several potentially fruitful areas of research for those interested in the role of NO and estrogen in the development of atherosclerosis [7,8].

References