Changes in Coronary Resistance Related to the Stages of the Female Life Cycle

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Backgrounds Estrogen is known to dilate the coronary vascular system mainly through nitric oxide (NO) release. However, it has not been determined whether or not this effect occurs equally throughout all stages of the female life cycle. We examined the changes in coronary flow properties in adolescent, adult and ovariectomized (OVX) female rats using the endothelial NO synthetase blocker, L-N (omega) nitroarginine (L-NNA).

Methods and Results Female rats were divided into 3 groups: adolescent (13 weeks, n=6), adult (19 weeks, n=8) and OVX (20 weeks, n=7, 12 weeks after oophorectomy). Coronary effluent was measured using the Langendorff non-working heart model before and 15 min after the use of L-NNA. In OVX rats, coronary effluent was significantly decreased in comparison with adolescent and adult rats (adolescent vs OVX: $p<0.001$; adult vs OVX: $p<0.05$). After treatment with L-NNA, coronary effluent was significantly higher in the adolescent group compared with the adult and OVX groups (adolescent vs adult: $p<0.01$; adolescent vs OVX: $p<0.0005$).

Conclusions Oophorectomy brought about an increase in coronary vascular resistance. L-NNA exacerbated coronary vascular resistance in relation to maturation. It is suggested that the effect of estrogen on vascular dilatation in adolescents is largely dependent on a non-NO pathway, whereas adults are largely dependent on an NO pathway. (Circ J 2006; 70: 478–481)

Key Words: Coronary circulation; eNOS; Estrogen; Female; Maturation

Coronary artery disease (CAD) is one of the most prevalent types of heart disease in the industrialized world! The incidence of myocardial infarction in women is lower than it is in men until they reach their mid-forties according to the Framingham Study.² The incidence of CAD in women increases after the age of 55 and it exceeds the incidence of CAD in men at over 75 years. Moreover, in regard to female hormones, the incidence of cardiovascular disease is greater in the postmenopausal period than in the premenopausal period.^{3–5} It is assumed that female hormones have protective effect in regard to CAD. It has already been reported that estrogen shows a number of beneficial effects on the cardiovascular system.^{6–13} Estrogen dilates coronary vessels by up-regulating endothelial nitric oxide synthetase (eNOS) by means of a genomic effect.^{4,15} eNOS has an essential role on coronary vasodilation; for example, a mutation on the eNOS gene is associated with coronary vasospasm.¹⁶ Coronary vasospasm occurs even in female adolescents with a suspected abnormality of the eNOS gene.¹⁷ However, the role of eNOS on arterial dilatation in female growth and aging remains uncertain. Furthermore, it has been reported in pigs that the dilation of the cerebral artery is dependent on prostanoïd in infancy, whereas it becomes more dependent on nitric oxide (NO) in adulthood, which means there is a kind of age-dependency for vasodilators.¹⁸ Therefore, the present study was designed to investigate the role of eNOS on coronary circulation in the various stages of the female life cycle using L-N (omega) nitroarginine (L-NNA; an

inhibitor of eNOS).

Methods

The Committee on Animal Research at the University of Tsukuba approved the experimental protocols. The rats were cared for in accordance with the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration of 1964.

Animal Preparation and Langendorff Perfusion

Female Wistar–Imamichi rats (Institute of Animal Reproduction, Ibaraki, Japan) were used. Rats were divided into 3 groups: adolescent group (13 weeks, body weight=250–300 g, n=6), adult group (19 weeks, body weight=280–320 g, n=8) and ovariectomized (OVX) group (20 weeks, body weight=340–410 g, n=7; 12 weeks after oophorectomy). An age of 13 weeks in Wistar–Imamichi rats was regarded as juvenile because their body weights were increasing and the estradiol cycle is established in 100% of the population. An age of 19 weeks was regarded as adult due to attainment maximum ovarian mass, as found in the literature.¹⁹ Because of the rapid change of estradiol cycle in female rat, the serum estrogen concentration of non-OVX rats was considered to correspond the average between the proestrus and estrus stages.²⁰ After anesthetization by diethyl ether inhalation, their hearts were excised and prepared for the Langendorff apparatus. The hearts were perfused with a modified Krebs–Henseleit (KH) solution, which contained 124 mmol/L NaCl, 6.8 mmol/L KCl, 1.3 mmol/L MgSO₄, 1.1 mmol/L KH₂PO₄, 2.5 mmol/L CaCl₂, 26 mmol/L NaHCO₃ and 5.6 mmol/L glucose. This was undertaken under a coronary pressure of 66 mmHg and 460±11 mmHg in oxygen tension. The hearts were kept at 37°C and stabilized for 20 min after the initial perfusion to avoid the effect of primary ischemia. Then, coronary flow

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Fig 1. Experimental protocol. Coronary effluent was measured 20 min after perfusion of KH solution (Series 1) and 15 min after the perfusion of L-NNA. KH, Krebs–Henseleit; L-NNA, L-N(omega) nitro-arginine.

Fig 2. Comparison of initial coronary effluent in 3 different female groups. Coronary flow of initial perfusion in different female rat groups indicated that oophorectomy was the causative factor exaggerating coronary vascular resistance.

Fig 3. Comparison of actual coronary effluent after 15 min use of L-NNA. Coronary effluent significantly decreased in the order of adolescent rats, adult rats and oophorectomy rats after the use of L-NNA. The flow in ovariectomized rats was affected by both oophorectomy and endothelial nitrite oxygen synthetase inhibition. L-NNA, L-N(omega) nitroarginine.

(ml/min) was continuously measured every 60 s.

Experimental Protocols (Fig 1)

Series 1 Coronary effluent was measured under stable conditions confirmed after 20 min of Langendorff perfusion.

Series 2 At 15 min after the initiation 100 μ mol/L of the eNOS inhibitor L-NNA, coronary effluent was measured.

Statistics

All variables were expressed as mean $\pm$ SD. All groups were analyzed for statistics using a Student's unpaired t-test

with Bonferroni correction. A difference of $p < 0.05$ was considered significant.

Results

Series 1

Coronary flow measurements were performed under conditions of acute estrogen withdrawal because the KH solution did not contain estrogen. In the OVX group, coronary effluent was significantly decreased in comparison with the adolescent and adult groups (adolescent: 10.3 ± 2.1 ml; Adult: 9.1 ± 2.9 ml; OVX: 6.4 ± 0.7 ml, respectively;

Fig 4. Coronary effluent after the use of L-NNA (represented as percentage from pre L-NNA treatment). When expressed as percentage, the reduction of coronary effluent was different with age. The flow in adolescent rats was significantly higher than in adult rats, regardless of the presence of ovary, after the use of L-NNA, but there was no significant difference between adult rats. L-NNA, L-N(omega) nitroarginine.

Fig 5. Flow volume of eNOS dependency at initial flow. eNOS dependent flow increased with maturation from 27% to 53% ($p < 0.05$). The percentage of eNOS dependent flow of oophorectomy rats was not significantly different regardless of the presence or absence of the ovary. eNOS, endothelial nitrite oxygen synthetase; L-NNA, L-N(omega) nitroarginine.

ADOLESCENT vs OVX: $p < 0.001$; adult vs OVX: $p < 0.05$. Oophorectomy brought about a significant increase in coronary vasoconstriction as compared with the other 2 groups. There was no significant difference between age groups with intact ovary (Fig 2).

Series 2

After the use of L-NNA, coronary effluent significantly decreased in of young female rats (adolescent group; 7.5 ± 1.9 ml/min), mature female rats (group adult; 4.8 ± 1.6 ml/min) and OVX rats (2.8 ± 1.6 ml/min) (adolescent vs adult: $p < 0.05$; adult vs OVX: $p < 0.05$; adolescent vs adult: $p < 0.0005$, respectively, Fig 3). When the results were expressed as percentages from pre-treatment of L-NNA, the coronary flow of young female rats was significantly preserved as compared to the adult and OVX groups (adolescent: $73 \pm 5\%$; adult: $53 \pm 13\%$; OVX: $45 \pm 12\%$, respectively; adolescent vs adult: $p < 0.01$; adolescent vs OVX: $p < 0.0005$, Fig 4).

Discussion

It is known that estrogen has 2 mechanisms in the relaxing of blood vessels. One is a genomic effect (chronic effect) and the other is a non-genomic effect (acute effect). The genomic effect of estrogen on eNOS is the enhancement of eNOS expression in vascular endothelial cells

through binding to estrogen receptors (ER). The non-genomic effect of estrogen on eNOS is the activation of eNOS and the increase of NO production by binding ER onto the vascular endothelial cell membrane.²¹ Another non-genomic effect of estrogen on vasodilation includes a direct effect of vascularizing smooth muscle cells with prostacyclin (PGI₂) and endothelium derived hyperpolarizing factor.²¹

As seen in the results of the present study, coronary flow in OVX rats significantly decreased compared with non-OVX adult rats and adolescent rats. It is indicated that oophorectomy (a 3 month absence of ovarian function) brought about coronary vasoconstriction due to the reduction of eNOS expression (genomic effect). In contrast, the effects of L-NNA on vascular tone were less significant in young female rats than in mature female rats, regardless of the presence or absence of the ovary. This indicated that vasodilation is more dependent on eNOS in adult rats than in adolescent rats. Oophorectomy decreased the eNOS dependent flow rather than the eNOS non-dependent flow (Fig 5).

It has recently been noted that the role of vasoactive agents does not have a constant effect throughout life!^{8,22,23} The cerebral arterial tone is regulated differently in neonates and adults. Cerebral arterial dilatation in newborn piglets is entirely prostanoid dependent, whereas it requires both NO and prostanoids in juveniles!⁸ Vasodilatation in newborns

is NO independent, but NO becomes more important for the regulation of vascular tone with maturation.

In a study of the endothelium-dependent dilatation of coronary arteries in regard to maturation in the male pig, the mRNA of eNOS was lower in juvenile pigs than in adult pigs.²⁴ Chatrath et al demonstrated that relaxation due to NO was greater in coronary arteries from adult compared with juvenile female pigs.²⁵ Moreover, relaxation was only significantly inhibited by cyclooxygenase in juveniles, suggesting that PGI₂ was predominant in coronary dilatation in juvenile male pigs.²⁴ In the case of a human adipose cell study, eNOS expression was more prominent in adults than in children.²⁶

These studies suggest that substances related to vasodilatation were not constant throughout life. In females, coronary vasodilatation is largely affected by estrogen as indicated previously.²⁷ However, even in the presence of estrogen, the subjects of the non-genomic effects of estrogen may be modulated differently with age.

When seeking a therapeutic target associated with vasodilatation and vasoconstriction in different stages of the female life cycle, precise explanations of vasoactive substances are essential.

Conclusions

Oophorectomy brought about an increase in coronary vascular resistance. L-NNA exacerbated coronary vascular resistance in relation to maturation. It is suggested that the effect of estrogen on vascular dilatation in adolescent rats is largely dependent on a non-NO pathway, whereas adult rats are largely dependent on an NO pathway.

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