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添付資料： 研究報告書

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2．研究テーマ

動脈管収縮弛緩におけるガス状血管弛緩因子の役割

3．成果の概要（100字程度）

動脈管における膜電位依存性カリウムイオンチャンネル(Kv)の発現をRNAレベルと蛋白レベルで明らかにした。Kvは胎生がすすむと減少することがわかった。

Kv発現におけるガス状血管弛緩因子の関与は少ないことがわかった。

4．研究組織

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—日中医学協会助成事業—

動脈管収縮弛緩におけるガス状血管弛緩因子の役割
—カリウムチャンネル発現への影響—

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Abstract

Oxygen-sensitive, voltage-gated potassium channels (Kv) may contribute to the determination of the membrane potential in smooth muscle cells of the ductus arteriosus (DA) and, thus, to regulation of contractile tone in response to oxygen. Developmental changes in Kv during gestation may be related to dilation of the DA during fetal life and closure of the DA after birth. This study investigated developmental changes in the expression of Kv in the DA and compared it with that of the pulmonary artery (PA) and the aorta (Ao). The role of gasotransmitter NO on the expression of Kv was examined by administering a potent nitric oxide synthase inhibitor, Nomega-nitro-L-argininemethyl ester (L-NAME), to a pregrant rat at day 19, 20 and 21 of gestation. The DA, PA and Ao were isolated from fetal rats at days 19 and 21 of gestation (term; 21.5 days). The expression of Kv1.2, Kv1.5, Kv2.1 and Kv3.1, putative oxygen-sensitive Kv channels which open in response to oxygen, was evaluated at both the mRNA and protein levels, using quantitative real-time PCR and immunohistochemistry. In the Kv family studied, Kv1.5 mRNA was expressed most abundantly in the DA, PA and Ao in both day 19 and day 21 fetuses. Although the expression levels of Kv 1.2, Kv1.5, Kv2.1, Kv3.1 did not change much with development in the PA and Ao, in the DA they decreased with development. L-NAME (10mg/kg) did not cause any changes in the expression of Kv 1.2, Kv1.5, Kv2.1, Kv3.1 in the DA in day 20 and day 21 fetus. The role of NO in dilating DA during fetal life may be minimal in the near-term fetal rat. The decrease in the expression of Kv channels may enhance DA closure after birth by eliminating the opening of Kv channels when oxygen increases.

Key words: Ductus arteriosus; Development; Gene expression; Oxygen; Potassium channel. Gasotransmitter

Introduction:

The ductus arteriosus (DA), a vital artery connecting the pulmonary artery (PA) and aorta (Ao) which diverts blood from the PA to the descending Ao during the fetal period, constricts in response to the increase in oxygen (O_2) tension in the blood at birth. The responses of blood vessels to changes in O_2 tension vary depending on the location of the blood vessels and on the developmental stages. The isolated small PA relaxed in response to an increase in O_2 tension, whereas the isolated main PA showed no response or only a slight constriction after an acute increase in O_2 tension. The isolated Ao showed no response to an increase in O_2-tension. However, the DA isolated from the mature fetus constricted markedly in response to an increase in O_2 tension.

Potassium (K) channels play a major role in maintaining the resting membrane potential of vascular smooth muscle cells, thus regulating vascular tone. It is known that some voltage-dependent K-channels (Kv) in arteries are O_2-sensitive. The opening of Kv channels in vascular smooth muscle cells causes membrane hyperpolarization, inactivates voltage-gated Ca^{2+} channels, and decreases the intracellular Ca^{2+} concentration, leading to vasorelaxation.

Only a few studies have reported the expression of Kv channels in the DA and there is no report of a study comparing the expression of Kv channels among the Ao, PA, and DA with advancing gestation during fetal life. As the DA closes while the PA and Ao remain open after birth, there might be a difference in the expression of oxygen-sensitive Kv channels between the DA, PA and Ao. Therefore, our aim was to determine the expression of Kv channels in the DA, PA and Ao at different gestational stages at the mRNA and protein levels.

Nitric oxide is known to dilate vessels including DA. The role of gasotransmitter NO on the expression of Kv remains unknown. It may .stimulate the expression of Kv, thus causing vasodilation during fetal life.

Materials and methods:

We used fetal rats at days 19 and 21 of gestation to examine the developmental expression of Kv1.2, Kv1.5, Kv2.1 and Kv3.1 in the DA, PA and Ao. Total RNAs of the DA, PA and Ao were isolated from pooled segments of 240 day 19 rat fetuses and 140 day 21 rat fetuses. The total RNA was reverse-transcribed into cDNA with random hexamers and MultiScribe™ Reverse Transcriptase (Fostacity, CA, U.S.A). The real-time PCR reaction was conducted with a Premix Ex Taq Kit (Takara Bio) using an ABI PRISM ™ 7300 Sequence Detection System (Applied Biosystems). For each sample analyzed, cDNA copy numbers were calculated on the basis of the results of the standard curve of the same run. The Kv cDNA copy numbers were normalized using the calculated GAPDH cDNA copy number of the same sample.

Tissues of the DA, PA and Ao excised from both day 19 and day 21 fetal rats were fixed in 4% cold

paraformaldehyde for histoimmunochemistry. Rabbit polyclonal antibodies (anti-Kv1.2, anti-Kv1.5, anti-Kv2.1 and anti-Kv3.1) were used.

Results:

The expression of Kv1.2 mRNA in the DA was less than that in the PA and the Ao on both day 19 and day 21 (Fig. 1). In both the PA and Ao, expression of Kv1.2 mRNA in the day 19 fetuses was similar to that in the day 21 fetuses. In the DA, however, the expression of Kv1.2 mRNA in the DA of the day 21 fetuses was less than that in the day 19 fetuses. In the day 19 fetuses, the expression of Kv1.5 mRNA was approximately equivalent in the DA, PA and Ao. In the day 21 fetuses, the expression of Kv1.5 mRNA in the DA was less than that in the PA and Ao .

In both the day 19 and day 21 fetuses, the staining of Kv1.2 was light in the DA, moderate in the PA and intense in the Ao . In the DA, the staining intensity of Kv1.2 in the day 21 fetus was lighter than that in the day 19 fetus.

The role of gasotransmitter NO on the expression of Kv was examined by administering a potent nitric oxide synthase inhibitor, Nomega-nitro-L-argininemethyl ester (L-NAME), to a pregrant rat at day 19, 20 and 21 of gestation. L-NAME (10mg/kg) did not cause any changes in the expression of Kv 1.2, Kv1.5, Kv2.1, Kv3.1 in the DA in day 20 and day 21 fetus.

Discussion:

In this study, we have demonstrated, for the first time, developmental changes in the expression of Kv1.2, Kv1.5, Kv2.1 and Kv3.1 in the DA, PA and Ao in rat fetuses at both mRNA and protein levels. Quantitative real-time PCR data showed a lower mRNA expression of Kv1.2, Kv1.5, Kv2.1 and Kv3.1 in the DA in day 21 fetuses than in day 19 fetuses, and these results corresponded well with the results of protein expression detected by immunohistochemistry. The decrease in the expression of Kv channels in the DA may reduce the opening effect of Kv channels when O_2 tension increases.

L-NAME (10mg/kg) did not cause any changes in the expression of Kv 1.2, Kv1.5, Kv2.1, Kv3.1 in the DA in day 20 and day 21 fetus. The role of NO in dilating DA during fetal life may be minimal in the near-term fetal rat.

In conclusion, the decrease in the expression of Kv channels, which open in response to oxygen, may enhance the DA closure after birth by eliminating the effect of the opening of Kv channels when oxygen increases.

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