Developmental Differences in the Maturation of Sarcoplasmic Reticulum and Contractile Proteins in Large Blood Vessels Influence Their Contractility

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Ductus arteriosus • mRNA expression • Contractile system • Development

Developmental changes in the contractile system of blood vessels such as the ductus arteriosus (DA), pulmonary artery (PA), and aorta (Ao) have not been investigated extensively. We assessed the developmental changes in the expression of genes that regulate vasoconstriction of fetal blood vessels.

DA, PA, and Ao were taken from rabbit fetuses at 21, 27, and 30 days of gestation (full term = 31 days) as well as 2-day-old rabbits. Total DA, PA, and Ao RNA were isolated from pooled segments. Expression of target mRNAs was quantified using absolute quantitative real-time PCR:

1. *Contractile proteins* Contractile activity in smooth muscles is determined primarily by the phosphorylation state of myosin regulatory light chain (MRLC). During muscle contraction, intracellular Ca^{2+} levels increase substantially, and binding of Ca^{2+} to calmodulin activates MLC kinase, which then phosphorylates MRLC. Expression of calmodulin and MLC kinase was not significantly different among the vessels. Expression of tropomyosin 2 was higher in DA compared to PA.
2. *Sarcoplasmic reticulum (SR)* Cytosolic Ca^{2+} levels are increased through Ca^{2+} release from SR and Ca^{2+} entry from the extracellular space via Ca^{2+} channels. Expression of SR cardiac-type ryanodine receptor (RYR) increased throughout fetal maturation and was much higher than skeletal-type RYR. Expression of SR calcium storage protein calsequestrin-2 increased with development in Ao but

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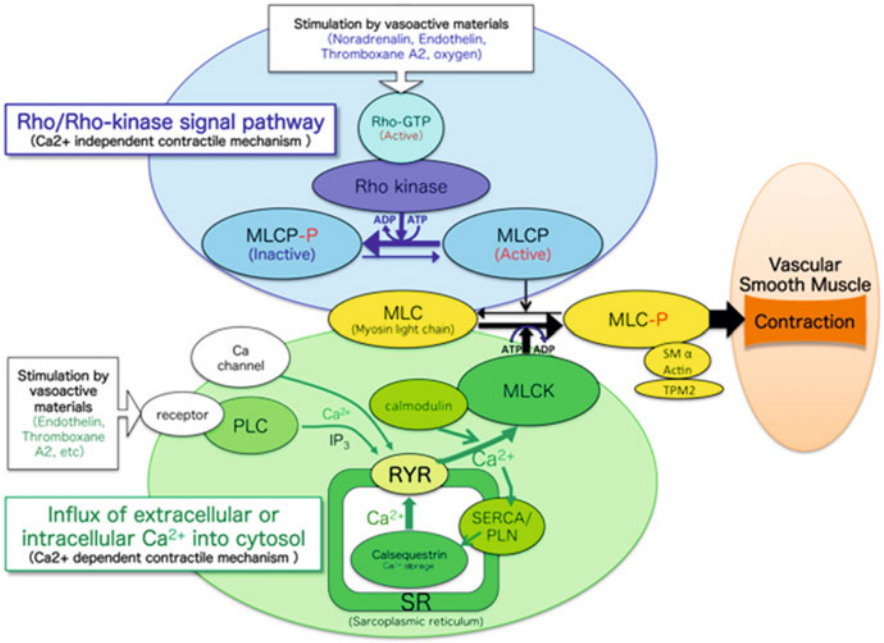

Fig. 36.1 Vascular smooth muscle contraction. Contractile activity in smooth muscle (SM) is determined primarily by the phosphorylation state of myosin regulatory light chain (MRLC). Increase in intracellular Ca^{2+} levels leads to Ca^{2+}-calmodulin binding, which activates MLC kinase (MLCK) to phosphorylate MRLC. Cytosolic Ca^{2+} is increased through Ca^{2+} release from the sarcoplasmic reticulum (SR) and Ca^{2+} entry from extracellular space via Ca^{2+} channels. The Ca^{2+}-independent Rho/Rho kinase pathway inhibits MLC phosphatase (MLCP) activity and promotes phosphorylation of MLC. *RYR* ryanodine receptor, *SERCA* sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase, *PLN* phospholamban, *TPM2* tropomyosin 2

not in DA and PA, with expression levels remaining very low in the latter two. Expression of SR Ca^{2+} pump regulator phospholamban increased with development in PA and Ao but remained very low in DA. The expression of SR genes differs significantly at development stages and is vessel dependent, indicating differential maturity of SR in fetal vessels.

3. *Rho/Rho-kinase* The Ca^{2+}-independent Rho/Rho kinase pathway inhibits MLC phosphatase activity and promotes phosphorylation of MLC. Expression of small GTPase RhoB and Rho kinase-1 was higher than that of RhoA and Rho kinase-2. The expression levels of these Rho/Rho kinase pathway genes were similar in fetal and newborn vessels.

In conclusion, contraction of the premature DA, PA, and Ao may be regulated predominantly by the Rho/Rho kinase pathway, owing to the poor expression of the component protein genes in the immature SR. DA contractile systems may be well developed compared with those of the surrounding PA and Ao (Fig. 36.1).

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