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Rapid communication

Expression of multidrug resistance protein 4 and 5 in the porcine coronary and pulmonary arteries

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Abstract

Guanosine 3′,5′-cyclic monophosphate (cGMP) has an important role in regulating vascular smooth muscle tone. We examined whether mRNA for multidrug resistance protein (MRP) 4 and MRP5, which were recently identified as ATP-dependent export pumps for cyclic nucleotides, is expressed in the porcine coronary and pulmonary arteries. The results showed that both arteries express mRNA for MRP4 and MRP5, and thus these proteins may be novel targets for the prevention and/or treatment of various cardiovascular diseases.

Keywords: cGMP; Multidrug resistance protein; Vascular vessel

Guanosine 3′,5′-cyclic monophosphate (cGMP) is an important second messenger in many biological systems including vascular smooth muscle. Intracellular cGMP levels are controlled by the rate of synthesis by guanylyl cyclase(s), degradation by phosphodiesterase(s) and extrusion from the cell by some transport system(s). The role of cyclic nucleotide transport system(s), unlike those of guanylyl cyclase(s) and phosphodiesterase(s), in the regulation of vascular smooth muscle functions remains to be established. Recently, multidrug resistance protein (MRP) 4 and MRP5 were identified as ATP-dependent export pumps for cyclic nucleotides (Jedlitschky et al., 2000; Chen et al., 2001). Both MRPs can transport adenosine 3′,5′-cyclic monophosphate and cGMP, and cGMP is the higher affinity substrate of these transporters (Jedlitschky et al., 2000; Chen et al., 2001). In the present study, we investigated the expression of MRP4 and MRP5 mRNA in the porcine coronary and pulmonary artery.

Pig hearts and lungs were obtained from a local slaughterhouse. The left descending coronary artery (main branch) and the intrapulmonary artery (the third branch of the main lobar artery) were carefully isolated. The arteries were immersed in ice-cold Krebs–Ringer bicarbonate buffer (in mM: NaCl, 119; KCl, 4.8; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 10) (pH = 7.4) and transferred to our laboratory. The arteries were cleared of connective tissue and cut into ring segments about 2 mm wide. Endothelium was mechanically removed by gently rubbing the luminal surface with a tapered swab.

The porcine vascular vessels were homogenized in TRIZOL reagent (Gibco BRL Life Technologies) and total RNA was extracted. Single-stranded cDNAs were synthesized from total RNA using oligo dT priming and Superscript II Reverse Transcriptase (Gibco BRL Life Technologies). Primers for the amplification of the MRP4 gene (sense 5′-CCATTGAAGATCTTCCTGG-3′; antisense 5′-GGTGTTCAATCTGTGTGC-3′) and those for the MRP5 gene (sense 5′-GGATAACTTCTCAGTGGG-3′; antisense 5′-GGAATGGCAATGCTCTAAAG-3′) were used (Kool et al., 1997). As a control for cDNA synthesis, β-actin-specific primers (sense 5′-GTGGGCCGCCCTAGGCACCA-3′; antisense 5′-TTAATGTCACGCACGATTTC-3′) were used. Polymerase chain reaction (PCR) was performed in a final volume of 50 μl containing 5 μl PCR buffer (10 ×), 4 μl dNTPs (2 mM), 1 μl of each sense and antisense primer (50 pmol/μl), 1 μl cDNA, and 0.25 μl AmpliTaq Gold DNA

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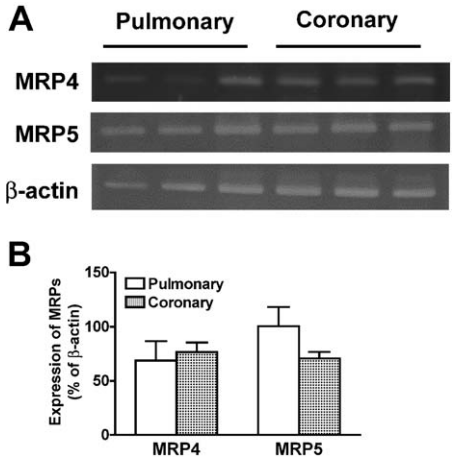

Fig. 1. Detection of MRP4, MRP5 and β-actin mRNA in porcine coronary and pulmonary arteries using RT-PCR. Representative examples (A) and quantitation of signals for MRP4 and MRP5 (B). The intensity of each band was quantified by densitometory: the data are normalized by β-actin mRNA. Data expressed as means ± S.E.M. obtained from three to four separate preparations.

polymerase (5 U/μl; Applied Biosystems), using a programmable thermal controller (PTC-100, MJ Research). The PCR conditions were initial denaturation for 12 min at 95 °C, followed by 42 cycles of 15 s at 94 °C, 30 s at 58 °C, and 45 s at 72 °C, and final extension for 10 min at 72 °C. Aliquots (10 μl) of the PCR products were separated on a 1.6% agarose gel and stained with 0.1% ethidium bromide. The density of the bands was analyzed by NIH image software.

As shown in Fig. 1, we were able to detect the expression of MRP4 and MRP5 mRNA in the porcine coronary and pulmonary arteries by reverse transcriptase (RT)-PCR. These results suggest that MRP4 and MRP5 are present in vascular smooth muscles, and thus that the cyclic nucleotide transport system(s) might contribute to the regulation of intracellular cGMP levels. Because cGMP plays an important role in regulating vascular functions, the transport system(s) may be a novel target for the prevention and/or treatment of various cardiovascular diseases.

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