

Investigations of Coxsackie-adenovirus-receptor (CAR) mRNA expression of rats during organ development and in cultured rat cardiomyocytes - Importance of CAR expression for adenovirus-mediated gene transfer into rat cardiomyocytes

7. SUMMARY

It was the aim of this study to investigate the rCAR1- and rCAR2-mRNA expression in different organs during peripartal organ development of rats and compare it with the rCAR1- and rCAR2-mRNA expression detected in juvenil and adult rats. In order to get inside into regulation of CAR in the cardial system rCAR1- and rCAR2-mRNA was investigated in neonatal rat cardiomyocytes dependend on cell density and culture time. Finally, impact of CAR for adenoviral gene transfer into cardiomyocytes was investigated by expression of hCAR in cultured neonatal rat cardiomyocytes.

The rCAR-isoforms-mRNAs were expressed in all investigated organs. However rCAR2-mRNA expression reached only approximately 10 % of expression of rCAR1-mRNA. However, exclusively in brain, changing of rCAR1- and rCAR2-mRNA expression during organ development was similar. Relative high expression of rCAR2-mRNA in the brain in the early prenatal development indicate that rCAR2 may have a special importance this course of brain development. Additionally investigation should be help to examine this.

Especially in brain, skeletal muscle, heart and kidney a stronger rCAR-mRNA expression was detectable during embyonal and peripartal development compare to expression found in juvenil and adult rats as measured by competitive RT-PCR. The decrease was most prominent in brain and skeletal muscle. No significant changes in rCAR1- and rCAR2-mRNA expression were found in the liver and the lung.

Investigation of cultured neonatal rat cardiomyocytes demonstrated that rCAR-mRNA was significant higher expressed if cells were seeded at a low density (rCAR1 up to 9.1 fold; rCAR2 up to 2.1 fold) compare to cells seeded at a high density if analyzed 24 hrs after isolation of the cells. Decrease of rCAR-mRNA expression was almost linear. Increasing of culture time for 48 hrs and 72 hrs did not result in further changing rCAR1-mRNA expression.

Comparatively high rCAR-mRNA expression in brain, skeletal muscle, heart and kidney during embryonal and neonatal organ development and in cultured neonatal rat cardiomyocytes seeded at low density (result in few cell-cell contacts), indicate that CAR may have importance in organ development, possibly act as a cell adhesion molecule and may be involved in pathfinding for cell-cell contact formation in several organs.

Investigation of cultured neonatal rat cardiomyocytes using a hCAR expressing AdV demonstrate that CAR is involved in adenovector attachment and influences over this way the gene transfer efficiency into cardiomyocytes. So it was shown that hCAR expressed on the surface of cardiomyocytes increased adenovector attachment and uptake as well as transgene expression severalfold.