RHOA-ROCK signalling is necessary for lateralization and differentiation of the sinoatrial node.

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Background: Disturbances in the normal electrical activation pattern of the heart are an important cause of mortality and morbidity in the pediatric and adult population. Up to date, the mechanisms responsible for conduction diseases like sick sinus syndrome remain unknown. RHOA, involved in cellular migration, proliferation and transcriptional regulation, is expressed within the developing cardiac conduction system in chick and disruption in adult mice results in arrhythmias including bradycardia and atrial fibrillation. How this occurs is largely unknown.

Aim: To assess the role of the RHOA-ROCK signaling pathway in the formation and differentiation of the sinoatrial node (SAN) during embryonic development.

Methods: The role of RHOA-ROCK signalling was studied using chemical inhibition (Y-27632) during chicken heart development. The electrogram and atrial activation patterns were studied by ex ovo electrophysiological recordings. The developing SAN area was characterized by gene expression analysis using quantitative real time polymerase chain reaction (qPCR) for known pacemaker genes and with immunohistochemical stainings.

Results: Early in development, the entire myocardium of the inflow area (sinus venosus) of the heart has pacemaker potential. This area includes a ‘transient left-sided’ SAN as well as the definitive right-sided SAN. The pacemaker potential was confirmed by ex ovo electrophysiological measurements, expression of the cation channel HCN4, lack of NKX2.5 and gene expression of the transcription factors SHOX2 and TBX3. Later in development, this pacemaker potential is restricted to the right-sided SAN both in function and in gene expression. Disruption of RHOA-ROCK signalling results in an immature sinus venosus myocardium, maintaining the overall pacemaker potential with a significant lower heart rate. The sinus venosus myocardium has aberrant left-right patterning shown by down-regulation of PITX2C at the left side, up-regulation of NKX2.5 at the right side and global up-regulation of ISL1 expression. ROCK inhibition also results in abnormal pulmonary vein development. In vitro experiments confirm the effect of Y-27632 is exclusive for sinoatrial cardiomyocytes.

Conclusions: Abnormalities in the RHOA-ROCK pathway result in aberrant right/left patterning, abnormal differentiation of the sinus venosus progenitor cell population and abnormal differentiation of the right-sided SAN as definitive pacemaker of the heart.