Expression of the I_{Kr} components KCNH2 (rERG) and KCNE2 (rMiRP1) during late rat heart development

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Abstract

To understand molecular mechanisms that regulate formation and maintenance of cardiac I_{Kr} (rapidly activating component of the delayed rectifier K^+ current), we have investigated the spatiotemporal expression pattern of two rat potassium voltage-gated channels, namely subfamily H (eag-related), member2 (KCNH2) (alias name: rERG) and Isk-related family, member2 (KCNE2) (alias name: rMiRP1) during late embryonic development by means of the *in situ* hybridization technique. KCNE2 is transcribed predominantly in atrial und ventricular myocardium at stages E14.5-E18.5dpc and only a minor signal emerged in the tongue at E16.5dpc. In contrast, KCNH2 transcripts appeared in a less confined pattern with intense signals in atrial and ventricular myocardium, somites, spinal cord, bowel system, central nervous system and thymus at stages E14.5-E18.5dpc. Non-cardiac expression even exceeds the intensity of the cardiac signal, indicating that KCNH2 contributes to K^+ currents in non-cardiac tissue as well. Transcription of the rat β-subunit KCNE2 is present in all regions of the fetal myocardium and co-distributes perfectly with transcription of the pore forming α-subunit KCNH2. It seems likely that KCNH2 and KCNE2 are linked to form cardiac I_{Kr} channels, associated to cardiogenesis and cardiomyocyte excitability.

Keywords: cardiac development; delayed rectifier; ERG; fetal expression; I_{Kr} current; MiRP1

Introduction

Repolarization in phase III of the cardiac action potential is dependent on the rapidly activating component of the delayed rectifier K^+ current (I_{Kr}). A recently isolated human cDNA, hERG (KCNH2) is structurally related to voltage-gated potassium channels, with six membrane-spanning segments (S1-S6), a pore-forming unit and a putative cyclic nucleotide binding domain (cNBD) and has been suggested to encode the α-subunit of cardiac I_{Kr} channels (Warmke and Ganetzky, 1994). Native I_{Kr} channels and KCNH2 channels differ in their functional behavior indicating an assembling with additional potassium channel subunits to establish the cardiac I_{Kr} current. (Sanguinetti *et al.*, 1995; Sanguinetti *et al.*, 1996). In this regard, Abbott *et al.* (1999) isolated a MinK related potassium channel gene, MiRP1 (KCNE2), which after coexpression with KCNH2 subunits, resemble the functional characteristics of native cardiac I_{Kr} channels in their gating, unitary conductance, regulation by potassium, and distinctive biphasic inhibition by the class III antiarrhythmic agent E-4031. Moreover, mutations in KCNH2 and KCNE2 are associated with the long QT syndrome (LQTS), a cardiac disorder, which causes a prolongation of the cardiac action potential leading to polymorphic ventricular arrhythmias and sudden cardiac death (Sanguinetti *et al.*, 1996; Abbott *et al.*,1999). Although the LQTS is not a frequent diagnosis, ventricular arrhythmias are very common with more than 100,000 Americans suddenly dying annually (Kannel, 1987) and an aberrant cardiac re-

polarization process is discussed as the underlying mechanism of these fatal arrhythmias, possibly triggered by variations in the delayed rectifier current I_{Kr} (Willich *et al.*, 1987; Tomaselli *et al.*, 1994; Nuss *et al.*, 1999; Tomaselli and Marban, 1999). Interestingly, gene expression analysis of I_{Kr} components revealed high abundant KCNH2 transcription in adult myocardium and pronounced level in brain, retina, thymus, adrenal gland, striated muscle, lung and cornea of adult rats (Wymore *et al.*, 1997). The KCNE2 subunit gene, however, was reported to be expressed in a more restricted fashion with high level of mRNA in heart and striated muscle of adult rats (Abbott *et al.*,1999), which might imply that formation and myocardial specificity of I_{Kr} is due to the small potassium channel subunit KCNE2. In this regard, Tinel *et al.* (2000) reported that KCNE2 is highly expressed in adult human brain, heart, striated muscle, pancreas, placenta, kidney, colon and thymus and is present on a lower level in liver, ovary, testis, prostate, small intestine and leucocyte.

Compared to the available knowledge at the adult level, little is known about transcription of I_{Kr}-components during embryonic development, a period during which important structural, physiological and biochemical changes occur. Electrophysiological analysis provided evidence that I_{Kr} is the prominent delayed rectifier current in cultured murine fetal cardiomyocytes, whereas I_{Ks} currents were only detectable in later-stage ventricular cells (Davies *et al.*, 1996). In the current study, we investigated the expression of the I_{Kr} forming components KCNH2 and KCNE2 during late embryonic development of the rat and found that both I_{Kr} forming components were expressed in the atrial and ventricular chambers of the fetal rat heart.

Material and Methods

Preparation of rat embryos

Wistar rats were mated over night. Vaginal plug was checked the next morning and noon of that day corresponded to embryonic age 0.5 dies post conceptionem (dpc). Pregnant rats were killed by cervical dislocation, the uterus was dissected out from the abdomen, rinsed in ice cold phosphate buffered saline (PBS), pH 7.4 and embryos of the desired age were isolated and instantly frozen on dry ice. Frozen sections (15 μm) of either sagital or thoracic transversal layers were prepared and thaw-mounted onto SuperFrost Plus slides (Menzel-Glaser).

All experimental procedures have been performed according to the recommendation from the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals.

Oligonucleotides

The following rat specific antisense DNA oligonucleotides were synthesized (Amersham Pharmacia Biotech): rat cardiac specific troponin I-probe: CCTG CTTCGCAATCTGCAGCATCAGAGTCTTCAACTGAA GTTTTCTGGAGG (51 mer, GenBank accession number X58499: position 236-287), rat KCNH2-specific probe: TTTTATCTTGGGTGCTATGATTTCCCGGTCA-CTGGTGGGTGAAGC (45 mer, GenBank accession number Z96106: position 1054-1098) and rat KCNE2-specific probe: GTTCGGCTGTTGTGTTCCTCCTCC-AGCTGTCCATATAAGTAATGA (45 mer, GenBank accession number: AF071003, position 90-134). No significant sequence homology was found between our rERG1 (KCNH2) probe and two further members of the rat ERG gene family, rERG2 and 3, respectively. In addition, the KCNE2 oligonucleotide used in this study shows no significant homology with sequences of the rat KCNE1 or rat KCNE3 genes.

In situ hybridization

Oligodesoxynucleotide probes were labeled at the 3'-end with [α-^{35}S]-dATP using terminal deoxynucleotidyl transferase (TdT-kit, Roche). The labeled probes were denaturated (5 min, 95°C) and 3×10^5 cpm were applied to 100 μl hybridization buffer. Control experiments were performed either by adding 100 × excess of unlabeled probe or using a labeled sense-strand probe. *In situ* hybridization was carried out in a humid chamber with 50% formamide, 4× SSC, 10% dextrane-sulphate for 10-14 h at 42°C (Wisden and Morris, 1994). Samples were rinsed in 1× SSC at room temperature (RT), washed for 20 min in 1× SSC at 60°C, 1min in 1× SSC at RT, 5 min in 0.1× SSC at RT, dehydrated in ethanol, air dried for 15 min and exposed to Kodak Biomax MR X-ray film for 3-6 days at RT. Pictures were taken using a Zeiss light-microscope (Axioplan) and processed in Adobe Photoshop.

Results

In situ hybridizations with radioactive oligonucleotides were performed on sagittal and transversal sections of rat embryos. To validate the experimental approach and to specify cardiac tissue within the complex anatomy of the rapidly developing embryo, a rat cardiac specific troponin I (cTnI) cDNA was used for in situ hybridization and proved to bind selectively to embryonic heart tissue from E14.5dpc to E18.5dpc (Figure 1A, 1D, 1G, 2A and 2D). The cTnI gene is exclusively and strongly expressed in all regions of the embryonic heart (ventricle, atrium, outflow tract) with a dense and evenly distributed signal, which remained un-

changed during late embryogenesis.

Transcripts of the rat I_{Kr} component KCNE2 (rMiRP1) were predominantly observed in the ventricular and atrial wall during embryonic stages E14.5 to E18.5dpc (Figure 1B, 1E, 1H, 2B and 2E) and a very weak signal appeared in the tongue at stages E16.5 and E18.5dpc (Figure 1E and 1H). The pattern of KCNE2 expression remained constant from stages E14.5 to E18.5dpc (Figure 1B, 1E, 1H, 2B and 2E) with myocardial expression level similar to cTnI level (Figure 1A, 1D, 1G, 2A and 2D). In the tongue, however, we observed a reduction of KCNE2 expression at E18.5dpc (Figure 1H).

In contrast to KCNE2, transcription of the pore-forming subunit KCNH2 (rERG1) displayed a more ubiquitous expression pattern with prominent signals in spinal cord, heart and brain tissue and a modest signal in somites at stage E14.5dpc (Figure 1C).
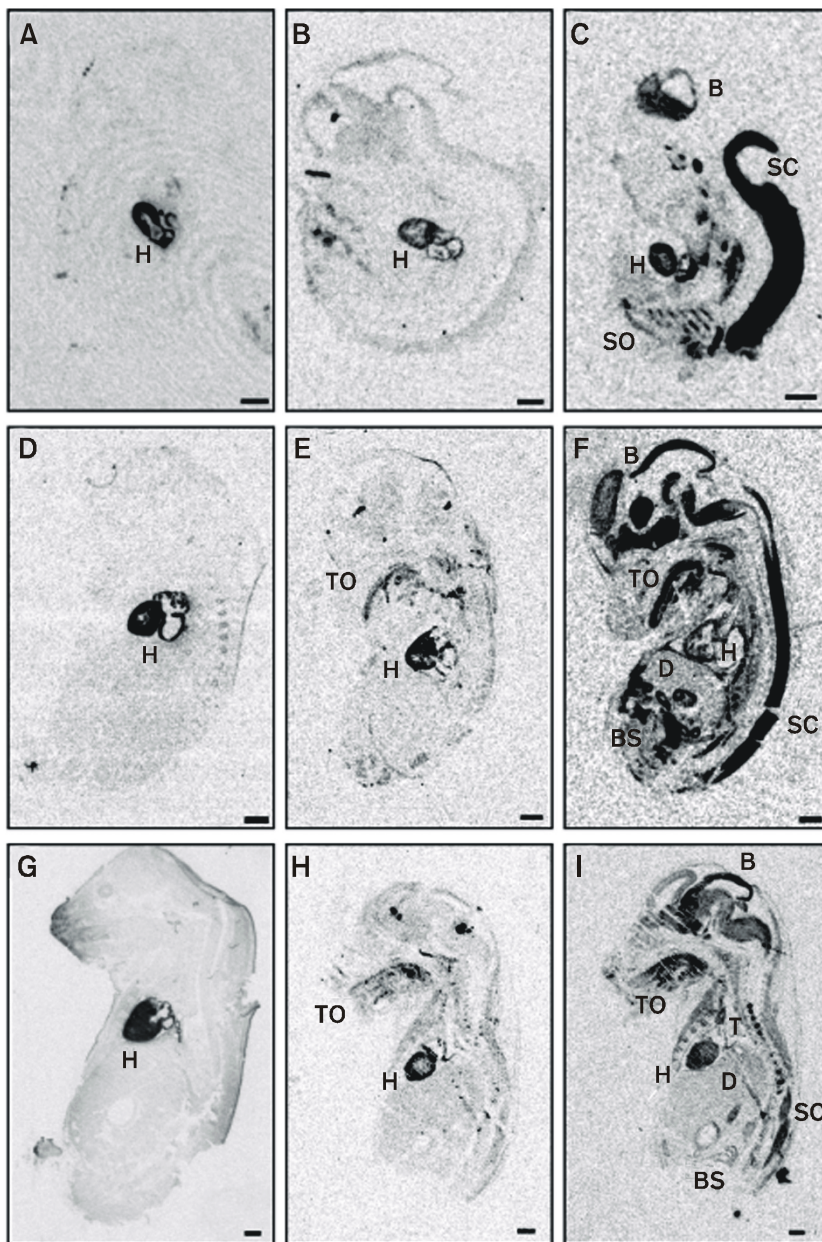

Figure 1. *In situ* hybridization of sagittal embryo sections (E14.5-E18.5dpc). Expression of cTnI is restricted to myocardial tissue in the course of embryonic development. Note the high abundant and exclusive cTnI signal in atrium, ventricle and outflow tract at embryonic stages E14.5dpc (A), E16.5dpc (D) and E18.5dpc (G). Similarly, KCNE2 signals are evenly distributed in ventricle, atrium and outflow tract at stage E14.5dpc (B). During further development, KCNE2 is continuously expressed in myocardium and only a weak signal appeared in the tongue at embryonic stage E16.5dpc (E). At stage E18.5dpc, KCNE2 expression remained constant in myocardium but declined in the tongue (H). A very different pattern was observed for KCNH2 at embryonic stage E14.5dpc with strong signals in spinal cord, myocardium and brain and less intense signals in somites (C). During developmental progress, additional KCNH2 signals appeared in diaphragm and bowel system at E16.5dpc (F) and in thymus at E18.5dpc (I). Interestingly, like KCNE2, the extra-cardiac expression of KCNH2 declined at stage 18.5dpc (I). Bar is 1 mm. B, Brain; BS, Bowel system; D, Diaphragm; H, Heart; SC, Spinal cord; SO, Somites; T, Thymus; TO, Tongue.
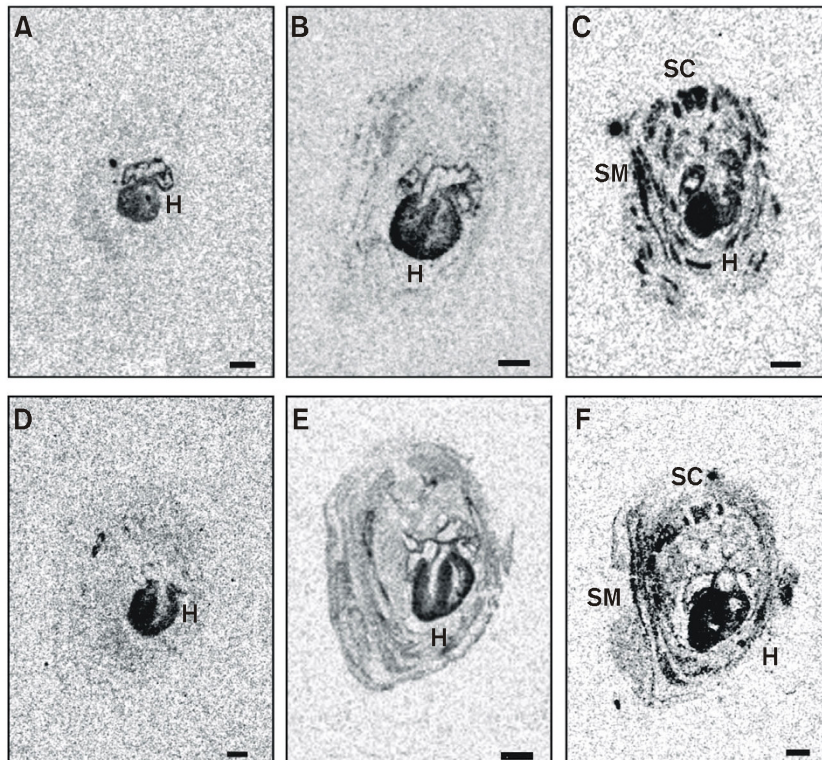
Figure 2. *In situ* hybridization on transversal sections (thorax) with cTnI, KCNE2 and KCNH2 oligonucleotides. Both stages, E16.5 dpc (A, B, C) and E18.5dpc (D, E, F), display dense and evenly distributed signals of cTnI (A, D), KCNE2 (B, E) and KCNH2 (C, F) in all parts and layers of the heart. Bar is 1 mm. H: Heart, SC: Spinal cord, SM: Striated muscle.

Moreover, KCNH2 expression was detected in the bowl system, diaphragma, skeletal muscle and tongue at stage E16.5dpc (Figure 1F and 2C) and in the thymus at stage E18.5dpc (Figure 1I).

Interestingly, KCNH2 transcription declined in non-cardiac tissue (Figure 1I and 2F) with the strongest reduction in spinal cord and brain at stage E18.5dpc (Figure 1I).

It seems noteworthy, that KCNE2 and KCNH2 transcripts appear on equivalent level and are evenly distributed throughout the ventricular and atrial wall at embryonic stages 16.5dpc (Figure 2A-C) and 18.5 dpc (Figure 2D-F) with a signal intensity comparable to the rat cardiac troponin I gene.

Discussion

I_{Kr} currents have been suggested to influence and stabilize the rhythm of heart contraction with respect to fetal heart development. This idea has been supported by the observation of embryotoxic effects in pregnant rats, where application of I_{Kr} specific channel blocker, dofetilide, has been described to strongly impair the rhythm of embryonic hearts with lethal effects (Spence *et al.*,1994; Webster *et al.*, 1996). To explore the expression pattern of the I_{Kr} forming components KCNH2 (hERG) and KCNE2 (MiRP1), we analysed, by means of *in situ* hybridiza-

tion, sagittal and transversal sections in late rat embryonic development.

Pronounced level of rat KCNH2 transcripts were observed homogeneously distributed in atrial and ventricular myocardium at embryonic stages E14.5 to E18.5dpc, an expression pattern identical to the cardiac expression in fetal mice (Franco *et al.*, 2001). Additionally, we detected KCNH2 expression in non-cardiac tissue with high level of mRNA in brain, spinal cord, somites, diaphragm, bowel system and thymus at stage E14.5-E18.5dpc.

A very similar distribution of KCNH2 transcripts was observed in adult rats (Wymore *et al.*, 1997) indicating that KCNH2 might contribute to potassium currents in non-cardiac tissue in embryonic and adult rats as well.

In contrast, fetal transcription of the rat KCNE2 gene revealed a pattern very different from the expression pattern of KCNH2 with strong and even signals in all layer of atrial and ventricular myocardium and a very weak signal in the tongue. At late-gestation, KCNE2 expression declined in non-cardiac tissue but remained high in the entire myocardium. In opposite to our findings, Franco *et al.* (2001) reported a selective expression of KCNE2 in atrial myocardium. However, given a central role of I_{Kr} channels during early cardiac development their observation suggest a diversity of β-subunits required to establish atrial and ventricular specific I_{Kr} channels

in mouse myocardium.

There is evidence that formation of human cardiac I_{Kr} channel required coexpression of KCNE2 and KCNH2 (Abbott *et al.*, 1999). As reported herein, transcription of the rat β-subunit KCNE2 in all regions of the fetal myocardium co-distributes perfectly with transcription of the pore forming β-subunit KCNH2. Thus, it seems likely that during rat fetal development KCNH2 and KCNE2 are linked to form cardiac I_{Kr} channels.

Acknowledgment

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