Volume 284, number 2, 152–154 FEBS 09827 June 1991

ADONIS 001457939100536Y

Developmental expression of cloned cardiac potassium channels

Steven L. Roberds[1] and Michael M. Tamkun[1,2]

[1]*Departments of Pharmacology and of* [2]*Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232-0615, USA*

Received 18 March 1991; revised version received 12 April 1991

Cardiac K^+ channels are responsible for repolarization of the action potential and are the targets of several antiarrhythmic drugs. This study examines the differential expression of six K^+ channel mRNAs during rat heart development. RK1 and RK2 K^+ channel transcripts were undetectable prior to 10 days after birth. In contrast, RK4 mRNA was present at equivalent levels from day 14 in utero to 20 days after birth. RK3 and RK5 were detected as early as 14 days in utero. These data indicate that K^+ channel expression in the heart is closely regulated and further argue for physiologically distinct roles for K^+ channel isoforms.

Potassium channel; Cardiac development; mRNA regulation

1. INTRODUCTION

K^+ channels are the most diverse group of voltage-gated ion channels, and cDNA molecules encoding numerous channel isoforms have been cloned [1–5]. K^+ channel diversity is generated by the expression of multiple genes [4,6], alternative splicing of transcripts arising from a single gene [1], and assembly of individual K^+ channel gene products into heteromultimers [7]. These isoforms are both structurally and functionally distinct. Electrophysiological studies [8] indicate that K^+ currents vary during development of the rat, suggesting that variation in the level of channel expression during development adds another dimension to K^+ channel diversity. At the molecular level, Beckh and Pongs, and Swanson et al. examined the pattern of expression of seven distinct K^+ channel mRNAs over time in neonatal and adult rat brain [4,9].

We have previously shown that the tissue-specific expression of K^+ channel isoforms is individually regulated [5]. In this report, we examine the developmental changes in cardiac expression of six voltage-gated K^+ channels: RK1–5 [5] and drk1 [3]. RK4, the only channel of the group present at a higher level in heart than brain [5], is also the only channel expressed at relatively constant levels from 14 days in utero to 20 days post partum. The other K^+ channels increase at different rates during development, indicating that expression of each channel is individually regulated.

Correspondence address: S. Roberds, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232-6600, USA. Fax: (1) (615) 322-7236

2. MATERIALS AND METHODS

Hearts were dissected from decapitated Sprague–Dawley rats of the indicated age and total RNA was isolated using the guanidinium thiocyanate method [10]. The μg of total RNA per lane were separated by electrophoresis through a 1% agarose, 3% formaldehyde gel in the absence of ethidium bromide. The gel was submerged for 5 min in 50 mM NaOH and 1.5 M NaCl, neutralized for 30 min in 1 M Tris pH 6.8 and 1.5 M NaCl, then transferred overnight to Nytran (Schleicher and Schuell) by capillary action in 20 × SSC (3 M NaCl, 300 mM sodium citrate, pH 7.0). Five min of base treatment results in optimal RNA transfer and hybridization based on control experiments performed in this laboratory (data not shown). RNA was crosslinked to the Nytran by irradiation with ultraviolet light. The gel was then stained with ethidium bromide to assess the extent of electrophoresis and efficiency of transfer. Filters were prehybridized overnight at 65°C in 20% formamide, 10% dextran sulfate, 4 × SSPE (600 mM NaCl, 40 mM NaH_2PO_4, 4 mM EDTA, pH 7.4), 5 × BFP (1 g/l bovine serum albumin, 1 g/l polyvinylpyrrolidone-40, 1 g/l Ficoll, 0.001% sodium azide), 0.1 mg/ml sonicated salmon sperm DNA, 0.2 mg/ml yeast RNA, and 5% SDS. Each filter was hybridized 24 h at 65°C in the same solution with 10^6 cpm/ml of cDNA probe. The K^+ channel cDNA fragments used as probes [5] were isoform-specific, as they did not cross-hybridize with any other clones. However, it is conveivable that a probe might recognize other, unidentified K^+ channel isoforms. Filters were washed twice for 30 min at 65°C with 3 × SSC and 1% SDS, twice with 1 × SSC and 1% SDS, and twice with 0.2 × SSC and 1% SDS before autoradiography.

3. RESULTS AND DISCUSSION

Total RNA was isolated from the hearts of rats 14 days in utero (E14 days), 20 days in utero (E20 days), 10 days post partum (P10 days), and 20 days post partum (P20 days). At E14 days, the heart has been beating for 4 days, and the aorta is separating from the pulmonary artery [11]. By E20 days, the heart is essentially in its adult anatomical form [11]. Parasympathetic (vagal) innervation of the heart occurs at approximately E13 days

Published by Elsevier Science Publishers B.V.

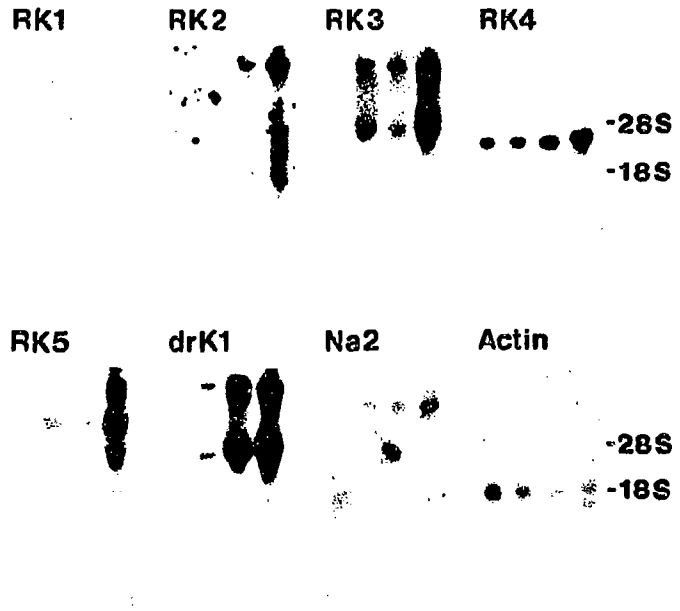

Fig. 1. Hybridization analysis of cardiac potassium channel isoform expression. RNA was isolated from animals 14 days in utero (E14), 20 days in utero (E20), 10 days post partum (P10), and 20 days post partum (P20), lanes from left to right, respectively. Following transfer to Nytran, each blot was hybridized with isoform-specific cDNA probes encoding the appropriate K^+ or Na^+ channel as described in Materials and Methods. The experiments were performed twice and yielded identical results. The positions of 28S and 18S ribosomal RNA are indicated. The filters probed with channel DNA were exposed for 21 days while the filter probed for actin was exposed for 90 min. All ion channel hybridizations were performed using original blots, while the β-actin hybridization was performed by reprobing the RK4 blot.

[12]. Functional sympathetic innervation to the sinus node occurs between E15 and E17 days [13], but the concentration of norepinephrine in total newborn rat heart is only 5% that of adult [12]. At P22 days, the pattern of adrenergic neuron distribution in the heart is finalized, although fibers increase in density and thickness until P35 days [12].

Fig. 1 shows the results of blot hybridization analysis using cDNA probes representing seven rat ion channels and chicken β-actin. RK1 transcripts are present at extremely low levels during all stages of development. However, RK1 does increase somewhat between P10 and P20 days. At this point, the RK1 probe hybridizes equally well to two transcripts of approximately 10 and 11 kb. Beckh and Pongs observed that RK1 mRNA increases between P7 and P30 days in the corpus striatum, inferior colliculus, and medulla-pons of the rat CNS [9]. Little RK1 expression was detected by these investigators in embryonic sensory ganglia or medulla.

RK2 mRNA (approximately 12 kb in length) is undetectable in utero. However, RK2 is detectable at low levels at P10 days and is much more abundant at P20 days. This suggests that RK2 may play a more important role in the maturing rat heart than in the prenatal heart. The increase in RK2 correlates with sympathetic innervation of the ventricles. However, it is not known at this time whether myocytes, neurons, or both are expressing RK2. RCK5, a K^+ channel clone nearly identical to RK2, increases in abundance from birth to P30 days in three regions of the rat CNS [9].

RK3 mRNA is present at E14 days and increases gradually during early development up to P20 days. Two transcripts of 4 and 12 kb are present in approximately equal amounts. Since the levels of these transcripts increase simultaneously, they may arise from a single gene. Use of alternative splice sites in untranslated regions, alternative transcription start sites, or alternative polyadenylylation sites could explain the presence of multiple hybridizing transcripts. Beckh and Pongs observed that RCK4, a K^+ channel clone similar to RK3, is present at relatively constant levels from birth through adulthood in the corpus striatum and inferior colliculus while in the medulla-pons region expression decreases with age [9]. Unlike RK1 and RK2, the regulation of RK3 expression appears to be different between heart and brain.

Table I

Expression of K^+ channel isoforms during rat heart and brain development

	RK1	RK2	RK3	RK4	RK5	drk1
Rat heart						
E14	–	–	+	+ + +	+	+
E20	–	–	+ +	+ + +	+	+ +
P10	+	+ +	+ +	+ + +	+	+ + +
P20	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Rat brain						
E18	–	–	+ +	ND	ND	ND
P1	–	+ +	+ + + +	+	ND	ND
P7	+	+ +	+ + +	ND	ND	ND
P30	+ + + +	+ + + +	+ +	+ + + +	ND	ND

Brain data for RK1-3 are from [9] (RCK1 = RK1, RCK5 = RK2, and RCK4 = RK3) and apply to the medulla-pons region only. Brain RK4 data are from [4] (K_v1 = RK4) and apply to whole rat brain. Transcript levels are standardized to the maximum observed. ND, not determined.

RK4 transcript levels are atypically constant during development with only a slight increase between P10 and P20 days. Interestingly, RK4 is the only channel studied here which is more prevalent in heart than brain [5]. While RK1, RK2, RK4, and drk1 are all delayed rectifier-like channels when expressed in *Xenopus oocytes* [2-4], only RK4 is present at high levels in the prenatal heart. The apparent increase in size of the 3.5 kb transcript at P20 days is an artifact of electrophoresis in that lane of the gel. When the same blot was reprobed with β-actin cDNA, the β-actin transcript also appeared slightly larger than in other lanes.

RK5 mRNA is present only at low levels as a 6 kb transcript through P10 days. At P20 days the abundance of RK5 message suddenly increases, and additional transcripts of 3.5 and 12 kb are detected. The 12 kb band is absent from brain tissue [5], suggesting that alternative transcript processing may be tissue-specific. Novel transcription start sites, polyadenylylation sites, or mRNA splice sites may be used in the 20-day-old rat heart. More investigation is needed to determine the mechanism and physiological significance of this observation.

Transcripts encoding drk1 are present at low levels in utero, and at high levels at both P10 and P20 days. Thus, drk1 is likely to play a much more significant role after birth than before. Rat brain Na^+ channel II mRNA, much like RK1, is present at low levels at all stages. However, a slight increase is noted between P10 and P20 days. Transcripts hybridizing to a full-length chicken β-actin cDNA probe [14] are present at comparable levels at all stages of development, but expression does decrease slightly from day P10. Therefore, channel expression is best standardized to total RNA loaded. Since β-actin is present only at very low levels in heart [15], the transcripts hybridizing to this cDNA probe most likely represent cardiac α-actin [15].

As summarized in Table I, regulation of ion channel expression in the developing heart is clearly complex, with each ion channel's pattern of expression being unique. In addition, the expression patterns differ between brain and heart. In brain RK3 is expressed at highest levels during embryonic development while in heart the highest levels of expression are observed at P20 days. While every ion channel examined is expressed in heart at its highest level 20 days post partum, only RK4 is expressed at a relatively constant level at all time points. One hypothesis suggested by these data is that RK4 plays an important role in the basic function of the heart and that other ion channels might take on various regulatory roles as the heart matures. Further understanding of the differences in function and cellular localization between K^+ channel isoforms is required to elucidate the physiological role of cardiac channel diversity. Once the physiological roles of these K^+ channel isoforms are better understood, the significance of complex ion channel regulation during cardiac development will be appreciated.

Acknowledgements: The authors thank Deborah Glover for technical assistance. This research was supported by NIH Grant GM 41325 (MMT) and was done during the tenure of a Grant-in-Aid award (MMT) from the American Heart Association and Winthrop Pharmaceuticals. SLR was supported by NIH Training Grant GM 07628 and an Advanced Predoctoral Fellowship in Pharmacology/Toxicology from the Pharmaceutical Manufacturers Association Foundation.

REFERENCES

[1] Schwarz, T.L., Tempel, B.L., Papazian, D.M., Jan, Y.N. and Jan, L.Y. (1988) Nature 331, 137-142.
[2] Stühmer, W., Ruppersberg, J.P., Schröter, K.H., Sakmann, B., Stocker, M., Giese, K.P., Perschke, A., Baumann, A. and Pongs, O. (1989) EMBO J. 8, 3235-3244.
[3] Frech, G.C., VanDongen, A.M.J., Schuster, G., Brown, A.M. and Joho, R.H. (1989) Nature 340, 642-645.
[4] Swanson, R., Marshall, J., Smith, J.S., Williams, J.B., Boyle, M.B., Folander, K., Luneau, C.J., Antanavage, J., Oliva, C., Buhrow, S.A., Bennett, C., Stein, R.B. and Kaczmarek, L.K. (1990) Neuron 4, 929-939.
[5] Roberds, S.L. and Tamkun, M.M. (1991) Proc. Natl. Acad. Sci. USA 88, 1798-1802.
[6] Wei, A., Covarrubia, M., Butler, A., Baker, K., Pak, M. and Salkoff, L. (1990) Science 248, 599-603.
[7] Ruppersberg, J.P., Schröter, K.H., Sakmann, B., Stocker, M., Sewing, S. and Pongs, O. (1990) Nature 345, 535-537.
[8] Aguayo, L.G. (1989) J. Physiol. 414, 283-300.
[9] Beckh, S. and Pongs, O. (1990) EMBO J. 9, 777-782.
[10] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K., eds. (1989) Current Protocols in Molecular Biology, Wiley, New York.
[11] Beaudoin, A. (1980) in: The Laboratory Rat (Baker, H.J., Lindsey, J.R. and Weisbroth, S.H., eds.) vol. II, pp. 75-103. Academic Press, New York.
[12] Pappano, A.J. (1977) Pharmacol. Rev. 29, 3-33.
[13] Shigenobu, K. (1990) in: Developmental Cardiology: Morphogenesis and Function (Clark, E.B. and Takao, A., eds.) pp. 273-289. Futura, Mount Kisco, NY.
[14] Cleveland, D.W., Lopata, M.A., MacDonald, R.J., Cowan, N.J., Rutter, W.J. and Kirschner, M.W. (1980) Cell 20, 95-105.
[15] Bains, W., Ponte, P., Blau, H. and Kedes, L. (1984) Mol. Cell. Biol. 4, 1449-1453.