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ScienceDirect
European Journal of Pharmacology 562 (2007) 130–131
www.elsevier.com/locate/ejphar

Rapid communication

Effect of experimental hypercholesterolaemia on K^+ channel α-subunit mRNA levels in rabbit hearts

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Received 11 December 2006; received in revised form 28 January 2007; accepted 1 February 2007
Available online 16 February 2007

Abstract

We investigated the effect of dietary cholesterol on gene transcription of delayed rectifier (I_{Kr} — ERG1 and I_{Ks} — KvLQT1) and transient outward ($I_{to,fast}$ — Kv4.2 and Kv4.3) potassium channel subunits in rabbit hearts using real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). While the level of Kv4.3 mRNA did not change, both Kv4.2 and ERG1 mRNAs were downregulated, whereas the level of KvLQT1 was increased in hypercholesterolaemic rabbits, indicating that hypercholesterolaemia altered ventricular K^+ channel α-subunit gene transcription.

Keywords: K^+ channel; mRNA expression; Hypercholesterolaemia

Hypercholesterolaemia is often associated with abnormal electrical activities in the myocardium characterized by longer QT interval in the ECG, afterdepolarizations and ventricular arrhythmias (Adamantidis et al., 1992; Liu et al., 2003). Nevertheless, no data are available whether the expression of K^+ channel α-subunits implicated in the development of arrhythmias, as summarized previously by Tristani-Firouzi et al. (2001), changes in hypercholesterolaemia. Therefore, the purpose of the present study was to evaluate the effects of diet-induced hypercholesterolaemia on gene transcription of these repolarizing outward currents in rabbit ventricles.

Adult, male New Zealand white rabbits were maintained on 1.5% cholesterol-enriched diet ($n=4$) over 8 weeks preceding the experiments as described previously (Szilvassy et al., 2001), yielding an increase in serum cholesterol level from 1.7±0.4 to 24.1±2.9 mmol/l. Control animals were given standard lab chow ($n=4$). All experiments were approved by the ethics committee of the Medical and Health Science Center of our University. Total RNA was extracted from left ventricular muscle samples (2–3/rabbit) using the RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the supplier's instructions. DNase-treated total RNA preparations (2 μg) were reverse transcribed by using random primers (Invitrogen) and Super-Script™ II reverse transcription kit (Invitrogen) according to the manufacturer's recommendations, followed by RT-PCR using ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Primer pairs and TaqMan probes for RT-PCR were designed by Primer Express software (Applied Biosystems) based on published rabbit mRNA sequences (http://www.ncbi.nlm.nih.gov/GenBank) for Kv4.2 (GenBank accession no. AF493547), for Kv4.3 (AF085170), for KvLQT1 (AJ291316), for ERG1 (U87513) and for 18S rRNA (X06778). The oligonucleotide sequences are available at http://biochemistry.med.unideb.hu/SBBG/OligoDB.htlm. PCR reaction was carried out in a 10 μl volume containing final concentrations of 1×Taq buffer (Fermentas), 0.1 mM dNTP mix (Fermentas), 2.5 mM $MgCl_2$ (Fermentas), 1×ROX reference Dye (Invitrogen), 500 nM forward and reverse primer each (Bio-Science), 100 nM TaqMan probe (Bio-Science), 1 μl cDNA and 0.25 units of Taq DNA polymerase (Fermentas). Thermal cycling conditions for amplification were 1 min at 94 °C, followed by 40 cycles of 15 sec at 94 °C, 60 sec at 60 °C (for ERG1 and Kv4.2), at 58 °C (for KvLQT1) or at 55 °C (for Kv4.3), respectively.

In present RT-PCR studies left ventricular mRNA expressions of ERG1, KvLQT1, Kv4.2, Kv4.3 were quantified from

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doi:10.1016/j.ejphar.2007.02.003

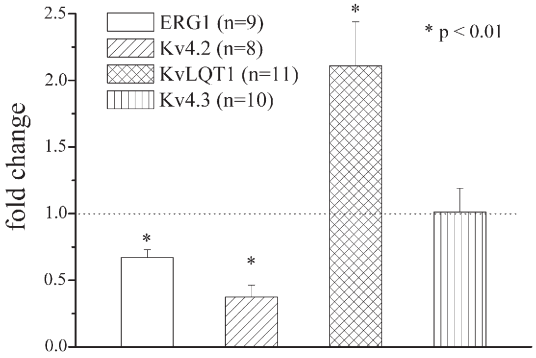

Fig. 1. Relative gene expression of potassium channel transcripts expressed as $2^{(-\Delta\Delta Ct)}$, where the threshold cycle difference (ΔCt) was determined as $Ct_{Kv} - Ct_{18S\ rRNA}$ for each sample and normalized to average ΔCt for untreated sample. The data are presented as mean±SEM. Statistically significant differences between the two groups were calculated by the Student's test for paired data. Number of experiments are also indicated in parenthesis.

control and hypercholesterolaemic rabbit hearts. We performed 8–11 RT-PCR measurements for each transcript in technical triplicates, using 4 animals from either group. Using the $2^{(-\Delta\Delta Ct)}$ method (Livak and Schmittgen, 2001), the data are presented as the fold change in gene expression normalized to 18S rRNA as an endogenous reference and relative to the untreated sample to permit comparison between the 2 groups. Most transcripts studied reached Ct values within the range of 27 to 34, as evaluated in the control and hypercholesterolaemic samples. Both Kv4.2 and ERG1 mRNA expression were markedly downregulated (0.38±0.13, $n=8$ and 0.67±0.06, $n=9$, respectively; $p<0.01$), whereas KvLQT1 mRNA was significantly upregulated (2.11±0.33, $n=11$; $p<0.01$) in samples from hypercholesterolaemic rabbits compared to those seen in controls (Fig. 1). However, Kv4.3 mRNA expression exhibited no difference (1.01±0.18, $n=10$) between the two groups (Fig. 1).

These results clearly show that there were significant downregulations of both Kv4.2 and ERG1 transcription along with increased KvLQT1 expression in the heart of hypercholesterolaemic rabbits. In contrast, Kv4.3 mRNA level was unaffected in cholesterol fed rabbits. Our present study using RT-PCR is the first to evaluate an association between diet-induced hypercholesterolaemia and alterations in the level of potassium channel transcripts in hearts of rabbits. Nevertheless, other genes producing a variety of potassium channel subunits, such as Kir, minK, or MIRP have not been examined, whether or not they are affected by cholesterol treatment. In addition, correlation of the mRNA levels with protein levels also need to be determined. We conclude that arrhythmogenic nature of hypercholesterolaemia may partially be mediated by a reduction in Kv4.2 and ERG1 with a concomitant elevation in KvLQT1 expression in rabbit hearts. It is known that a decrease in repolarizing potassium currents prolongs action potential that triggers arrhythmias through generating early afterdepolarizations, a basic mechanism of arrhythmogenesis (Nabauer and Kaab, 1998). As this rabbit model mimics human hypercholesterolaemia, these findings may provide fundamental significance in comprehension as well as in the research of heart diseases caused by hypercholesterolaemia.

Acknowledgements

This study was supported by GVOP2005-3.3.1-05/1-2005-05-0069/3.0 and by Ceramed. We thank Dr János Kádas and Dr Istvan Szatmari for their helping in RT-PCR experiments.

References

Adamantidis, M.M., Moreau-Guedon, L., Martin-Nizard, F., Sqalli-Houssaini, H., Duriez, P., Fruchart, J.C., Dupuis, B., 1992. Oxidized low density lipoproteins exert arrhythmogenic effects in rabbit Purkinje fibers. Biochem. Biophys. Res. Commun. 182, 548–554.

Liu, Y.B., Wu, C.C., Lu, L.S., Su, M.J., Lin, C.W., Lin, S.F., Chen, L.S., Fishbein, M.C., Chen, P.S., Lee, Y.T., 2003. Sympathetic nerve sprouting, electrical remodeling, and increased vulnerability to ventricular fibrillation in hypercholesterolemic rabbits. Circ. Res. 92, 1145–1152.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. Methods 25, 402–408.

Nabauer, M., Kaab, S., 1998. Potassium channel down-regulation in heart failure. Cardiovasc. Res. 37, 324–334.

Szilvassy, Z., Csont, T., Pali, T., Droy-Lefaix, M.T., Ferdinandy, P., 2001. Nitric oxide, peroxynitrite and cGMP in atherosclerosis-induced hypertension in rabbits: beneficial effects of cicletanine. J. Vasc. Res. 38, 39–46.

Tristani-Firouzi, M., Chen, J., Mitcheson, J.S., Sanguinetti, M.C., 2001. Molecular biology of K(+) channels and their role in cardiac arrhythmias. Am. J. Med. 110, 50–59.