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Profiling of cAMP and cGMP phosphodiesterases in isolated ventricular cardiomyocytes from human hearts: Comparison with rat and guinea pig

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ABSTRACT

Aims: Phosphodiesterases (PDEs) are key enzymes controlling cAMP and cGMP levels and spatial distribution within cardiomyocytes. Despite the clinical importance of several classes of PDE inhibitor there has not been a complete characterization of the PDE profile within the human cardiomyocyte, and no attempt to assess which species might best be used to model this for drug evaluation in heart disease.
Main methods: Ventricular cardiomyocytes were isolated from failing human hearts of patients with various etiologies of disease, and from rat and guinea pig hearts. Expression of PDE isoforms was determined using RT-PCR. cAMP- and cGMP-PDE hydrolytic activity was determined by scintillation proximity assay, before and after treatment with PDE inhibitors for PDEs 1, 2, 3, 4, 5 and 7. Functional effects of cAMP PDEi were determined on the contraction of single human, rat and guinea pig cardiomyocytes.
Key findings: The presence and activity of PDE5 were confirmed in ventricular cardiomyocytes from failing and hypertrophied human heart, as well as PDE3, with ventricle-specific results for PDE4 and a surprisingly large contribution from PDE1 for hydrolysis of both cAMP and cGMP. The total PDE activity of human cardiomyocytes, and the profile of inhibition by PDE1, 3, 4, and 5 inhibitors, was modelled well in guinea pig but not rat cardiomyocytes.
Significance: Our results provide the first full characterisation of human cardiomyocyte PDE isoforms, and suggest that guinea pig myocytes provide a better model than rat for PDE levels and activity.

Introduction

The cyclic nucleotides cAMP and cGMP were first discovered over four decades ago, yet recent years have seen a huge increase in our understanding of these second messengers. Once formed, cyclic nucleotides spread rapidly throughout the cytosol. Yet much experimental evidence exists to show that cyclic nucleotide signals produced by different receptors can be confined to discrete "compartments" within the cell, and thus two different stimuli that both increase cyclic nucleotide levels can have different effects (Fischmeister et al., 2006). Phosphodiesterases (PDEs) are the only enzymes that breakdown cAMP and cGMP and terminate the cyclic nucleotide signal, and as such are prime candidates for modulators of spatial distribution (Lugnier, 2006). PDEs 1, 2, 3, 10 and 11 are dual-substrate, hydrolysing cAMP and cGMP; PDEs 4, 7 and 8 are cAMP-specific; and PDEs 5, 6 and 9 are cGMP-specific (Lugnier, 2006).

Cardiomyocyte PDE activity is of great interest to the pharmaceutical industry. Inhibiting PDE3 can provide acute inotropic support to failing hearts, however, the long-term use may increase patient mortality (Amsallem et al., 2005; Nony et al., 1994). Screening for unwanted effects of new cardiac drugs on PDE3 is therefore a priority, since these agents will be targeted at a population with existing heart disease.

Most work on PDE expression in the heart has used whole heart or tissue, rather than cardiomyocytes, thus allowing the possibility that some PDE activity may come from fibroblasts or vascular cells rather than cardiomyocytes themselves (Maurice et al., 2003). This is particularly true for human myocardium: the small number of attempts made to localize PDE isoforms to the human cardiomyocyte have been by immunofluorescence staining, which is limited by the availability of selective antibodies for the PDE isoforms (Vandeput, et al., 2007; Nagendran et al., 2007). In the present study we used pure preparations of isolated adult human ventricular myocytes to determine isoform and subtype-specific expression of PDEs 1–7, cyclic AMP and cGMP hydrolyzing activity and effects of cyclic AMP-PDE inhibition on contractile function.

The complex relationship between isoform-specific PDE inhibition, spatially confined rises in cyclic AMP or cGMP and the function effects on basal or β-adrenoceptor-stimulated contraction has been extensively characterized using the isolated ventricular myocyte (Fischmeister et al., 2006; Nikolaev et al., 2006). However, the choice of animal species has not been optimized to reflect PDE isoform

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balance in the myocyte. Previous studies using ion-exchange chromatography have identified PDEs 1–4 in rat (Dubois et al., 1993; Bode, Kanter, and Brunton, 1991) and guinea pig heart (Bethke et al., 1992), with PDE1 being claimed to reside in the non-myocyte fraction of rat heart (Bode et al., 1991). Reports of PDE5 expression and activity in whole cardiac tissues have been variable (Maurice et al., 2003; Wallis et al., 1999). The present paper also compares directly the PDE profile of the failing human ventricular myocytes with that of two commonly used animal models, the rat and guinea pig, from which myocytes may be easily isolated.

Methods

Tissue samples

Left ventricular samples were obtained from adult male Sprague–Dawley rats (250–400 g) and male Dunkin–Hartley guinea pigs (500–700 g). Human myocardium was obtained from explanted failing hearts, or during surgical resection of patients with obstructive hypertrophic cardiomyopathy, under ethical approvals from the Royal Brompton and Harefield Trust. In some cases patients had been implanted with a left ventricular assist device some months before the final transplant operation. A summary of the patient details, the areas of the heart studied, and the experiments they were used for is shown in Table 1.

Cardiomyocyte isolation

Cardiomyocytes were isolated using enzymatic methods as described previously from rat (Harding et al., 1988), guinea pig (Brown et al., 1990) and human (Brown et al., 1990) ventricle. Immediately following digestion, cells were washed twice with low calcium (LC) buffer (NaCl 120.0, KCl 5.4, $MgSO_4$, pyruvate 5.0, glucose 20.0, taurine 20.0, HEPES 10.0, nitrilotriacetic acid 5.0, $CaCl_2$ 0.05 mM, pH 7.4). Low speed centrifugation at $27\times g$ for each wash ensured that only the relatively heavy cardiomyocytes were pelleted, excluding any smaller cells that might have survived the digestion process. Cell digests were also visually checked to ensure that few or no non-cardiomyocytes were present. For RTPCR and SPA, cells were snap-frozen in liquid nitrogen and stored at −80 °C until use. For functional measurements of cell shortening, cells were used immediately following isolation.

Table 1
Patient details.

Sample number	AREA	Disease	Sex	Age	Experiments conducted			Other
					PCR	SPA	Myocyte contraction	
1	IVS	HOCM	F	63			IBMX	
2	LV	IHD	M	41	Y	Y	IBMX, MIL	LVAD
3	IVS	HOCM	M	48			IBMX, ROL	
4	RV	CONGENITAL HD	F	38			ROL	
5	RV	CONGENITAL HD	M	22			IBMX, MIL, ROL	
6	IVS	HOCM	M	42			IBMX	
7	IVS	HOCM	M	58		Y	ROL	
8a	RV	DCM	M	57	Y	Y	MIL	ICD
8b	LV	DCM	M	57		Y		
9	RV	IHD	M	51			MIL, ROL	LVAD
10	LV	IHD	M	52			ROL	
11	RV	DCM	M	20			ROL	
12	IVS	HOCM	M	58			ROL	
13	LV	IHD	M	57			UK90234	PACEMAKER
14	RV	Myocarditis	F	27		Y		
15	RV	DCM	M	53	Y			
16	RV	CF	F	26	Y			
17	LV	DCM	M	34			UK90234	
18	RV	IHD	M	49			UK90234	

LV: left ventricle; RV, right ventricle; IVS, interventricular septum; HOCM, hypertrophic obstructive cardiomyopathy; IHD, ischaemic heart disease; HD, heart disease; DCM, idiopathic dilated cardiomyopathy; CF, cystic fibrosis; SPA, scintillation proximity assay; IBMX, iso butyl methyl xanthine; MIL, milrinone; ROL, rolipram; LVAD, left ventricular assist device; ICD, implantable cardiodefibrillator.

RTPCR

RNA isolation

RNA was isolated from cardiomyocytes with the "Promega RNAgents Total RNA isolation System", using isopropanol extraction. Cells were first resuspended in ice-cold denaturing solution and lysed using a Dounce glass homogenizer, before extracting the RNA as per the manufacturer's instructions.

Reverse transcription

The "Platinum Taq" kit from Applied Biosystems was used. The cDNA reaction was carried out in a thermal cycler as follows: 25 °C for 10 min, 48 °C for 30 min, 95 °C for 5 min, 4 °C until tubes removed. If not used immediately cDNA was stored at −80 °C.

Polymerase chain reaction

PCR was carried out on the generated cDNA using "Platinum Taq" DNA polymerase from Invitrogen, as per manufacturer's instructions. The same primers were used for all three species, with the exception of PDE4C, where different primers for human and rodent were used. Primer sequences were as follows (all 5′–3′, F = forward primer, R = reverse primer):

PDE1A (F) CCACTTTGTGATCGGAAGTC
PDE1A (R) TTCTGCTGAATGATGTCCACC
PDE1B (F) CAGGGTGACAAGGAGGCAGAG
PDE1B (R) GACATCTGGTTGGTGATGCC
PDE1C (F) TCTCAAAGGATGACTGGAGG
PDE1C (R) GCTTCTCTGTCACCCTGTC
PDE2A (F) CCTCCTGTGACCTCTCTGACC
PDE2A (R) TGAACTTGTGGGACACCTTGG
PDE3A (F) TCACAGGGCCTTAACTTACAC
PDE3A (R) GGAGCAAGAATTGGTTTGTCC
PDE3B (F) CCTCAGGCAGTTTTATACAATG
PDE3B (R) TGCTTCTTCATCTCCCTGCTC
PDE4A (F) GTGGAGAAGTCTCAGGTGGG
PDE4A (R) TGGAACTTGTCAGGCAGGG
PDE4B (F) TAGAAGATAACAGGAACTGG
PDE4B (R) GCAATGTCTATGTCAGTCTC
PDE4C (rodent) (F) ACGTGGCGTACCACAACAGC
PDE4C (rodent) (R) TACCGCGAGGTGATGGTTCTC
PDE4C (human) (F) TGATCTGAGCAACCCCACCAAG
PDE4C (human) (R) GGTGAGGTCTGAGGGACTTCG
PDE4D (F) GGATAATGGAGGAGTTCTTCC
PDE4D (R) CGATTGTCCTCCAAAGTGTCC
PDE5A (F) CCCTGGCCTATTCAACAACGG
PDE5A (R) GTGGGTCAGGGCCTCATACAG
PDE7A (F) TGGACAAGCCAAGTGTATGCTG
PDE7A (R) TTTAAGTAACAGTGCATGGCC

PCR was carried out in a thermal cycler as follows: 94 °C for 2 min, then the following three steps for 34 cycles: 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, a final step of cooling to 4 °C. PCR products were used immediately or stored overnight at 4 °C before use. PCR products were run on a 1.2% agarose gel at 120 mA for ~60 min, and visualized under UV light using ethidium bromide staining. β-actin was used as a housekeeping control.