Multiple cyclic nucleotide phosphodiesterases (PDEs) belonging to four families (PDE1 to PDE4) hydrolyze cAMP in cardiac cells, but the functional significance of this diversity is not well understood. The goal of this study was to characterize the involvement of different PDEs in excitation-contraction coupling in cardiomyocytes. For this, sarcomere shortening and Ca2+ transients were recorded simultaneously in rat ventricular myocytes field stimulated at 0.5 Hz with an IonOptix system. Selective inhibition of PDE2 with Bay 60-7550 (Bay, 100 nM) or PDE4 with Ro-201724 (Ro, 10 μM) had no effect on basal cell contraction, whereas selective inhibition of PDE3 with cilostamide (Cil, 1 μM) or β-adrenergic stimulation with isoprenaline (Iso, 1 nM) increased myocyte shortening. Inhibition of PDE4 potentiated the response to Cil and Iso, showing that PDE4 becomes important when cAMP is prestimulated. Similar results were obtained on Ca2+ transients. cAMP measurements by FRET in beating cardiomyocytes indicate that ISO strongly increases cAMP levels. Effects of selective PDE inhibitors are under investigation. These results show that PDE2, PDE3 and PDE4 differentially regulate excitation-contraction coupling in cardiomyocytes.
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J016
INHIBITION OF THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 4, MRP4 PROMOTES CARDIAC HYPERTROPHY

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Cyclic nucleotides mediated signaling determines the regulation of many cardiac function. It is generally admitted that the cyclic nucleotides degradation is due to phosphodiesterases. Recently, cAMP and cGMP were also shown to be extruded by the cell via an active efflux transporter, called MRP4. We investigated whether inhibition of MRP4 may independently control cyclic nucleotides levels and modify functions related to cyclic nucleotides signalling pathways in cardiac myocytes.

Methods — We used silencing RNA or adenovirus encoding MRP4 shRNA (Ad-ShMRP4) to inhibit MRP4 expression in rat cardiomyocytes in vitro. We then performed physiological evaluation of cardiac morphology and function in knock-out MRP4 mice. As a model of adrenergically induced cardiac hypertrophy, miniosmotic pumps containing isoproterenol delivering 20 ìg/g/day for 2 weeks were implanted subcutaneously into 3-months old MRP4KO and wild type mice. As a model of physiological hypertrophy, animals were housed in a cage with free access to a running monitored wheel.

Results — MRP4 is present in human, mouse and rat cardiomyocytes and over-expressed in case of increased cardiac intracellular cAMP. Using a FRET technique MRP4 inhibition was shown to increase intracellular cAMP level. Adult rat cardiomyocytes infected with Ad-shMRP4 demonstrated a significant increase in the calcium current density and in cell size compared to cells infected with the negative control Ad-shLuciferase, suggesting an activation of the cAMP/PKA pathway. While unchallenged young (3 months) MRP4KO mice displayed normal cardiac parameters, MRP4KO mice progressively developed significant cardiac hypertrophy by 9-12 months of age (HW/BW: 5.15 vs 4.46 in WT mice). Isoproterenol-treated MRP4KO mice displayed a significant increase in cardiac hypertrophy compared to stimulated WT mice (HW/BW: 6±0.38 vs 5.29±0.34; p=0.001). In contrast to the regulation of pathological cardiac hypertrophy, MRP4 inhibition did not affect the physiological cardiac growth response associated with physical training (HW/BW: 4.83±0.01 vs 4.84±0.13, p=NS), indicating the absence of a regulatory role of MRP4 in physiological cardiac hypertrophy.

Conclusion — These results reveal a unique and important function for MRP4 in stress-dependent cardiac growth by controlling cyclic nucleotides signalling pathways in cardiomyocytes.

J017
THE RHO/RAC EXCHANGE FACTOR VAV2 CONTROLS NITRIC OXIDE–DEPENDENT RESPONSES IN VASCULAR SMOOTH MUSCLE CELLS

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The regulation of arterial contractility is essential for blood pressure control. The GTase RhoA promotes vasoconstriction by modulating the cytoskeleton of vascular smooth muscle cells. Whether other Rho/Rac pathways contribute to blood pressure regulation remains unknown. We have previously demonstrated that vav2 null mice suffered from serious defects in the cardiovascular system of, including tachycardia, systemic arterial hypertension, extensive cardiovascular remodelling, heart fibrosis, and loss of kidney homeostasis. By studying this hypertensive knockout mouse lacking the Rho/Rac activator Vav2, we have discovered a new pathway composed of Vav2, the GTase Rac1, and the serine/threonine kinase Pak that is critical for nitric oxide–triggered blood vessel relaxation and normotension. This pathway mediates the Pak–dependent inhibition of phosphodiesterase type 5, a process that favors the inactivation of the RhoA pathway and the depolymerization of the F–actin cytoskeleton in vascular smooth muscle cells. The inhibition of phosphodiesterase type 5 requires its physical interaction with autophosphorylated Pak1 but, unexpectedly, occurs without detectable transphosphorylation events between those two proteins. The administration of phosphodiesterase type 5 inhibitors prevents the development of the hypertension and the cardiovascular disease in Vav2–deficient animals, demonstrating the key role of this signaling route in blood pressure regulation. Taken together, these results unveil the cause of the cardiovascular phenotype of Vav2 knockout animals, identify a new Rac1/Pak1 signaling element, and provide a mechanistic framework to better understand blood pressure control in physiological and pathological states.

J018
THE DEUBIQUITINASES USP33 AND USP20 COLLABORATIVELY REGULATE BETA2 ADRENERGIC RECEPTOR RECYCLING AND RESENSITIZATION

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Chronic agonist stimulation of the β2 Adrenergic Receptors (β2ARs) leads to their lysosomal trafficking and degradation. Previous studies demonstrated that agonist-induced β2AR ubiquitination is necessary for lysosomal targeting and degradation of the receptor. We have now found that the de-ubiquitinating enzymes USP33 and USP20 are recruited to the β2AR complexes, by using cellular co-immunoprecipitation assays. This led to our hypothesis that USP33