Title	Epac1-selective cyclic-AMP analog 8-pCPT-2'-O-Me-cAMP induces vasodilation on porcine coronary artery in endothelium-independent pathway
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P041

Topic: Epac1-selective Cyclic-AMP Analog 8-pCPT-2'-O-Me-cAMP Induces Vasodilation On Porcine Coronary Artery In Endothelium-Independent Pathway

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Background: Exchange protein directly activated by cyclic AMP (cAMP) 1, Epac1, is a newly discovered cAMP target which acts beyond the classical cAMP effector protein kinase A (PKA). The identification of Epac1 overthrows the concept that cAMP activatez single effector. Epac1 is a guanine nucleotide exchange factor (GEF) probably activates small G-protein Rap1. Epac1 ubiquitously expresses in all tissues and therefore should play important roles in cellular signaling. Appreciation of the potential importance of Epac1 has come with the current development of Epac1-selective-cAMP analogs for discriminating the activation from PKA to Epac1. Among those analogs, 8-pCPT-2'-O-Me-cAMP (8-pCPT) is the most potent Epac1 activator available (K_d=2.2 μM) with a low affinity to PKA (K_d=200-300 μM). 8-pCPT was revealed to induce aorta and airway relaxation, whereas the underlying mechanism remains unclear. Moreover, to date no comparable data exist on the role of Epac1 in porcine vascular system. Current study aimed to investigate the effect of 8-pCPT in isolated porcine coronary vasodilatation.

Methods: Hearts from either sexes of pig (50-70kg) were collected from local slaughter house right before experiment. Right coronary arteries were isolated and the surrounding connective tissue was removed before being cut into 2-3mm rings. For endothelium-independent experiments, the endothelial layer was removed mechanically. Rings were mounted on organ chamber and immersed in physiological solution (Krebs-Henseleit, 37^{o}C, 5%O_2). Change in isometric tension was recorded with the basal tension being adjusted to 2g. Indomethacin (COX1-inhibtior, 10 μM, EtOH) and SQ22536 (adenylyl cyclase inhibitor, 100 μM, H_2O) were added to all chambers for 30 minutes to eliminate COX derivatives and endogenous cAMP respectively. Rings were then challenged with thromboxane A2 analogue U46619 (30 nM, MeOAc) and direct relaxation was examined by cumulative addition of 8-pCPT (10 nM- 100 μM, H_2O) in the presence of different blockers. 8-pCPT was also used to incubate with rings prior to the contraction-relaxation responds induced by cumulative addition of forskolin (adenylyl cyclase activator), 6-MB (selective PKA activator) or levcromakalim (K_{ATP} channel opener) to investigate the potentiated effect of Epac1. Data is presented in % of relaxation with 30 nM U46619 contraction. U46619 was also used to construct cumulative dose-respond contraction curve in the presence or absent of 8-pCPT. Data is presented in %of contraction to 60mM KCl contraction. In addition the artery rings underwent protein extraction for immunoblotting against anti-Epac1 antibody (Epac (A-5)), rat aortae (12-weeks male Sprague-Dawley (SD)) were used as control.

Results: Epac (A-5) antibody detected one discrete protein band ~99kDa in porcine coronary artery smooth muscle extraction. Up to 100 μM 8-pCPT counteract U46619 contraction in rings with endothelium (-89.7%±8.7, n=5) and without endothelium (-95.6%±13.6, n=5) which the half-maximal concentration is 37.8 μM (±12.5). Hence all the rings in the following experiments were endothelium-removed for us to focus on the effect in smooth muscle layer. However incubation of BFA (Rap/GEF fixer, 30 μM, DMSO) and GGTI-298 (Rap1 inhibitor, 10 μM, DMSO) did not reverse 8-pCPT induced relaxation (n=1-3). Also GGTI-298 showed no effect on forskolin-induced, PKA independent (with Rp-8-Br-cAMPS (PKA inhibitor, 100 μM, H_2O)) vasodilation (10 nM-10 μM) (n=3). Also 8-pCPT (100 nM-30 μM) did not alter 6-MB (10 μM- 1 mM, H_2O) (n=3) or levcromakalim (10 nM- 10 μM, DMSO) (n=7) -induced vasodilation. Nevertheless, 8-pCPT (30 μM) significantly reduced the maximal contraction to U46619 (1 nM- 1μM) by 19.7% (±6.6, n=6, $P<0.01$) while the same inhibition do not occur under the incubation with Y27632 (Rho-kinase inhibitor, 10 μM, H_2O)

Summary: The results obtained suggested that Epac1 induces direct relaxation in porcine coronary artery via endothelium-independent pathway whereas does not undergo Rap1. Though theoretically we cannot exclude that Eapc1 activates Rap1 in porcine coronary artery but it is less possible that Rap1 participates in smooth relaxation. Although reports suggested that Epac1 modulate PKA or K_{ATP} channels activity, we did not observed such relationship in porcine coronary artery smooth muscle. Interestingly Epac1 modulate RhoA/Rho-kinase dependent smooth muscle contraction, suggested that RhoA/Rho-kinase is one of the possible targets of Epac1. Beyond the above findings, the complete mechanism of Epac1-induced vasodilation is still unclear, it is room for determination of the unrecognized role of other proposed downstream effectors of Epac1 such as Rac, Rap2b, AMPK and Akt, in the action of vasodilation in porcine coronary artery.

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