

A₁ Adenosine Receptor-Induced Modulation Of Transglutaminase 2 Activity In H9c2 Cardiomyoblasts

The A₁ adenosine receptor (A₁R) is a member of the adenosine receptor group of G-protein-coupled receptors, which also includes A_{2A}, A_{2B} and A₃. The A₁R is G_{i/o}-protein linked and is involved in neuro- and cardioprotection. Stimulation of this receptor reduces the cardiac infarct size similarly to ischaemic preconditioning as well as activating protein kinases such as PKC, ERK1/2 and p38 MAPK, which have been implicated in cardioprotection (1). Transglutaminase-2 (TG2) is a multifunctional enzyme regulated by Ca²⁺ and protein kinases (e.g. PKA) and capable of mediating intracellular signalling by acting as a GTP-binding protein (G_{αh}) (2). TG2 has been implicated in signalling via the α₁-adrenergic receptor and in some instances, has been shown to be necessary for cardioprotection (3). The aims of this study are to determine whether TG2 is activated by the A₁R and if so, which protein kinase(s) are involved.

Mitotic rat H9c2 cardiomyoblasts were subjected to the selective A₁R agonist N⁶-cyclopentyladenosine (CPA) and TG2 activity measured *in vitro* (4) and *in-situ* (5). H9c2 cells were pre-treated with the selective A₁R antagonist DPCPX (1 μM: 30 min), G_{i/o} protein inactivating pertussis toxin (100 ng/ml⁻¹:16h) or TG2 inhibitors Z-DON (150 μM: 60min) and R283 (200 μM: 60min). Where indicated, cells were also treated with the protein kinase inhibitors Ro 31-8220 (PKC inhibitor, 10 μM), PD 98059 (MEK1/2 inhibitor, 50 μM), SP 600125 (JNK1/2 inhibitor, 20 μM) and SB 203580 (p38 MAPK inhibitor, 20 μM) for 30 min prior to stimulation with CPA (100nM for 10 min). Results represent mean ± S.E.M. and p values <0.05 were considered statistically significant.

CPA (control TG2 activity 30.71 ± 2.48 vs CPA-induced 72.30 ± 1.65; n=6) produced transient increases in TG2-catalysed biotin-cadaverine incorporation activity, peaking at 10 min. Furthermore, CPA (p[EC₅₀] = 8.87 ± 0.17; n=6) stimulated a concentration-dependent increase in amine incorporation activity. Responses to CPA-induced TG2 activity were blocked by the application of the above mentioned inhibitors (Table 1).

Table 1: Effects of inhibitors on CPA-induced TG2 activity.

	Inhibitor							
	DPCPX	ZDON	R283	PTX	PD98059	Ro 31-8220	SB203580	SP600125
Control (Basal TG2 activity)	64.95 ± 5.45			22.37 ± 2.12			38.35 ± 6.83	36.34 ± 6.18
100 nM CPA	98.49 ^{a**} ± 11.65			32.65 ^{a*} ± 3.28			66.11 ^{a*} ± 8.78	62.04 ^{a*} ± 6.71
CPA + Inhibitor	45.95 ^{b****} ± 5.67	30.70 ^{b****} ± 5.41	15.37 ^{b****} ± 2.43	17.63 ^{b***} ± 2.03	15.89 ^{b****} ± 1.88	19.86 ^{b**} ± 2.36	41.64 ^{b*} ± 4.72	29.35 ^{b**} ± 4.63

*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001, (a) versus control and (b) versus 100 nM CPA alone. Data obtained from four independent experiments.

In summary, these findings have shown for the first time that the A₁R regulates TG2 activity via PKC and a range of protein kinase pathways.

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