A cooperative cardioprotective effect of adenosine A_1 and A_{2a} receptor agonism in ischemia-reperfusion damage in the isolated perfused mouse heart

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Adenosine A_1 agonist (CPA) ameliorates necrotic damage in a cardiac cell line after simulated ischemia by a cooperative effect on adenosine $A_{2A/2B}$ receptors[1]. In the present study, we investigated whether this cooperative effect was seen in mouse hearts using a Langendorff preparation with an ischemia-reperfusion protocol. Langendorff isolated perfused CD1 A_{2A} knockout (KO) and wild-type (WT) mouse hearts were subjected to no-flow global ischemia (30 min) and reperfusion (60 min) at constant pressure (80 mmHg). Cardiac troponin I (cTn I) and lactate dehydrogenase (LDH) release were measured by collecting cardiac effluent before ischemia and at the end of reperfusion. Adenosine A_1 and A_{2A} cooperative cardioprotective signalling was assessed by extracellular signal-regulated kinase (ERK) 1/2 phosphorylation. Both wild-type and A_{2A}KO mice were treated with adenosine A_1 agonist CPA (100nM) added at reperfusion, either in presence of adenosine A_1 antagonist (DPCPX, 1µM) or adenosine A_{2A} antagonist (ZM241385, 50nM) or adenosine A_{2B} antagonist (MRS1754, 100nM). A significant increase in the infarct size was observed in A_{2A}KO compare to WT mice (26.75 ± 1.78 and 18.69 ± 5.21 % of area at risk respectively, $p<0.05$). CPA treatment significantly reduced infarct size in WT animals. Treatment with ZM241385 worsened left ventricular developed pressure (LVDP) recovery in WT but, as expected, not in A_{2A}KO mice (72.02 ± 22.50% and 17.60 ± 2.83% change from baseline for CPA vs. 5.48 ± 5.48 and 41.86 ± 19.34% change from base line for CPA + ZM241385 in WT and A_{2A}KO mice at 70 min respectively, $p<0.05$). In contrast, MRS1754 worsened LVDP recovery in both A_{2A} KO and WT mice (72.02 ± 22.50% change from baseline for CPA vs. 5.06 ± 3.68% change from baseline for CPA + MRS1754 in WT mice at 70 min, $p<0.05$). Conversely, adenosine A_1 antagonist (DPCPX) caused a bigger reduction in contractility (dP/dt_{max}), in A_{2A} KO compare to WT mice. In WT mice CPA and CPA + CGS21680 significantly reduced cTn I level (2.84 ± 0.59 and 4.97 ± 0.08 ng/ml vs. 11.99 ± 1.06 ng/ml in control, $p<0.05$). In A_{2A}KO CPA + CGS21680 did not reduced the cTn I release. In WT mice LDH release reduced significantly (4.73 ± 0.04, 6.68 ± 0.51% cytotoxicity in CPA and CPA + CGS21680 groups vs. 14.03 ± 1.92% cytotoxicity in control group, $p<0.05$), however in A_{2A}KO mice no significant difference was observed. In WT mice CPA treatment increased ERK1/2 phosphorylation (1.53 ± 0.26 pERK/ERK1/2 in CPA vs. 0.83 ± 0.06 pERK/ERK1/2 in control, $p<0.05$), which was significantly reversed by DPCPX and ZM241385 (0.92 ± 0.16 and 0.83 ± 0.05 pERK/ERK1/2 respectively, $p<0.05$). Overall the beneficial cooperative effects of CPA were inhibited by DPCPX, ZM241385 and MRS1754. In conclusion, the data suggests that in wild-type mice both adenosine A_{2A} and A_{2B} receptor antagonists worsen functional recovery in the presence of adenosine A_1 agonist. However, in the A_{2A} KO mice, an adenosine A_{2B} antagonist worsened functional recovery but as expected the adenosine A_{2A} antagonist did not. In the isolated perfused mouse heart, A_1-mediated cardioprotection requires a cooperative activation of A_2 receptors, presumably via endogenous adenosine.

[1.] Urmaliya et al, Cardiovasc Pharmacol 53(5):424-433 (2009)