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Heart perfusion with NO donor S-nitrosoglutathione induces cardiomyocyte apoptosis in mitochondrial permeability transition-dependent manner

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Increased nitric oxide levels may induce cytochrome c release and caspase activation, but the mechanism is not clear. We found that 10 min of heart perfusion with S-nitrosoglutathione (GSNO) substantially inhibited the mitochondrial respiratory chain, induced cytochrome c release from mitochondria and caused an about 3-fold increase in caspase-3-like protease activity. GSNO-affected mitochondrial respiration was restored on addition of exogenous cytochrome c to the incubation medium, suggesting that mitochondrial inactivation was mainly due to the loss of this protein. The effect of GSNO was completely prevented by adding cyclosporin A, a selective inhibitor of mitochondrial permeability transition, to the perfusion buffer: after perfusion of hearts together with GSNO and cyclosporin A there were neither a decrease in the mitochondrial state 3 respiration rate nor an increase in caspase activity. To conclude GSNO may induce cardiomyocyte apoptosis mediated by mitochondrial permeability transition.

Key words: S-nitrosoglutathione, mitochondria, cytochrome c, apoptosis

INTRODUCTION

Recent evidence indicates that different heart disorders, including ischemia, may cause an increase in nitric oxide concentration from nanomolar to micromolar levels [1]. Mitochondria are the main cellular targets of nitric oxide: they reversibly inhibit cytochrome c oxidase [2, 3], and nitric oxide derivatives may irreversibly damage many enzymes of the mitochondrial oxidative phosphorylation system [4–6]. Mitochondria play an essential role in myocardial cell metabolism, and mitochondrial processes may influence cell viability and death pathways. Thus, if glycolysis is also inhibited or is insufficient to compensate for mitochondrial respiration, nitric oxide can induce necrosis [7]. Recent evidence indicates that one of the main pathways of the activation of apoptosis-specific proteases is mediated by mitochondria which may release apoptogenic proteins, mainly cytochrome c [8]. It has been shown that high nitric oxide concentrations can induce cytochrome c release and caspase activation [9], but the mechanism is not clear.

MATERIALS AND METHODS

Wistar rat hearts were used in experiments. Heart perfusion (37 °C, 10 min) was performed on a Langendorf system using Krebs–Henseleit solution (11 mM glucose, 118 mM NaCl, 25 mM $NaHCO_3$, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.7 mM $MgSO_4$ and 0.7 mM Na-piruvate, pH 7.2) supplemented with 1 mM nitrosating NO donor S-nitrosoglutathione (GSNO) or GSNO together with 0.1 μM cyclosporin A. For control, hearts were perfused without specific additions.

Mitochondrial fractions were isolated from perfused hearts by standard procedures of homogenisation and subsequent differential centrifugation [10]. To prepare cytosolic fractions for measurements of caspase activity, the perfused hearts were homogenated in a medium containing 300 mM sucrose, 20 mM Tris, 1 mM EGTA, pH7.7 (2 °C), and the postmitochondrial supernatans were centrifuged 1 h × × 100000 g to obtain cytosolic fractions.

Mitochondrial activity was investigated by measuring respiratory rate with a Clarck type electrode in the mitochondrial incubation medium that contained 110 mM KCl, 10 mM Tris-HCl, 5mM KH_2PO_4, 50 mM creatine, 1 mM $MgCl_2$, 1 mM piruvate, 1 mM malate, pH 7.2 (37 °C). Mitochondrial state 3 respiration rate was obtained by adding 1 mM ATP, which was converted to ADP by mitochondrial creatine kinase.

Caspase-3-like protease activity in cytosolic fractions was determined spectrophotometrically by mea-

suring the caspase-3 specific substrate DEVD cleavage rate. Cytosolic protein (1mg/ml concentration in incubation medium) was incubated (37 °C) with 0.1 mM colorimetric caspase-3 substrate *z*-DEVD-*p*NA in incubation buffer that contained 50 mM HEPES, 10% sucrose, 1 mM $MgCl_2$, 1 mM ATP, pH 7.4 (37 °C).

Data were expresed as means ± S. E. of at least 3 separate experimets. Statistical comparison between experimental groups was performed using Student's t test. Statistical significance was assumed at $p < 0.05$.

RESULTS AND DISCUSSION

We found that 10 min of heart perfusion with S-nitrosoglutathione (GSNO) substantially inhibited mitochondrial state 3 (substrate- and ADP-stimulated) respiration rate with substrate pyruvate + malate (Table). The effect of GSNO was completely prevented by adding cyclosporin A, a selective inhibitor of mitochondrial permeability transition, to the perfusion buffer. The GSNO-affected respiration rate was restored after addition of exogenous cytochrome c (Table) to the mitochondrial incubation medium, suggesting that mitochondrial inactivation was mainly due to the loss of this protein. After perfusion with GSNO and cyclosporin A, stimulation of respiration by exogenous cytochrome c was twice lower than in mitochondria isolated from only-GSNO-treated hearts (Table).

These results suggest that GSNO inhibits the respiratory chain due to permeability transition-induced cytochrome c release from mitochondria. Once released from mitochondria to the cytoplasm, cytochrome c may activate caspases and induce apoptosis. Consequently, we measured caspase activity in cytosolic fractions isolated from hearts perfused with GSNO. We found GSNO to cause an about 3-fold increase in caspase-3-like protease activity (Table). Cyclosporin A prevented GSNO-induced activation of caspases suggesting that this activation is due to mitochondrial permeability transition.

In conclusion, heart perfusion with GSNO induces a mitochondrial permeability transition-dependent inhibition of mitochondrial respiration, cytochrome c release, and subsequent caspase activation.

Table. **Mitochondrial state 3 respiration and caspase activity after heart perfusion with NO donor S-nitrosoglutathione**
GSNO – S-nitrosoglutathione, CsA – cyclosporin A, cyt c – cytochrome c
* – statistically significant difference as compared to control;
\# – statistically significant effect of cyclosporin A;
& – statistically significant effect of cytochrome c.
n = 3–4.

Parameter / Effectorrate,	Mitochondrial respiration ngatom O / min mg		Caspase activity, nmol / min mg
	– cyt c	+ cyt c	
No effector	395 ± 13	586 ± 17	0.0273 ± 0.002
GSNO	204 ± 21*	371 ± 28*&	0.0870 ± 0.019*
GSNO + CsA	502 ± 64#	870 ± 103#	0.0180 ± 0.010#

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References

1. Beckman J S. Circ Res 1999; 29: 870–1.
2. Torres J, Darley-Usmar V, Wilson M T. Biochem J 1995; 312: 169–73.
3. Giuffre A, Sarti P, D'Itri E, Buse G, Soulimane T, Brunori M J Biol Chem 1996; 271: 33404–8.
4. Cassina A, Radi R. Arch Biophys Biochem 1996; 328: 309–16.
5. Clementi E, Brown G C, Feelisch M, Moncada S. Proc Natl Acad Sci USA 1998; 95: 7631–6.
6. Riobo N A, Clementi E, Melani M, Boveris A, Cadenas E, Moncada S, Poderoso J J. Biochem J 2001; 359: 139–45.
7. Leist M, Single B, Naumann H, Fava E, Simon B, Kuhnle S, Nicotera P. Exp Cell Res 1999; 249: 396–403.
8. Hengartner M O. Nature 2000; 407.
9. Andreka P, Zang J, Dougherty C, Slepak TI, Webster KA, Bishpric NH. Circ Res 2001; 88: 305–312.
10. Borutaite V, Morkuniene R, Budriunaite A et al. J Mol Cell Cardiol 1996; 28: 2195–2201.

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ŠIRDIES PERFUZIJA NO DONORU S-NITROZOGLUTATIONU SUKELIA NUO MITOCHONDRIJŲ PRALAIDUMO PRIKLAUSANČIĄ KARDIOMIOCITŲ APOPTOZĘ

S a n t r a u k a

Vystantis įvairių formų širdies sutrikimams, taip pat sergant ischemija pastebėta, kad azoto monoksido koncentracija gali padidėti nuo nanomolių iki kelių mikromolių. Pagrindinis azoto monoksido taikinys ląstelėje yra mitochondrijos. Nustatyta, kad mitochondrijos gali dalyvauti sukeliant ląstelės apoptozę, todėl NO sąlygoti mitochondrijų pažeidimai gali nulemti apoptotinį mirties kelią, tačiau mitochondrijų pažeidimų mechanizmai bei jų reikšmė sukeliant apoptozę nėra išaiškinti. Šiame darbe nustatėme, kad NO donoras S-nitrozoglutationas sukelia nespecifinį mitochondrijų pralaidumą, dėl kurio mitochondrijos netenka citochromo c, sumažėja kvėpavimo grandinės aktyvumas. Dėl S-nitrozoglutationo sukelto nespecifinio pralaidumo į citoplazmą išėjus citochromui c yra aktyvuojamos kaspazės.