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The novel H_2S-donor 4-carboxyphenyl isothiocyanate promotes cardioprotective effects against ischemia/reperfusion injury through activation of mitoK_{ATP} channels and reduction of oxidative stress

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ABSTRACT

The endogenous gasotransmitter hydrogen sulphide (H_2S) is an important regulator of the cardiovascular system, particularly of myocardial function. Moreover, H_2S exhibits cardioprotective activity against ischemia/reperfusion (I/R) or hypoxic injury, and is considered an important mediator of "ischemic preconditioning", through activation of mitochondrial potassium channels, reduction of oxidative stress, activation of the endogenous "anti-oxidant machinery" and limitation of inflammatory responses. Accordingly, H_2S-donors, i.e. pro-drugs able to generate exogenous H_2S, are viewed as promising therapeutic agents for a number of cardiovascular diseases. The novel H_2S-donor 4-carboxy phenylisothiocyanate (4CPI), whose vasorelaxing effects were recently reported, was tested here in different experimental models of myocardial I/R.

In Langendorff-perfused rat hearts subjected to I/R, 4CPI significantly improved the post-ischemic recovery of myocardial functional parameters and limited tissue injury. These effects were antagonized by 5-hydroxydecanoic acid (a blocker of mitoK_{ATP} channels). Moreover, 4CPI inhibited the formation of reactive oxygen species. We found the whole battery of H_2S-producing enzymes to be present in myocardial tissue: cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (MPST). Notably, 4CPI down-regulated the post-ischemic expression of CSE.

In Langendorff-perfused mouse hearts, 4CPI reduced the post-ischemic release of norepinephrine and the incidence of ventricular arrhythmias. In both rat and mouse hearts, 4CPI did not affect the degranulation of resident mast cells.

In isolated rat cardiac mitochondria, 4CPI partially depolarized the mitochondrial membrane potential; this effect was antagonized by ATP (i.e., the physiological inhibitor of K_{ATP} channels). Moreover, 4CPI abrogated calcium uptake in the mitochondrial matrix.

Finally, in an in vivo model of acute myocardial infarction in rats, 4CPI significantly decreased I/R-induced tissue injury.

In conclusion, H_2S-donors, and in particular isothiocyanate-based H2S-releasing drugs like 4CPI, can actually be considered a suitable pharmacological option in anti-ischemic therapy.

1. Introduction

The gasotransmitter hydrogen sulphide (H_2S) is a pleiotropic and ubiquitous mediator which influences almost all the functions of the mammalian body [1]. Among these, the regulation of

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the cardiovascular system is an important role of this gasotransmitter [2,3]. H_2S is biosynthesized by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) [4], and by the cooperation between cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (MPST) [4–6]. The pattern of distribution and localization of these enzymes is quite complex and they may coexist in cardiovascular tissues [7–9].

H_2S exhibits cardioprotective activity against ischemia/reperfusion (I/R) or hypoxic injury and is considered an important mediator of "ischemic preconditioning" (IPC), a self-defence cardioprotective mechanism against myocardial I/R injury. The mechanisms of action accounting for this cardioprotective activity are heterogeneous and not yet completely understood. Mitochondrial ATP-sensitive potassium channels ($mitoK_{ATP}$) are well-known effectors of ischemic preconditioning [10]. Their activation by H_2S is likely to be a relevant cardioprotective mechanism [11], since the anti-ischemic effects of H_2S are largely antagonized by blockers of mitochondrial potassium channel [12]. Other mechanisms have also been proposed to explain the cardioprotective activity of H_2S. Antiapoptotic responses play a role in cardioprotection against I/R injury, and are due to the H_2S-induced triggering of pathways of intracellular signalling, such as PI3K/Akt, PKC and ERK 1/2, and the Nrf-2-mediated antioxidant machinery [13]. Even the inhibition of type-5 phosphodiesterase by H_2S plays a potential role. In fact, the intracellular rise of cGMP, and the consequent cGMP-dependent protein kinase activation, trigger downstream effectors of ischemic preconditioning [14], upregulate CSE levels and promote further H_2S production [15].

Exacerbation of I/R-induced tissue injury can be also due to an intense inflammatory response, triggered by the degranulation of resident heart mast cells and the release of cytokines, growth factors, chemokines, and other pro-inflammatory mediators [16]. Remarkably, cardiac mast cells contain renin and the release of this proteolytic enzyme from cardiac mast cells contributes to the I/R-associated heart injury [17].

Noteworthy, H_2S was reported to inhibit antigen-induced degranulation of rat basophile leukemic RBL-2H3 cells (a well known mast-cell like model) and murine bone marrow-derived mast cells, suggesting an inhibitory role of this gasotransmitter in mast cell-mediated inflammatory responses [18,19]. Therefore, a possible H_2S-mediated inhibition of the degranulation of resident heart mast cells may be a further (and poorly investigated) mechanism contributing, at least in part, to the overall cardioprotective activity.

Given the intriguing biological activity of H_2S in the cardiovascular function, natural and synthetic H_2S-donors, i.e. pro-drugs able to generate exogenous H_2S, are viewed as promising cardioprotective agents [20–22]. 4-carboxy phenyl-isothiocyanate (4CPI, Fig. 1) is a H_2S-releasing compound, known to evoke vasorelaxing responses in isolated rat aorta and to increase coronary flow in isolated rat hearts [23]. Moreover, 4CPI was shown to cause membrane hyperpolarization in human aortic smooth muscle cells through the activation of Kv7 potassium channels, which play a role in H_2S-induced vasodilation [24]. Noteworthy, Kv7 channels (in particular, Kv7.4) have been recently recognized in heart mitochondria, where they appear to play a cardioprotective role [25].

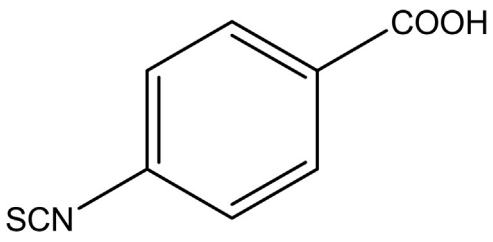

Fig. 1. Chemical structure of 4CPI.

Although the vascular effects of the novel H_2S-donor 4CPI have been elucidated, its potential cardioprotective activity is yet to be evaluated. Hence, the aim of this study was to investigate the effects of 4CPI in different experimental models of I/R in isolated hearts and of acute myocardial infarction in vivo, by evaluating well-known markers of I/R-induced myocardial injury (i.e., reduced myocardial contractility, ventricular arrhythmias, cell death, oxidative stress). The involvement of some relevant mechanisms of action, such as activation of mitochondrial ion channels and/or inhibition of degranulation of resident heart mast cells, was also tested in isolated cardiac mitochondria and isolated hearts, respectively.

2. Materials and methods

2.1. Substances

4CPI (Fluorochem Ltd, Hadfield, UK) was dissolved (10^{-2} M) in dimethylsulfoxide (DMSO), and further diluted in bi-distilled water. 5-hydroxy decanoic acid (5HD; Sigma–Aldrich, Milano, Italy) was dissolved in bidistilled water. Tetraphenylphosphonium chloride (TPP^+Cl^-, Sigma–Aldrich, Milano, Italy) was dissolved in bi-distilled water and 2,3,5-triphenyltetrazolium chloride (TTC, Sigma–Aldrich. Milano, Italy) was dissolved (1%, p/w) in phosphate buffer (pH 7.4). Olygomicin, 2,4-dinitrophenol (DNP), carbonyl cyanide m-chlorophenylhydrazone (CCCP) and valinomycin were purchased from Sigma–Aldrich (Milano, Italy), dissolved in pure DMSO (10 mM) and further diluted in bi-distilled water.

2.2. Pharmacological procedures

All the experimental procedures were carried out following the guidelines of the European Community Council Law 2010/63 and have been approved by the Committee for animal experimentation of the University of Pisa. All procedures on mice were approved by Weill Cornell Medicine Institutional Animal Care and Use Committee.

2.3. Langendorff-perfused rat hearts

Male Wistar rats (260–350 g) were treated with an i.p. injection of different increasing doses of 4CPI 0.072 mg/kg, 0.24 mg/Kg, 0.72 mg/kg and 2.4 mg/kg or their vehicle (DMSO). After 2 h, all the animals were anaesthetized with sodium pentobarbital (100 mg/kg i.p.) and heparinized (100 UI i.p.) to prevent blood clotting. When required from experimental procedure, 5HD (10 mg/kg i.p.) was administrated 20 min before 4CPI (0.24 mg/kg)-treatment.

After opening the chest, hearts were quickly excised and placed in a 4 °C Krebs solution (composition mM: $NaHCO_3$ 25.0, NaCl 118.1, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2 \times 2H_2O$ 1.6, KH_2PO_4 1.2, glucose 11.5), equilibrated with 95% O_2 and 5% CO_2, to stop contraction and reduce oxygen consumption. Rapidly, the ascending aorta was cannulated and the hearts were mounted on a Langendorff apparatus, and then perfused with Krebs solution (thermostated at 37 °C and continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2) at constant pressure (70–80 mmHg). The above procedure was completed within 2 min. A water-filled latex balloon connected to a pressure transducer (Bentley Trantec, mod 800, UgoBasile, Comerio, Italy) was introduced into the left ventricle via the mitral valve and the volume was adjusted to achieve a stable left ventricular end-diastolic pressure of 5–10 mmHg during initial equilibration. After 30 min of equilibration, hearts were subjected to 30 min of global ischemia (no flow). Thereafter, hearts were reperfused for 120 min. Functional parameters were continuously recorded during the whole experiment. At the end of reperfusion hearts were