Effect of desferrioxamine on reperfusion damage of rat heart mitochondria

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Summary

Ischaemia of the myocardium leads to necrosis unless oxygen supply is restored but it has only recently been realised that reperfusion is not without danger. The greatest rate of myocardial damage, as measured by mitochondrial function, occurred during the first 5 minutes of reperfusion in rat hearts subjected to normothermic ischaemic cardiac arrest. Addition of desferrioxamine to the perfusate after 5 minutes of reperfusion did not reverse the mitochondrial damage. It is therefore concluded that desferrioxamine prevents mitochondrial damage caused by ischaemia-reperfusion but does not reverse the damage already present.

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Necrosis is the ultimate outcome of myocardial ischaemia unless oxygen delivery is restored in time. However, reperfusion of ischaemic tissue is not without danger, in fact it has been shown that it adds insult to injury.[1,2] Circumstantial evidence suggests that this reperfusion injury — independent of ischaemic injury — is caused by the generation of oxygen-derived radicals, i.e. superoxide anions, hydrogen peroxide and hydroxyl radicals.[1,2]

Oxygen-derived radicals are produced during the course of normal cell metabolism but are short-lived because of inactivation by protective mechanisms. These protective mechanisms are of two kinds: (*i*) enzymatic, e.g. superoxide dismutase, catalase and glutathione peroxidase; and (*ii*) non-enzymatic, e.g. vitamins C and E, and β-carotenes.[3] These protective mechanisms have sufficient capacity to cope with the normal metabolic generation of oxygen-derived radicals. Superoxide radicals, if not immediately neutralised, can, through the release of iron from ferritin[4] and other large proteins,[5] generate the highly reactive hydroxyl radical via the Haber-Weiss and Fenton reactions.[6]

The superoxide radicals implicated in ischaemia-reperfusion injury of the myocardium may have their origin in increased

generation — more than the normal protective mechanisms can cope with — or may occur as a result of decreased activity of the free radical scavenger mechanisms, or both. Evidence suggests that both are responsible.[3] Moreover it has been demonstrated that during ischaemia iron is released from the chelated iron fraction (and therefore available in reperfusion) and thus contributes substantially to the free radical-induced injury through the Haber-Weiss and Fenton reactions.[7]

We have demonstrated,[7] using rat hearts, that desferrioxamine in the perfusate during ischaemia and the reperfusion period protects the myocardium against reperfusion injury as measured by the change in mitochondrial function. Using mitochondrial function as index of reperfusion injury, we undertook to determine at what time during reperfusion after myocardial ischaemia the greatest rate of change in mitochondrial function occurred. In continuation of our previous work,[7] in which we demonstrated that desferrioxamine in the perfusate prevented the mitochondrial damage caused by ischaemia-reperfusion, we therefore investigated the effect of addition of desferrioxamine to the perfusate after this period of damage.

Material and methods

Male (500 - 600 g) and female (300 - 400 g) Wistar rats were used. The animals were allowed free access to food and water before experimentation.

Krebs-Henseleit bicarbonate buffer[8] containing 2,5 mM calcium and 10 mM glucose was used for perfusion. Where indicated, this solution also contained 0,76 mM desferrioxamine (Desferal). The perfusion solution in all instances was equilibrated with an O_2/CO_2 (95%/5%) gas mixture.

After the rats were decapitated, the heart was removed and arrested in cold saline (4°C). (Hearts that were not subjected to perfusion were termed unperfused.) The aorta was mounted on an aortic cannula and the heart perfused retrogradely without recirculation for 10 minutes at 37°C against water pressure of 90 cm. During this period the left atrium was connected to an atrial cannula to allow for 5-minute atrial perfusion at a pressure of 10 cm water ('the working heart model').[9] Hearts treated as above were referred to as controls. All other hearts were subjected to normothermic ischaemic cardiac arrest (NICA) with or without subsequent reperfusion, which was established by simultaneously clamping both the aortic and atrial cannulae after the control perfusion period.[9]

After NICA coronary pressure was restored by unclamping the tube connected to the aortic cannula.[9] Retrograde perfusion was performed for 66% of the total reperfusion period. After this period of retrograde perfusion, perfusion in the 'working heart mode' was performed for the rest of the reperfusion period.

After termination of the experiments, the hearts were plunged into ice-cold isolation medium (0,18 mM KCl plus 0,01M ethylenediamine tetra-acetic acid (EDTA) with the pH adjusted to 7,4 with tris base at 4°C), homogenised using a Polytron PT homogeniser, and the mitochondria isolated as described previously.[10]

Oxidative phosphorylation was determined polarographically using a Gilson oxygraph with glutamate as substrate. The incubation medium contained 0,25M sucrose, 10 mM tris-HCl (pH 7,4), 8,5 mM K_2HPO_4 and 5 mM glutamate (tris buffer, pH 7,4) at 25°C to which 3 mg mitochondrial protein was added. To measure the Q_{O_2} (state 3), 500 nmol adenosine phosphate (ADP) was added to initiate the reaction. The following measurements were taken: (*i*) ADP/O (nmol ADP consumed/nmol oxygen consumed); (*ii*) Q_{O_2} (state 3) (nmol oxygen consumed in the presence of ADP/mg mitochondrial protein/min); and (*iii*) oxidative phosphorylation rate (OPR) (nmol ADP consumed/mg mitochondrial protein/min), i.e. Q_{O_2} (state 3) × ADP/O.[11]

To establish the period of maximum rate of mitochondrial damage during reperfusion, hearts from female rats were subjected to either 15 minutes or 20 minutes of NICA followed by no reperfusion, 5 minutes, 10 minutes or 20 minutes reperfusion after which the hearts were processed and mitochondrial function measured.

For the second part of this study, hearts from male rats were subjected to one of the following experimental conditions: (*i*) control perfusion: (*a*) without Desferal, (*b*) with Desferal; (*ii*) 20 minutes NICA: (*a*) without Desferal, (*b*) with Desferal; (*iii*) 20 minutes NICA followed by 5 minutes reperfusion; (*iv*) (*iii*) followed by: (*a*) 5 minutes reperfusion with Desferal, (*b*) 30 minutes reperfusion with Desferal; and (*v*) 20 minutes NICA with Desferal in perfusate followed by 5 minutes reperfusion without Desferal.

Statistical analysis. All values were expressed as mean ± SEM and, where applicable, the unpaired Student's *t*-test was calculated and the level of significance was set at $P < 0,05$.

Results

NICA for 15 minutes did not significantly alter mitochondrial function, but a further 5 minutes of NICA decreased mitochondrial function significantly. The maximum rate of additional mitochondrial damage occurred in the first 5 minutes of reperfusion, thereafter mitochondrial damage did not significantly decrease for up to a further 20 minutes of reperfusion (Table I).

NICA with Desferal in the perfusate for 20 minutes significantly prevented mitochondrial damage (Table II: experimental conditions (*ii a*) and (*ii b*)). Addition of Desferal during

TABLE I. EFFECT OF NICA FOLLOWED BY DIFFERENT PERIODS OF REPERFUSION ON MITOCHONDRIAL FUNCTION

			15 min NICA			20 min MICA		
Experiment	Time (min)	*N*	ADP/O	Q_{O_2} (state 3)*	OPR*	ADP/O*	Q_{O_2} (state 3)*	OPR*
Unperfused		8	2,95 ± 0,08	117,89 ± 4,24	347,76 ± 13,83	2,95 ± 0,08	117,89 ± 4,24	347,76 ± 13,83
Control		8	2,93 ± 0,08	120,73 ± 5,16	353,74 ± 18,38	2,93 ± 0,08	120,73 ± 5,16	353,74 ± 18,38
Reperfusion	0	9	2,96 ± 0,09	116,48 ± 10,40	344,78 ± 20,73	2,93 ± 0,09	93,74 ± 4,50†	275,15 ± 18,13†
Reperfusion	5	8	2,92 ± 0,10	75,82 ± 4,80†‡	221,69 ± 19,14†‡	2,60 ± 0,12‡	77,34 ± 5,08†*	207,23 ± 11,99†‡
Reperfusion	10	7	2,82 ± 0,06	70,62 ± 4,50†‡	199,09 ± 13,27†‡	2,76 ± 0,12‡	75,00 ± 7,39†‡	205,63 ± 20,88†‡
Reperfusion	20	8	2,88 ± 0,16	62,01 ± 12,17†‡	178,59 ± 12,13†‡	2,01 ± 0,46	67,07 ± 4,01†‡	184,92 ± 11,50†‡

*Statistically significant difference ($P < 0,05$).
†Treated v. unperfused.
‡Reperfused v. not reperfused (*iii*).

TABLE II. EFFECT OF DESFERAL ON MITOCHONDRIAL REPERFUSION DAMAGE

	Experimental conditions*	*N*	ADP/O†	Qo_2 (state 3)†	OPR†
	Unperfused (—)	8	2,94 ± 0,03	88,36 ± 4,60	259,78 ± 16,73
i	**(*a*) Control (—)**	7	2,94 ± 0,03	86,40 ± 4,61	254,46 ± 15,55
	(*b*) Control (+)		2,95 ± 0,05	88,41 ± 5,06	260,81 ± 14,84
ii	**(*a*) 20 min NICA (—)**	6	2,83 ± 0,04‡	75,51 ± 4,01	213,14 ± 11,10‡
	(*b*) 20 min NICA (+)	4	2,95 ± 0,04	92,46 ± 3,76§	272,76 ± 14,55§
iii	**20 min NICA (—)§ 5 min reperfusion (—)**	8	2,69 ± 0,01‡§¶	62,75 ± 4,03‡§¶	168,80 ± 19,29‡§¶
iv	**(*a*) (*iii*) + 5 min reperfusion (+)**	6	2,76 ± 0,04‡	60,63 ± 5,00‡	168,94 ± 18,55‡
	(*b*) (*iii*) + 30 min reperfusion (+)	8	2,81 ± 0,05‡‖	52,09 ± 6,18‡	146,37 ± 19,87‡
v	**20 min NICA (+)§ 5 min reperfusion (—)**	4	2,91 ± 0,03**	56,98 ± 5,32‡	165,81 ± 18,46‡

* (—) = without Desferal
(+) = with Desferal
† Statistically significant difference ($P < 0,05$):
‡V. unperfused.
§NICA (+) v. NICA (—).
¶(*iii*) v. (*ii*) (*a*).
‖(*iv*) v. (*iii*).
**(*v*) v. (*iii*).

the 20-minute NICA period only did not prevent the reperfusion damage in the first 5 minutes of reperfusion (Table II: experimental conditions (*iii*) and (*iv*)). Reperfusion with Desferal did not improve mitochondrial impairment due to ischaemia followed by reperfusion (Table II: experimental condition (*iii*) v. (*iv*) and (*iv b*)).

From our experimental results we conclude that the greatest rate of mitochondrial damage occurs during the first 5 minutes of reperfusion. This damage was not prevented by the inclusion of Desferal in the perfusate during the NICA period, and, furthermore, this reperfusion damage was not reversed by subsequent reperfusion with Desferal-containing solution.

Discussion

Using rat hearts subjected to NICA as an experimental model of ischaemic injury it was shown that Desferal in the perfusate prevents or delays ischaemic mitochondrial injury and subsequent reperfusion mitochondrial damage.[7,10] Following this work, certain questions remained. Firstly, at what period after the start of reperfusion the greatest rate of mitochondrial damage occurred. The first part of our experiment established that the greatest rate of mitochondrial damage occurred during the initial 5 minutes of reperfusion (Table I).

This result was used in the planning of the second part of our study; the effect of Desferal in the perfusate on prevention and alleviation of the reperfusion mitochondrial injury. The results (Table I) show that ischaemia caused by NICA only starts to affect mitochondrial function significantly after more than 15 minutes. Moreover, this deterioration of mitochondrial function is prevented by the inclusion of Desferal in the perfusate during NICA, but when Desferal was omitted from the reperfusion solution the hearts previously perfused with Desferal-containing solution during NICA showed a greater rate of mitochondrial damage within 5 minutes (Table II: (*ii b*) v. (*v*)). This rate was of such a degree that the level of mitochondrial injury after 5 minutes reperfusion was similar to that of hearts not perfused with Desferal (Table II: experimental conditions (*iii*) and (*v*)). Thus prior perfusion during NICA with Desferal-containing solution did not prevent mitochondrial damage due to reperfusion. To determine whether Desferal had any effect on mitochondrial damage caused by reperfusion we perfused hearts, subjected to 20 minutes of NICA and 5 minutes reperfusion without Desferal, for 5-minute or 30-minute periods with Desferal in the reperfusion solution. Results showed that this procedure did not significantly improve damaged mitochondrial function (Table II: experimental conditions (*iv a*) and (*iv b*) compared with (*iii*)).

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