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Suppression of Myocardial Mitochondrial Respiratory Function in Acute Failing Hearts Made by a Short-term Ca^{2+} Free, High Ca^{2+} Coronary Perfusion

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M. TAKAKI, D. D. ZHAO, L. Y. ZHAO, J. ARAKI, M. MORI AND H. SUGA. Suppression of Myocardial Mitochondrial Respiratory Function in Acute Failing Hearts Made by a Short-term Ca^{2+} Free, High Ca^{2+} Coronary Perfusion. *Journal of Molecular and Cellular Cardiology* (1995) 27, 2009–2013. We made acute cardiac failure in excised cross-circulated canine hearts by a new coronary perfusion protocol consisting of Ca^{2+} free Tyrode perfusion for the first 10 min, high Ca^{2+} (16 mmol/l) Tyrode perfusion for the next 5 min, and normal Tyrode perfusion for the last 5 min interrupting blood cross circulation. After 50 min from the blood recirculation, left ventricular contractility was stably depressed to 60% of control. We studied mechanoenergetics of these acute failing hearts for the next 1–3 h. Then, we prepared mitochondria from these excised failing hearts and the support dogs' normal hearts to examine their mitochondrial respiratory function by the respiratory control index (RCI) and the oxygen consumption rate in state III (State III O_2). RCI and State III O_2 were significantly smaller in the failing hearts than in the normal hearts. However, sham protocol consisting of normal Tyrode coronary perfusion for 20 min did not affect RCI and State III O_2. These results revealed that the mitochondrial respiratory function was moderately impaired in these acute failing hearts made by the new short-term Ca^{2+} intervention. However, no ultrastructural injuries of mitochondria were detected in these failing hearts.

KEY WORDS: Ca^{2+} paradox; Ca^{2+} overload; Mitochondria; Respiratory function; Respiratory control index; Heart.

Introduction

We studied myocardial mitochondrial respiratory function in acute failing hearts made by a newly developed short-term Ca^{2+} free, high Ca^{2+} coronary perfusion (Ca^{2+} depletion-Ca^{2+} overload combination) protocol in the excised cross-circulated canine hearts (Araki *et al.*, 1995). This perfusion was designed to make acute failing hearts by Ca^{2+} overload without hypoxia and ischaemia. We finally succeeded in producing myocardial dysfunction by a combination of short-term coronary Ca^{2+} free perfusion and high Ca^{2+} perfusion without ischaemia (Araki *et al.*, 1995). We assessed mechanoenergetics of the failing heart by the E_{max} (an index of contractility)-PVA (pressure-volume area; total mechanical energy)-Vo_2 (oxygen consumption) framework (Suga, 1990). These acute failing hearts decreased E_{max} to about 60% of control, but did not decrease the contractile efficiency

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(inverse slope of the Vo_2-PVA relation). We considered that this new protocol effectively elicited Ca^{2+} overload leading to acute cardiac failure (Araki *et al.*, 1995). However, intriguingly, the mechanoenergetics of this acute failing heart (Araki *et al.*, 1995) was qualitatively different from those post-ischaemic stunned (Ohgoshi *et al.*, 1991), acidotic (Hata *et al.*, 1994a) and post-acidotic stunned hearts (Hata *et al.*, 1994b). Therefore, we examined mitochondrial respiratory function to characterize energetic aspects of this acute cardiac failure at a subcellular level.

Materials and Methods

Two mongrel dogs were anesthetized with pentobarbital sodium (25 mg/kg, i.v.) after premedication with ketamine hydrochloride (50 mg per dog, i.m.) in each experiment. The details of the heart preparation have been described elsewhere (Ohgoshi *et al.*, 1991; Namba *et al.*, 1994; Araki *et al.*, 1995). Briefly, a heart was excised from a (donor) dog without any interruption of coronary circulation and was cross-circulated with systemic arterial blood of the other (support) dog which was artificially ventilated. Temperature of the heart was monitored and maintained constant near 36°C throughout the experiment. Arterial pH, Po_2 and Pco_2 of the support dog were maintained within their physiological ranges.

Main protocol (eight hearts)

After a control period of 2–3 h cross circulation with blood, the Ca^{2+} free, high Ca^{2+} coronary perfusion protocol was carried out as follows. The blood perfusion was switched to Ca^{2+} free Tyrode solution for 10 min followed by high Ca (16 mmol/l) Tyrode solution for 5 min, both under a hydrostatic pressure of 80–90 cmH_2O. The high Ca^{2+} perfusion was switched to normal Tyrode solution for 5 min to avoid contamination of the support dog by the high Ca^{2+} solution. Then, the blood cross circulation was restored.

Auxiliary protocol 1 (five hearts)

To examine the effects of cross circulation *per se*, the cross circulation was maintained without any Tyrode perfusion for 4–5 h.

Auxiliary protocol 2 (five hearts)

As sham protocol, cross circulation was interrupted by normal Tyrode perfusion for 20 min to examine the effects of Tyrode perfusion *per se*. In both main and sham protocols, the Tyrode solutions were drained off without returning to the support dog to avoid haemodilution and Ca^{2+} imbalance in the support dog. Ca^{2+} concentration was different among the normal Ca^{2+} (1.8 mmol/l), Ca^{2+} free (0 mmol/l) and high Ca^{2+} (16 mmol/l) Tyrode solutions. All solutions were gassed with 5% CO_2-95% O_2.

Left ventricular myocardial mitochondria were prepared from both the excised crosscirculated hearts which had been subjected to the main and auxiliary protocols and the intact normal hearts of the support dogs. A differential centrifugation was used according to the method of Hatefi *et al.* (1961). Immediately after the mitochondrial preparation, oxygen consumption of the mitochondria was measured polarographicaly with an oxygen electrode installed in a closed cell (UC-12; Central Kagaku, Tokyo, Japan) at 25°C (see Acknowledgements). The incubation medium contained 0.3 M mannitol, 10 mmol/l KH_2PO_4, 2.5 mmol/l $MgCl_2$, and 0.25 mmol/l ethylenediaminetetra acetate (EDTA) with pH 7.4 in a total of 0.7 ml. First, 0.03 ml of mitochondrial suspension (15.6 ± 3.7 mg protein/ml) was added and then 0.2 M succinic acid (0.03 ml) was added as a substrate into the incubation medium. After 2 min, 10 mmol/l adenosine diphosphate (ADP) (0.03 ml) were added. The rate of oxygen consumption in State III (State III O_2) and the respiratory control index (RCI) were determined. State III O_2 was calculated from mitochondrial oxygen consumption in n atoms (1 mole $O_2 = 2$ atoms O_2) of oxygen consumed per min per mg mitochondrial protein during State III respiration. RCI (dimensionless) was taken as the ratio between the rates of oxygen consumption before and after the addition of adenosine diphosphate (ADP) (Nishinaka *et al.*, 1992). Succinate was chosen as a metabolic substrate. For electron-microscopic examination, tissue specimens of left ventricular myocardium were removed from both the cross-circulated heart and the support dog's heart at the end of the mechanoenergetic study. The tissues were dissected into small pieces (1 mm × 1 mm) and fixed by immersion for 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The tissue pieces were then washed in the same buffer for 6 h and post-fixed for 2 h in 1% OsO_4 in 0.1 M cacodylate buffer, pH 7.4, dehydrated with ethanol, and embedded in Epon. Thin sections