ELSEVIER

European Journal of Pharmacology 279 (1995) 105–107

ejp

Short communication

Is endothelin-1 release at reperfusion of the ischaemic human heart due to cold-induced displacement of endothelin from binding sites?

Anders Franco-Cereceda [a,*], Gunilla Barr [b], Anders Öwall [b], Jan Liska [a], Jan M. Lundberg [c]

[a] *Department of Thoracic Surgery, Karolinska Hospital, S-171 76 Stockholm, Sweden*
[b] *Department of Cardiothoracic Anaesthetics and Intensive Care, Karolinska Hospital, Stockholm, Sweden*
[c] *Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden*

Received 30 March 1995; accepted 4 April 1995

Abstract

Following reperfusion of ischaemic human hearts subjected to cold (4° C) cardioplegia during coronary bypass surgery, there was an increase in cardiac outflow of endothelin-1 but not the pro-peptide big endothelin-1. Furthermore, specific endothelin-1 binding in human lung membrane preparations was displaced by incubation in buffer medium at 4° C. The present results thus indicate that cold-induced displacement of endothelin-1 binding, rather than increased synthesis, may explain the cardiac release of endothelium-1 following ischaemia during heart surgery in which cold cardioplegia has been used.

Keywords: Endothelin; Heart; Ischemia; Receptor displacement

1. Introduction

The vascular endothelium synthesizes the coronary vasoconstrictor peptide endothelin-1 through enzymatic cleavage of the propeptide big endothelin-1 (Yanagisawa et al., 1988; Franco-Cereceda, 1989). Increased plasma levels of endothelin-1 have been reported in a variety of cardiovascular disorders although the data on the possible cardiac release of endothelin-1 during myocardial ischaemia are conflicting (see Tonnessen et al., 1993; Franco-Cereceda et al., 1994). Acute myocardial infarction is associated with increased peripheral plasma levels of both endothelin-1 and big endothelin-1 (Miyashi et al., 1989). In order to evaluate a possible co-release of endothelin-1 and big endothelin-1 from the human heart we have measured the cardiac outflow of these peptides from the ischaemic heart during coronary bypass surgery.

2. Materials and methods

This study was approved by the Ethics Committee of the Karolinska Hospital. Four males (66 ± 3 years) with stable angina pectoris underwent coronary bypass surgery with extracorporeal circulation (for 84 ± 15 min). Hypothermia (32° C) and cardiac ischaemia were induced by aortic cross-clamping (44 ± 11 min) in combination with intermittent, cold cardioplegia (4° C) administered approximately every 10 min. Arterial and coronary sinus blood samples were obtained before cardioplegia was given and 1, 5, 15 and 30 min after release of the aortic cross-clamp. Coronary sinus blood flow was measured at each sampling time point by thermodilution. Intracoronary outflow of the peptides was calculated as previously described for neuropeptide Y (Franco-Cereceda et al., 1990). Endothelin-1- and big endothelin-1-like immunoreactivity was determined by radioimmunoassay (for details, see Weitzberg, 1993). In human lung preparations, specific endothelin-1-binding was determined as previously described (Hemsén et al., 1990). Briefly, the membranes were

* Corresponding author. Fax: -8-32 27 01.

SSDI 0014-2999(95)00213-8

incubated at 37° C for up to 180 min. Cold-induced displacement of endothelin-1 binding was studied by cooling the buffer solution to 4° C for 5, 30 and 60 min after equilibrium had been reached at 37° C. Data were calculated as difference of specific binding at 4° C compared to corresponding time points at 37° C.

3. Results

Basal arterial and coronary sinus plasma concentrations of endothelin-1-like immunoreactivity were 4.2 ± 0.4 and 3.8 ± 0.1 fmol $\times$ ml^{-1}, respectively, while the corresponding levels of big endothelin-1-like immunoreactivity were 9.6 ± 0.9 and 9.5 ± 1 fmol $\times$ ml^{-1}, respectively. Reperfusion of the ischaemic hearts was associated with elevated concentrations of endothelin-1-like immunoreactivity, in both arterial and coronary sinus plasma (maximal increase to 5.1 ± 0.5 and 5.5 ± 0.3 fmol $\times$ ml^{-1}, respectively). There was a positive gradient (i.e. a net outflow of endothelin-1) over the heart at 5 and 15 min, while no influence on the arterial or coronary sinus levels of big endothelin-1-like immunoreactivity was observed. The coronary sinus blood flow increased from a basal of 120 ± 30 ml $\times$ min^{-1} prior to ischaemia, to 195 ± 50 ml $\times$ min^{-1}, 265 ± 42 ml $\times$ min^{-1}, 202 ± 51 ml $\times$ min^{-1} and 146 ± 6 ml $\times$ min^{-1} at 1 min, 5 min, 15 min and 30 min after aortic declamping, respectively. Thus, correlated to coronary sinus blood flow, there was a marked, significant increase in cardiac outflow of endothelin-1-like immunoreactivity at 5 min and 15 min reperfusion while the outflow of big endothelin-1-like immunoreactivity remained unchanged throughout the reperfusion period (Fig. 1).

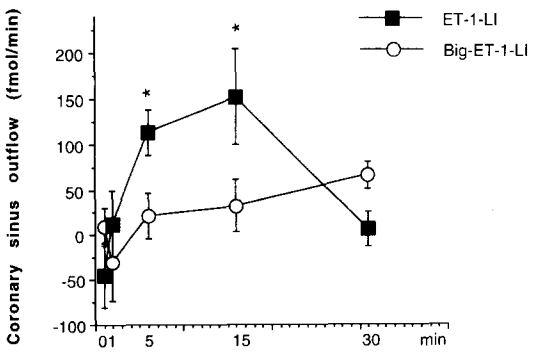

Fig. 1. Cardiac release of endothelin-1 like immunoreactivity (ET-1-LI; ■) and big endothelin-1 like immunoreactivity (Big ET-1-LI; ○) following reperfusion after 44 ± 11 min of global ischaemia induced by aortic cross-clamping and cardioplegia in four patients undergoing coronary bypass surgery. Data are given as fmol $\times$ min^{-1} and presented as means $\pm$ S.E.M. * $P <$ the Friedman test for several related samples.

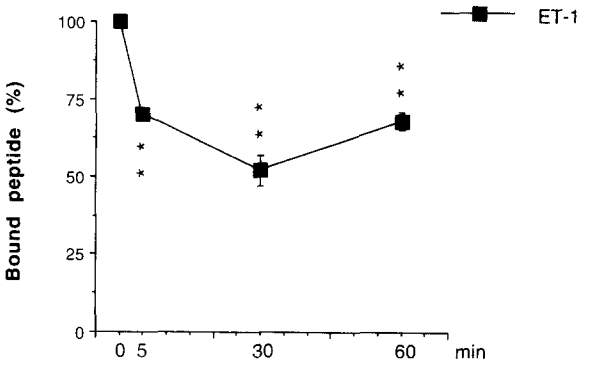

Fig. 2. Dissociation of [^{125}I]endothelin-1 binding (ET-1) in membrane preparations from the human lung. When equilibrium was reached, the incubation buffer was cooled to 4° C for 5, 30 or 60 min. Values represent means $\pm$ S.E.M. ($n = 6$ experiments in each group). * * $P < 0.01$, Mann-Whitney U-test. Bound peptide refers to difference of specific binding at 4° C compared to corresponding time points at 37° C.

High affinity binding sites for endothelin-1 were present in the lung with specific binding representing more than 80% of total binding ($n = 6$). The binding was time-dependent and reached equilibrium within 120 min and remained stable for up to 180 min in control experiments ($n = 6$). Cooling the incubation buffer solution to 4° C led to a displacement of specific endothelin-1 binding with 30%, 48% and 32% at 125 min, 150 min and 180 min, respectively ($n = 6$ in each group; $P < 0.01$ at all times compared to controls, Mann-Whitney U-test) (Fig. 2). The non-specific binding was unchanged by cooling.

4. Discussion

The present study demonstrates that aortic declamping after coronary bypass surgery is associated with increased cardiac outflow, suggesting release, of endothelin-1 but not of big endothelin-1. Prolonged cardiac ischaemia has been shown to cause severe coronary endothelial injury (see Tonnessen et al., 1993). However, the observed cardiac release of endothelin-1 is unlikely to represent a simple washout of accumulated peptide from deteriorating endothelial cells, or de novo synthesis, since big endothelin-1 overflow was not changed. Recently, endothelin receptor antagonism has been demonstrated to increase the circulating levels of endothelin-1, but not big endothelin-1 (Löffler et al., 1993) and the plasma levels of endothelin-1 increased following cold pressor test (Fyhrqvist et al., 1990). In analogy, the present observations that cooling the incubation buffer leads to displacement of endothelin-1 binding from lung membranes indicates that the conditions associated with cardioplegia and hypothermia lead to 'release' of endothelin-1 from tissue

binding sites. Neuropeptide Y is released from the heart during coronary bypass surgery (Franco-Cereceda et al., 1990) but this is apparently not due to cold-induced displacement of neuropeptide Y binding, since no such effect was observed in isolated spleen membrane preparations (unpublished data). Although big endothelin-1 was present in higher concentrations and has a longer plasma half-life than endothelin-1 (7 min for big endothelin-1 versus 1.5 min for endothelin-1, in man in vivo; Hemsén et al., 1995), no influence on plasma levels or coronary sinus outflow of big endothelin-1 was detected, indicating that extracorporeal circulation and reperfusion of the ischaemic heart are not associated with release of big endothelin-1. The possible influence of endothelin-1 displacement on the hyperaemia observed at reperfusion of the hearts after cardiac surgery remains to be further evaluated.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council (9895), the Heart-Lung Foundation, the Wallenberg Foundation, the Thuring Foundation, the M. Bergvall Foundation, the Swedish Society of Medicine, the Salus Foundation and funds from the Karolinska Institute.

References

Franco-Cereceda, A., 1989, Endothelin- and neuropeptide Y-induced vasoconstriction of human epicardial coronary arteries in vitro, Br. J. Pharmacol. 97, 968.

Franco-Cereceda, A., A. Öwall, A. Sollevi, G. Settergren and J.M. Lundberg, 1990, Release of neuropeptide Y and noradrenaline from the human heart after aortic occlusion during coronary artery surgery, Cardiovasc. Res. 24, 242.

Franco-Cereceda, A., A. Ericsson, P. Sellei, J. Vaage, G. Valen and J.M. Lundberg, 1994, Endothelin release at reperfusion of the porcine ischaemic heart in relation to noradrenaline and neuropeptide Y, Acta Physiol. Scand. 151, 541.

Fyhrqvist, F., O. Saijonmaa, K. Metsärinne, I. Tikkanen, K. Rosenlöv and K. Tikkanen, 1990, Raised plasma levels of endothelin-1 following cold-pressor test, Biochem. Biophys. Res. Commun. 169, 217.

Hemsén, A., A. Franco-Cereceda, R. Matran, A. Rudehill and J.M. Lundberg, 1990, Occurrence, specific binding sites and functional effects of endothelin in human cardiopulmonary tissue, Eur. J. Pharmacol. 191, 319.

Hemsén, A., G. Ahlborg, A. Ottoson and J.M. Lundberg, 1995, Metabolism of big endothelin-1 in the human circulation in relation to production of endothelin-1, Regul. Pept. 355, 287.

Löffler, B.M., V. Breu and M. Clozel, 1993, Effect of endothelin receptor antagonists and of the non-peptide antagonist Ro 46-2005 on endothelin levels in rat plasma, FEBS Lett. 333, 108.

Miyashi, T., M. Yangisawa, T. Tomizawa, Y. Sugishita, N. Suzuki, M. Fukino, R. Ajisaki, K. Goto and T. Masaki, 1989, Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction, Lancet ii, 53.

Tonnessen, T., P.A. Naess, K.A. Kirkeboen, J. Offstad, A. Ilebekk and G. Christensen, 1993, Release of endothelin from the porcine heart after short term coronary artery occlusion, Cardiovasc. Res. 27, 1482.

Weitzberg, E., 1993, Circulatory responses to endothelin-1 and nitric oxide, Acta Physiol. Scand. 148 (Suppl. 611), 1.

Yanagisawa, M., H. Kiruhara, S. Kimura, Y. Tomobe, M. Kobyashi, Y. Mitsui, Y. Yazaki, K. Goto and T. Masaki, 1988, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 332, 411.