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Pergamon

Clinical Hemorheology, Vol. 16, No. 5, pp. 701–711, 1996

0271-5198/96 $15.00 + .00

PII S0271-5198(96)00070-5

9TH EUROPEAN CONFERENCE ON CLINICAL HAEMORHEOLOGY
Siena, Italy 28 June - 1 July 1995

A.L. COPLEY LECTURE

ENDOTHELIN-1 IN CARDIOVASCULAR PHYSIOLOGY

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(Accepted by T. Di Perri on 26.08.96)

ABSTRACT

Endothelin-1 (ET-1) is a 21 amino acid peptide endowed with a a powerful vasoconstrictor activity. The molecular structure and biological activities of endothelin were explored in several studies starting soon after its discovery in 1988, but only recently has the availability of specific ET-1 antagonists allowed its physiological activities to be explored. It is now becoming evident that circulating ET-1 plays a key role in the continuous adaptation of vessel wall tone, of both resistence and capacitance vessels. On the other hand, in addition to the ability to control blood flow, ET-1 synthesis is in turn controlled by blood flow itself, thus making the connection between ET-1 and peripheral circulation particularly close. This review will focus on recent knowledge about the physiological role of circulating endothelin-1. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In 1989 bovine endothelial cells were found to produce a 21-aminoacid peptide endowed with a powerful vasoconstrictor activity (1). The peptide was called endothelin, its primary structure was defined and the gene cloned (2). Availability of the synthetic peptide resulted in the development of reliable and accurate radioimmunological assay methods so that the number of studies on endothelin quickly grew (3,4), and the lists of the cell types able to produce endothelin, of the biological effects of the peptide, and of the pathophysiological conditions where endothelin concentration was increased, rapidly expanded. Pharmacological studies, based on the intraarterial

KEY WORDS:
Endothelin physiology, Haemodynamics, Vasoactive substances, Dynamic forces, Gene regulation.

The infusion of exogenous big endothelin-1 into the brachial artery caused either a dose dependent forearm vasoconstriction and an increase in the concentration of endothelin-1 and of the inactive CTF. All these effects were blunted when exogenous big endothelin-1 was infused in the presence of an ECE inhibitor (phosphoramidon) (8).

The first evidence that endogenous ET-1 contributes to the maintenance of the physiological arterial tone in vivo was given by Mc Mahon *et al.* (24) who showed that the injection of an ECE inhibitor (phosphoramidon) in rats caused a significant blood pressure reduction. Successively, the same finding was obtained also in humans by showing that the infusion of phosphoramidon (30 nmol/min) into the brachial artery caused a progressive forearm vasodilation, reaching a maximum increase of 40% after 40 min infusion (8). The role of endogenous ET-1 in the maintenance of basal resistance vessel tone in man was well demonstrated by the infusion of a specific ETA receptor antagonist, BQ123 (25,26). BQ123 (100 nmol/min, 3 mg/h) caused a progressive vasodilation with a maximum increase in forearm blood flow of about 65% when infused alone and abolished the vasoconstriction when co-infused with ET-1 (8). The same authors also showed that endogenous ET-1 is mainly degraded in loco by neutral endopeptidase because the infusion into the human brachial artery of a selective inhibitor of neutral endopeptidase, thiorphan (30nmol/min), caused a forearm vasoconstriction with a maximum decrease in blood flow of about 20% (8).

ET-1 antagonists also helped to define the level of activity of ET-1 in the vascular tree. ET-1 exerts its prevalent activity at the level of small resistance and pre-capillary vessels because the injection into human skin of either a selective ET-A receptor blocker (PD 147953 $10^{-8\text{-}10}$ mol) or an ET-A and ET-B receptor antagonist (PD145065 10^{-8} mol), produced a marked vasodilation with a significant local increase in blood flow (27). Thus, clear evidence exists that ET-1 causes a tonic vasocostriction of small resistance vessels in physiological conditions.

In several pathophysiological conditions such as congestive heart failure (28,29,30,31) and myocardial infarction (32,33) ET-1 concentration in plasma shows a persistent three-four fold increase. Therefore, it is important to know whether these long-lasting increases of plasma ET-1 concentration have some pathophysiological consequences or rather a down regulation of the circulatory response occurs. Studies performed by infusing exogenous ET-1 in humans cannot answer this question because ET-1 was infused either for short periods of time (34) or at high doses, thus obtaining plasma concentrations significantly higher than those observed in pathophysiological disorders (18,19,20,21). Recently, the long term effects of a stable increase of plasma ET-1 concentration were assessed by Wilkins *et al.* (35) by injecting 2.5 ng/kg/min of ET-1 in dogs for 8 days which resulted in plasma concentrations 2-3 times baseline. In these conditions ET-1 infusion caused peripheral vascular resistance and blood pressure to increase within about 2 hours, followed by a decrease of cardiac output after about 6 hours and these haemodynamic effects remained significant without adaptation of the cardiovascular response, over the following 8 days of infusion (35). Thus, a 2-3 times increase in plasma ET-1 concentration causes haemodynamic effects which are not extinguished until the ET-1 concentration increase persists.

More recently, the effects of the ET-1 inhibition in a clinical condition characterized by a 3-4 fold increase of ET-1 plasma concentration were studied by Kiowsky *et al* (36). The administration of an ET-1 receptor antagonist (bosentan, 100 mg per os) in patients with chronic heart failure resulted in a significant reduction of blood pressure and systemic vascular resistance. This effect was paralleled by a 2-fold increase in plasma ET-1 concentration probably due to displacement of

blood flow and inversely related to the radius of the vessel, causes changes of shape and orientation of endothelial cells, both aimed to mitigate the cell effect of the force itself (47)
Some responses of the endothelial cells to mechanical forces are extremely rapid whereas other adaptative responses require minutes to hours to achieve a steady state. The former include the burst in cyclooxygenase activity (52), inositol triphosphate generation (53) and increase in cytosolic calcium concentration (54), whereas the latter involve gene expression, cytoskeleton redistribution and cell shape and morphology changes (55).
Both stretch and shear stress control the ET-1 synthesis by endothelial cells. In cultured bovine endothelial cells, changes in the intracellular tension increased the synthesis of ET-1 (56,57). Vessel wall stretch has been reported to increase the ET-1 synthesis also in vivo because infusion of isotonic fluids at increasing rates (44) caused an augment of plasma ET-1 with a pattern which is closely dependent on the increases of venous distension: the larger venous distension, the larger increase in ET-1 concentration. Stretching is able to induce ET-1 synthesis also in other tissues in addition to endothelium. In in vivo experiments an increased expression of ET-1 mRNA was observed in the left ventricle during the development of myocardial hypertrophy induced by aortic banding in rats (58). A specific ET-1 antagonist (BQ123) blocked the increase in the left ventricular weight (58), thus suggesting a role for ET-1 in the development of myocardial hypertrophy (59,60,61).
The effects of shear stress on ET-1 synthesis are different depending on the different levels of shear. In bovine cultured endothelial cells, the increase of shear stress from 0 up to 5-10 dynes/cm^2 caused an increased production of ET-1 (62). The first cell response to shear stress was an increase in the intracellular Ca^{2+} concentration followed by a depolymerization of actin with a transition from F-actin to G-actin (63). The increase in G-actin is probably the final cause of the increase in the ET-1 mRNA expression (64). At higher shear values (15-20 dynes/cm^2) ET-1 synthesis was inhibited (65,66) probably due to the increased nitric oxide formation induced by the high shear stress. Nitric oxide in turn inhibits ET-1 synthesis (67). That the inhibition of ET-1 synthesis induced by high shear values was nitric oxide-dependent was indicated by the dose-dependent reversion operated by L-MNNA (an inhibitor of nitric oxide synthase), and by the potentiation operated by iso-buthyl-methyl-xanthine (which potentiates the cGMP mediated nitric oxide activity). Moreover, the inhibition of ET-1 synthesis at high shear values can be reverted by methylene blue which directly inhibits NO (67).

CONCLUSIONS

The physiological activity of ET-1 in the regulation of peripheral blood flow has been recognized in the last few years, thanks to the development of specific ET-1 antagonists. Human experiments showed not only that ET-1 is crucial in the maintenance of peripheral vascular tone, but also that it participates in the continuous adaptation of the tone of capacitance vessel to volemic changes. Therefore, ET-1 appears not only as a powerful vasoconstrictor responsible for or worsening a clinical condition, but rather as a complex system provided with a relevant physiological role, dynamically controlled by both humoral and mechanical factors and closely cooperating with nitric oxide in the continuous adaptation of vascular bed.

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administration of endothelin-1 (ET-1), frequently achieving plasma concentrations several fold higher than those usually found in plasma, contributed to the development of the knowledge of the effects of ET-1, but could not clarify the ET-1 physiological functions. Only recent availability of specific ET antagonists (5) offered a relevant tool to investigate the physiological role of ET-1 in cardiovascular physiology. It is also becoming evident that not only ET-1 maintain blood flow by tonically contributing to peripheral vascular resistance, but that in turn the synthesis of ET-1 is controlled by blood flow itself, through the action of different mechanical forces.
The aim of the present report is to review the recent knowledge of the physiological activity of ET-1 in the regulation of peripheral blood flow.

ENDOTHELIN-1

Endothelin-1 (ET-1) is one of a family of three isopeptides and is the predominant isopeptide generated by the vascular endothelium (2,4). The other two members, endothelin-2 and endothelin-3, are more difficult to detect and are probably less important in human cardiovasculature. ET-1 is generated from a 38-amino acid precursor, big endothelin-1, through the cleavage of the C-terminal fragment (CTF) operated by a unique "endothelin converting enzyme" (ECE), a membrane bound phosphoramidon sensitive, thiorphan-insensitive neutral metalloprotease (6,7). On the other hand, ET-1 degradation seems to be mainly due to thiorphan-sensitive neutral endopeptidase (8).
ET-1 exerts its action through binding to at least two receptors, ET-A and ET-B, both of which have been isolated by in vitro expression of cloned human cDNA (9,10). The major receptor subtype causing vasoconstriction is the ET-A receptor expressed on vascular smooth muscle cells (11). ET-B receptor is mainly expressed on endothelial cells where it mediates the release of nitric oxide (NO) (12), but is also expressed on smooth muscle cells of capacitance and resistance vessels where it contributes to ET-A mediated vasoconstriction (13,14). The vasoconstrictor activity of ET receptors located on smooth muscle cells appears also from the more powerful vasoconstriction induced by application of ET-1 on the extraluminal side of a vessel (already evident at 10^{-10} M) rather than on the luminal side on endothelial cells (15,16). Moreover, most of ET-1 produced in the endothelium is released abluminally to interact with vascular smooth muscle, while only about one third reaches circulating blood where it can be measured by radioimmunoassay techniques (17).
Haemodynamic response to systemic administration of exogenous ET-1 and particularly the increase in blood pressure (18) and forearm and systemic resistance (18,19) suggests a main level of activity at resistance vessels (18,19,20,21). This suggestion is also supported by the observation that ET-1 injection in isolated rat heart caused a prevalent constriction of arterioles with a diameter which is 20 μm lower, rather than in the larger ones (22,23).

ROLE OF ET-1 IN MAINTAINING ARTERIAL TONE

An important progression in the knowledge of the possible physiological role of ET-1 was the recent demonstration of the existence in human forearm resistance vessels of an ECE activity (8).

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