

## Diversity of endothelium-derived vasoconstricting factors — arachidonic acid metabolites<sup>1</sup>

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### ABSTRACT

Vascular endothelium releases vasoconstricting and/or vasorelaxing substances. Here, we report the diversity of endothelium-derived vasoconstricting factors (EDCFs), arachidonic acid metabolites, and discuss the pathophysiological significance. In the canine basilar artery and the rabbit intrapulmonary artery, acetylcholine-induced contractions (ACh-induced EDC) are due to endothelial thromboxane A<sub>2</sub> (TXA<sub>2</sub>) (TXA<sub>2</sub>-type). The ACh-induced EDC in the rabbit coronary artery is due to endothelial leukotrienes (LTs) (LTs-type). In addition, in the rat coronary artery, nicotine and noradrenaline (NAd)-induced EDCs are due to endothelial COX-metabolites (COX metabolite-type). These arachidonic acid metabolites derived from endothelium (activation by vasoactive substances including ACh, NAd and nicotine) cause a contraction of vascular smooth muscle cells and may disturb the local circulation. These EDCFs (TXA<sub>2</sub>, LTs and COX-metabolites) may be involved in the pathophysiology of cardiovascular immuno-inflammatory diseases.

### INTRODUCTION

Furchgott found that ACh causes an endothelium-dependent relaxation (EDR) in the rabbit thoracic aorta<sup>[1]</sup>. Since then, extensive studies to determine endothelium-derived vasorelaxing factors (EDRF) have been carried out. Finally, NO was identified as an EDRF<sup>[2,3]</sup>. In addition to NO, PGI<sub>2</sub> also belongs to an EDRF. Although the exact chemical structure has not been determined yet, there is another type of EDRF, an endothelium-derived hyperpolarizing factor (EDHF)<sup>[4]</sup>. There are many reports on the pathophysiological significance of

EDRF in terms of the cardiovascular system. The NOS (NO synthase) activity of vascular preparations in SHR is lower than that of WKY<sup>[5-7]</sup>. In addition, the NO-mediated EDR in experimental diabetes mellitus rat by streptozotocin is also lower than that of control rat<sup>[8]</sup>. In contrast to the EDRF, we found that ACh caused an endothelium-dependent contraction (EDC) in the canine basilar artery and in the rabbit intrapulmonary artery and TXA<sub>2</sub> is an endothelium-derived vasoconstricting factor (EDCF) for EDC<sup>[9-12]</sup>. In addition to this type of EDCF (arachidonic acid metabolites), Yanagisawa *et al* have found another type of EDCF (peptide) from culture medium and have identified that endothelin (ET) is an EDCF<sup>[13]</sup>. Furthermore, it has been reported that superoxide anion is also an EDCF in the canine cerebral artery<sup>[14]</sup>. The present review deals with the diversity of the EDCF (especially in terms of arachidonic acid metabolites) in various arterial preparations and dis-

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cusses the possible pathophysiological significance.

**EDCF: THROMBOXANE A<sub>2</sub>(TXA<sub>2</sub>)-TYPE (CANINE BASILAR ARTERY)**

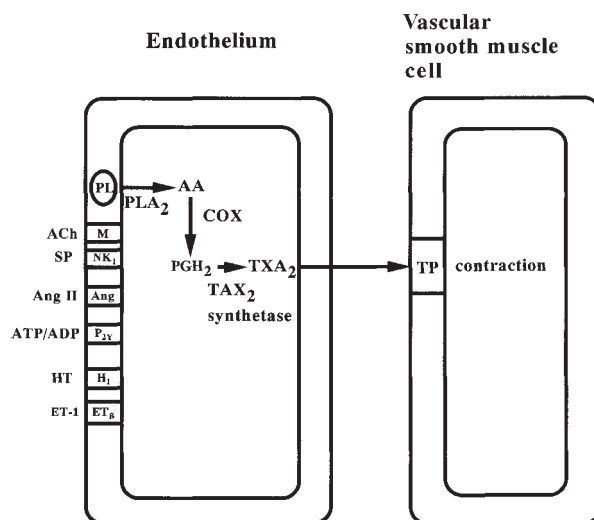
In the canine basilar artery, ACh caused a contraction and in order to obtain the steady contraction, the repetitive applications of ACh (about 6 times) was essential. The onset of contraction was delayed with a 20-30 s after application of ACh. This contraction was nearly abolished by rubbing of the intimal surface and the removal of endothelium was confirmed by electron microscopy<sup>[12]</sup>. This result indicates that ACh-induced contraction in the canine basilar artery is endothelium-dependent. The EDCF was analysed pharmacologically using several enzymatic inhibitors and receptor antagonists. ACh-induced EDC was attenuated by cyclooxygenase (COX) inhibitors (aspirin and indomethacin), indicating the involvement of COX metabolites in EDC. The EDC was also attenuated by TXA<sub>2</sub> synthetase inhibitor (OKY-046) and TXA<sub>2</sub> receptor antagonists (S-1452 and ONO-3708). These results indicate that ACh-induced contraction is due to endothelial TXA<sub>2</sub>. In addition to ACh, tested vasoactive substances including angiotensin I and II, ATP, histamine and bradykinin also caused the EDC and its EDCF is TXA<sub>2</sub><sup>[15]</sup>. This type of EDC is named as TXA<sub>2</sub>-type EDC. In addition to canine basilar artery, the rabbit intrapulmonary artery also showed EDC and TXA<sub>2</sub> was EDCF<sup>[16]</sup> (Fig 1).

It has been well documented that in addition to being a potent vasoconstrictor, TXA<sub>2</sub> is a potent aggregator of platelets. If the endothelium produces and releases TXA<sub>2</sub> as an endothelium-derived factor into vascular bed, the local circulation may be affected with the decrease in circulation (contraction of vascular smooth muscle cells) and the aggregation of platelet may also disturb the local circulation.

**EDCF: LEUKOTRIENE(LT)-TYPE (RABBIT CORONARY ARTERY)**

As shown in the previous section, we observed the EDC in the canine basilar artery but it is possible that such EDC was just an exceptional case. In order to generalize the EDC and EDCF, many other arterial preparations were examined in the following sections. In rabbit coronary artery, ACh caused a EDR in the preparation precontracted by PGF<sub>2α</sub> (in the absence of NOS inhibitor). In contrast to EDR, ACh caused EDC

**EDCF: TXA<sub>2</sub>-type**



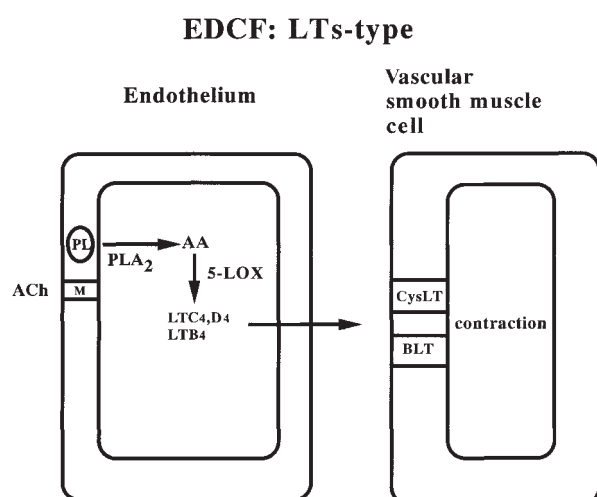
**Fig 1. Schematic presentation of endothelium-dependent contraction (EDC). EDCF: TXA<sub>2</sub>-type. PL: phospholipids. PLA<sub>2</sub>: phospholipase A<sub>2</sub>. AA: arachidonic acid. COX: cyclooxygenase. PGH<sub>2</sub>: prostaglandin H<sub>2</sub>. TXA<sub>2</sub>: thromboxane A<sub>2</sub>. TP: thromboxane A<sub>2</sub> receptor.**

in the resting tension under the presence of NOS inhibitor (*L*-NAME)<sup>[17]</sup>. EDR due to endothelial NO may be masked EDC. The EDCF for EDC was analysed under the presence of NOS inhibitor. EDC was attenuated by COX inhibitors (aspirin and indomethacin), 5-lipoxygenase (5-LOX) inhibitors (L 663,536 and BAY x1005), leukotriene antagonists (ONO-1078 and SK&F 104353), but not by TXA<sub>2</sub> synthetase inhibitor (OKY-046) and TXA<sub>2</sub> antagonist (ONO-3708). From these results, it was suggested that the EDCF for ACh-induced EDC in the rabbit coronary artery was due to LTs. LTC<sub>4</sub>, D<sub>4</sub> are well known as bronchi spasmogen and LTB<sub>4</sub> is known as a chemotactic agent for neutrophils (Fig 2).

Among the arachidonic acid metabolites, only TXA<sub>2</sub> and LTs were considered to be the EDCF. However, further test addressing the EDCF of the other species and other regions of vascular beds revealed that in addition to TXA<sub>2</sub> and LTs, COX metabolites (chemically unidentified yet) of arachidonic acid could be EDCF.

**EDCF: COX-METABOLITES-TYPE (RAT CORONARY ARTERY)**

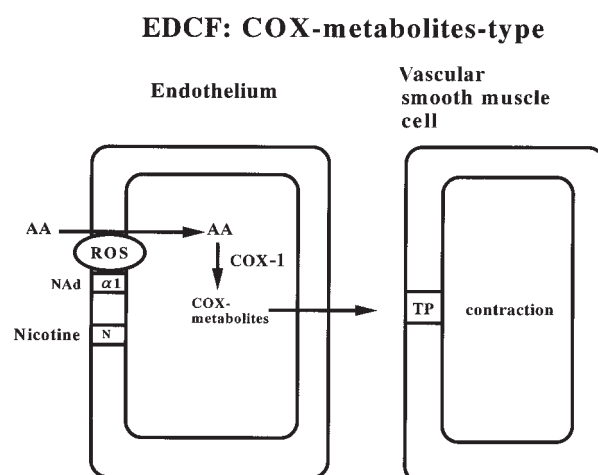
The other typical EDC was the rat coronary artery. In the rat coronary artery, nicotine and NAd caused EDC and this EDC was enhanced by the presence of NOS inhibitor (*L*-NAME) and arachidonic acid<sup>[18,19]</sup>. As



**Fig 2.** Schematic presentation of endothelium-dependent contraction (EDC). EDCF: LTs-type. PL: phospholipids. PLA<sub>2</sub>: phospholipase A<sub>2</sub>. AA: arachidonic acid. 5-LOX: 5-lipoxygenase. LTC<sub>4</sub>, D<sub>4</sub>: leukotriene C<sub>4</sub>, D<sub>4</sub>. LTB<sub>4</sub>: leukotriene B<sub>4</sub>. CysLT: leukotriene C<sub>4</sub>, D<sub>4</sub> receptor. BLT: leukotriene B<sub>4</sub> receptor.

described in the previous section, EDC counters act with EDR and the predominance of EDC or EDR was dependent on the tonus of the preparations. When it was precontracted by PGF<sub>2α</sub>, the EDR was dominant and when it was at resting tension, the EDC was dominant. The EDC was attenuated potently by COX-1 inhibitor (flurbiprofen) but slightly by COX-2 inhibitor (nimesulide). In addition, the other inhibitors, TXA<sub>2</sub> synthetase inhibitor (OKY-046), TXA<sub>2</sub> receptor antagonist (ONO-3708) and 5-LOX inhibitor (ZM 230487) did not affect EDC. These results indicate that nicotine-induced EDC in the rat coronary artery is mainly due to COX-1 metabolites but not by TXA<sub>2</sub> and 5-LOX metabolites<sup>[18,19]</sup> (Fig 3).

In addition to TXA<sub>2</sub> and LTs, other arachidonic acid metabolites can also be EDCF. Indeed, the third type of EDCF was found in the rat coronary artery.



**Fig 3.** Schematic presentation of endothelium-dependent contraction (EDC). EDCF: COX-metabolites-type. AA: arachidonic acid. COX: cyclooxygenase. TP: thromboxane A<sub>2</sub> receptor. ROS: reactive oxygen species.

The EDC in the rat coronary artery was not susceptible to TXA<sub>2</sub> synthetase inhibitor and TXA<sub>2</sub> receptor antagonist as well as 5-LOX inhibitors. These facts clearly indicate that TXA<sub>2</sub> and 5-LOX metabolites (LTC<sub>4</sub>, D<sub>4</sub> and LTB<sub>4</sub>) may be eliminated from EDCF in the rat coronary artery, but COX metabolites can be considered as EDCF, since the EDC was nearly abolished by COX inhibitors.

In other species (monkey) and other regions of arteries (rat mesenteric artery, rat common carotid artery, rat intrapulmonary artery), all tested preparations showed the EDC with or without the presence of NOS inhibitor and such EDCFs belonged to arachidonic acid metabolites (eg, TXA<sub>2</sub>-type, LTs-type, COX-metabolites-type or the mixed-type) (Tab 1). These endothelium-derived arachidonic acid metabolites may affect the local microcirculation and exacerbate ischemia.

**Tab 1.** Species and agonist differences of EDCFs.

Preparation	Agonist	EDCF
Canine basilar artery	ACh	TXA <sub>2</sub>
Rabbit coronary artery	ACh	LTs
Rat intrapulmonary artery	Bradykinin	COX-metabolites, LTs
Rat intrapulmonary artery	ATP	TXA <sub>2</sub> , LTS

## PERSPECTIVES

In this article, the endothelium-dependent responses in various arterial preparations, especially the diversity of endothelium-dependent contractions and their endothelium-derived vasoconstricting factors (arachidonic acid metabolites), were reviewed and their possible pathophysiological significance is discussed in below. As shown, there are at least three types of EDCFs (arachidonic acid metabolites), TXA<sub>2</sub>-type, LTs-type, and COX-metabolites-type. In most vascular preparations, EDC is masked by EDR and EDC appears under the low activity of endothelial NO (under NOS inhibition). In an exceptional case, EDC in canine basilar artery appears without the inhibition of NOS. So far, the effects of COX inhibitor, TXA<sub>2</sub> synthetase inhibitor and TXA<sub>2</sub> antagonist on systemic pressure were not known but NOS inhibitor (*L*-NAME) increased the systemic pressure in the rat. As far as systemic pressure is concerned, the NO system may play an important role. Indeed, endothelial NO activity in SHR is lower than that of WKY, suggesting that the loss of endothelial NO may be involved in hypertension<sup>[5-7]</sup>. In addition to experimental hypertension, the endothelial dysfunction (loss of NO activity) may be involved in experimental diabetes mellitus<sup>[8]</sup>. When the loss of endothelial NO (loss of EDRF) occurs, endothelial EDCF (arachidonic acid metabolites) could be correspondingly predominant. The local circulation may be disturbed by the increase in functional EDCF (eg, TXA<sub>2</sub>, LTs and COX-metabolites) and may trigger a cascade leading to chronic circulatory damage-induced immuno-inflammatory process (Tab 2). Interestingly, aging also leads to a decrease in the endothelial NO production<sup>[20]</sup>. Subsequently, the endothelial EDCF could become predomi-

nant in an age-related manner. In aging conditions, the local circulation may be deteriorated and followed by the cellular damage-induced immuno-inflammatory process.

In conclusion, the role of EDRF (NO relaxing factor) in normal circulatory conditions is more important than reducing role of EDCFs (eg, TXA<sub>2</sub>, LTs and COX-metabolites). The relative increase in the functional EDCFs and the decrease in the functional EDRF may induce the disturbance of the local circulation and trigger the adhesive interactions between endothelium and neutrophils and then accelerate the local immuno-inflammatory processes.

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**Tab 2. EDCF and action types.**

EDCF	Action
TXA <sub>2</sub>	Contraction Platelet aggregation
LTs	Contraction Permeability Chemotaxis
COX-metabolites	Contraction

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