A Diffusible Substance(s) Mediates Endothelium-Dependent Contractions in the Aorta of SHR

Di Yang, Michel Félotou, Nigel Levens, Ji Nan Zhang, Paul M. Vanhoutte

Abstract—A modified bioassay system was designed to demonstrate the diffusible nature of endothelium-derived contracting factor(s) released by acetylcholine in the aorta of spontaneously hypertensive rat. In “sandwich”-like layered preparation, isometric tension was recorded from a bioassay strip (without endothelium) in the presence of N^\text{\textsuperscript{\textregistered}}-nitro-l-arginine and tetrahydrobiopterin to selectively potentiate endothelium-dependent contractions. A donor strip (with or without endothelium) was stitched on the bioassay tissue so that it did not directly contribute to the recorded contractions. Acetylcholine induced contractions that occurred only when the donor strip was with endothelium. Superoxide dismutase did not affect but catalase and the combination of superoxide dismutase plus catalase significantly decreased the endothelium-dependent contraction. The contractions in the layered preparations were abolished when the donor strip with endothelium was treated previously with valeryl salicylate, an irreversible cyclooxygenase-1 inhibitor, but remained unaffected when the bioassay strip was treated with the compound. Previous treatment of the bioassay strip alone with S 18886 abolished the contractile response, whereas treatment of the donor strip with endothelium by the selective TP receptor antagonist only produced a moderate inhibition. These results indicate that in the aorta of spontaneously hypertensive rats, endothelium-dependent contractions to acetylcholine involve a diffusible substance(s) released by the endothelium. The production of this contracting factor(s) requires the activation of endothelial cyclooxygenase-1, and its action the activation of TP receptors on the vascular smooth muscle cells. (Hypertension. 2003;41:143-148.)

Key Words: cyclooxygenase ■ endothelium-dependent contraction ■ tetrahydrobiopterin ■ free radicals ■ rats, spontaneously hypertensive ■ receptors, thromboxane

The endothelium-derived contracting factors (EDCF) identified so far include superoxide anions, endoperoxides, thromboxane A\text{\textsubscript{2}}, and endothelin-1.\textsuperscript{1} In the aorta of the spontaneously hypertensive rat (SHR), endothelium-dependent contractions to acetylcholine involve reactive oxygen species that activate the cyclooxygenase-1 pathway with the production of endoperoxide(s), which stimulate TP receptors on the aortic vascular smooth muscle.\textsuperscript{2} Of the products of the cyclooxygenase pathway, endoperoxide(s) (in particular prostaglandin H\text{\textsubscript{2}}) are likely candidates as EDCF in the SHR aorta.\textsuperscript{1,3-5} This conclusion is supported by the observations that inhibitors of cyclooxygenase-1 and TP receptor antagonists abolish the endothelium-dependent contraction but that inhibitors of thromboxane synthase fail to do so.\textsuperscript{2,3,5,6} The release of endoperoxides is larger in the aorta of SHR than in that of normotensive Wistar-Kyoto rat (WKY); this phenomenon is associated with a greater expression of cyclooxygenase-1 in the SHR aorta.\textsuperscript{3} In addition, the aortas from SHR without endothelium show a higher sensitivity to exogenous prostaglandin H\text{\textsubscript{2}} than those of normotensive control rats.\textsuperscript{5} Both endothelial cells and smooth muscle cells express cyclooxygenase and TP receptors.\textsuperscript{7,8} The precise location of cyclooxygenase-1 and TP receptors activated during endothelium-dependent contractions is unknown. There is no direct evidence at hand showing that EDCF is a diffusible factor. Inhibitors of nitric oxide (NO) synthase augment endothelium-dependent contractions to acetylcholine in the SHR.\textsuperscript{9,10} This potentiation is augmented further by exogenous tetrahydrobiopterin, an essential cofactor of endothelial NO synthase.\textsuperscript{10} The mechanism underlying this effect of tetrahydrobiopterin involves superoxide generation by the autoxidation of tetrahydrobiopterin. This potentiation is selective for the endothelium-dependent contractions observed in SHR in the presence of L-arginine analogues, inhibitors of the NO synthase.\textsuperscript{10} This effect of tetrahydrobiopterin in the presence of an inhibitor of NO synthase makes it potentially a useful tool to bioassay EDCF. The purpose of this study was to design a bioassay system permitting the detection of the EDCF in SHR aorta and thus to determine the location (endothelium or smooth muscle) of cyclooxygenase-1 and TP receptors,
The donor strips could be with or without endothelium and were small metal stirrups were sewed to the two rims of the preparation. The tension was recorded, the endothelium was removed and two experiments were performed in the presence of oxygenated modified Krebs-Ringer solution. One stirrup was connected with acetylcholine (10⁻⁵ mol/L) to mimic the experimental (equilibration period and the experimental protocol) was shortened as much as possible and lasted between 120 to 150 minutes. All the experiments were performed in intact rings with endothelium incubated for 60 minutes with various inhibitors of endothelium (valproic acid, 10⁻⁴ mol/L; dehydrogenase, 10⁻⁴ mol/L; and catalase were purchased from Sigma Chemical Co.) and tetrahydrobiopterin (10⁻⁴ mol/L).

To rule out any contribution of the donor strip in the contractile response recorded from the sandwich preparation, some preliminary experiments were performed. The tension of the intact donor preparation was recorded directly from the donor strip without endothelium in the absence of KC(6) (60 mmol/L). The strain was obtained directly from the donor strip in additional experiments in the presence of KC(6) (60 mmol/L) or valproic acid (10⁻⁴ mol/L) or tetrahydrobiopterin (10⁻⁴ mol/L) or catalase (10⁻⁴ mol/L) or dehydrogenase (10⁻⁴ mol/L). Under those two experimental conditions, there was no measurable contraction in response to KC(6) (60 mmol/L).

In the bioassay, a diffusible substance, strips from the aorta of normotensive Wistar-Kyoto rats were prepared in the same reference to the axis of the transducer. The rings were stretched progressively to a passive tension of 2 g and contracted twice with the bioassay strip. To minimize these two experimental pitfalls, the concentration of the two stimuli was placed in organ chambers filled with the bioassay strip and contracted twice with KC(6) (60 mmol/L). A passive tension of 2 g was recorded. The duration of the whole experiment (equilibration period and the experimental protocol) was shortened as much as possible and lasted between 120 to 150 minutes.

The rings were stretched progressively to reach the optimal point of the drug's relaxation to acetylcholine (10⁻⁵ mol/L) in the bioassay experiments. The concentration of the two stimuli was placed in organ chambers filled with the bioassay strip and contracted twice with KC(6) (60 mmol/L). A passive tension of 2 g was recorded. The duration of the whole experiment (equilibration period and the experimental protocol) was shortened as much as possible and lasted between 120 to 150 minutes.

To rule out any contribution of the donor strip in the contractile response recorded from the sandwich preparation, some preliminary experiments were performed. The tension of the intact donor preparation was recorded directly from the donor strip in additional experiments in the presence of KC(6) (60 mmol/L) or valproic acid (10⁻⁴ mol/L) or tetrahydrobiopterin (10⁻⁴ mol/L) or catalase (10⁻⁴ mol/L) or dehydrogenase (10⁻⁴ mol/L). Under those two experimental conditions, there was no measurable contraction in response to KC(6) (60 mmol/L).

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In preliminary experiments, to confirm whether or not this layered sandwich-like bioassay system was designed to identify an inhibitor of the bioassay strip was placed in organ chambers containing control solution of the following composition (mmol/L): NaCl 118, KCl 4.7, Glucose 11, MgSO4 1.17, CaCl2 2.5, KHCO3 12, NaHCO3 25,0, and adenosine diphosphate (ADP) 0.1 mmol/L. The concentration of the two stimuli was placed in organ chambers filled with the bioassay strip and contracted twice with KC(6) (60 mmol/L). A passive tension of 2 g was recorded. The duration of the whole experiment (equilibration period and the experimental protocol) was shortened as much as possible and lasted between 120 to 150 minutes.

The postulated irreversible antagonist of TP receptors, benextramine (S18886), administered intra-arterially to inhibit cyclooxygenase in the bioassay experiments, did not alter the bioassay system's response to acetylcholine in the hypertensive vascular wall.

In certain experiments, the blood vessels were cut into rings (4 mm in length), suspended in organ chambers containing control solution of the following composition (mmol/L): NaCl 118, KCl 4.7, Glucose 11, MgSO4 1.17, CaCl2 2.5, KHCO3 12, NaHCO3 25,0, and adenosine diphosphate (ADP) 0.1 mmol/L. The concentration of the two stimuli was placed in organ chambers filled with the bioassay strip and contracted twice with KC(6) (60 mmol/L). A passive tension of 2 g was recorded. The duration of the whole experiment (equilibration period and the experimental protocol) was shortened as much as possible and lasted between 120 to 150 minutes.
Acetylcholine produced contractions only when donor tissue was with endothelium. This experiment demonstrates that endothelium-dependent contraction to acetylcholine involved a transferable mediator.

In the configuration of the conventional layered preparation (“intact” assay strip without endothelium, donor strip with or without endothelium), the addition of KCl (60 mmol/L) produced contractions of 0.72±0.03 and 0.66±0.03 g in the aortic strip associated with a donor with (n=34) or without (n=26) endothelium, respectively. After washing out the KCl, the addition of N\textsuperscript{6}-nitro-L-arginine (10\textsuperscript{-4} mol/L) plus tetrahydrobiopterin (10\textsuperscript{-4} mol/L) produced a transient contraction that represented 62±11% and 42±3% of the reference KCl contraction in the aortic strip associated with a donor with (n=34) or without (n=26) endothelium, respectively. In the presence of these drugs, acetylcholine (10\textsuperscript{-4} to 10\textsuperscript{-2} mol/L) evoked significant contractions when the donor tissue was with endothelium. The maximal contraction to acetylcholine (10\textsuperscript{-4} mol/L) was 45.7±3.8% (n=28) of the reference contractions induced by KCl (60 mmol/L), whereas in preparations with a donor tissue without endothelium the changes in tension were significantly less (8.9±1.4%, n=20, P<0.05, (Figure 1).

Involvement of Reactive Oxygen Species

In layered preparations (donor strips with endothelium), acute exposure (5 minutes before the application of acetylcholine) to superoxide dismutase (120 U/mL) did not affect significantly the contractions to acetylcholine (10\textsuperscript{-4} mol/L). However, catalase (1200 U/mL) and the combination of superoxide dismutase plus catalase significantly reduced the responses to acetylcholine (Figure 2).

Under the same experimental conditions, the presence of deferoxamine (10\textsuperscript{-4} and 10\textsuperscript{-3} mol/L) produced a significant inhibition of the acetylcholine-induced contraction but only at the highest dose tested (Figure 2), whereas the presence of mannitol (10\textsuperscript{-3} and 5×10\textsuperscript{-3} mol/L, n=4 and 5, respectively) did not significantly affect the response to acetylcholine (data not shown).

**Figure 1.** Isometric tension recording during effects of acetylcholine in “sandwich”-like layered preparations with (upper) or without (lower) endothelium from SHR aorta in the presence of N\textsuperscript{6}-nitro-L-arginine (10\textsuperscript{-4} mol/L) and tetrahydrobiopterin (10\textsuperscript{-4} mol/L). Papaverine was given at the end of the experiments to achieve full relaxation. Addition of tetrahydrobiopterin produced a transient contraction in both “sandwich”-like layered preparations with (upper) or without (lower) endothelium, linked to generation of superoxide anion caused by autoxidation of pteridine. Acetylcholine produced contractions only when donor tissue was with endothelium. This experiment demonstrates that endothelium-dependent contraction to acetylcholine involved a transferable mediator.

**Figure 2.** Effect of acute in vitro administration of scavengers of reactive oxygen species (left) and an iron chelator (right) on response to acetylcholine (10\textsuperscript{-4} mol/L) in layered preparations (donor strip with endothelium) from SHR aorta in the presence of N\textsuperscript{6}-nitro-L-arginine (10\textsuperscript{-4} mol/L) and tetrahydrobiopterin (10\textsuperscript{-4} mol/L). Left, Effect of superoxide dismutase (SOD, 120 U/mL), catalase (1200 U/mL), or the combination of SOD plus catalase. Catalase and the combination of catalase plus superoxide dismutase produced a significant inhibition of the endothelium-dependent contraction to acetylcholine. Right, Effect of deferoxamine (10\textsuperscript{-4} and 10\textsuperscript{-3} mol/L). Deferoxamine at the highest concentration tested produced a significant inhibition of endothelium-dependent contraction to acetylcholine. For the sake of clarity, respective controls have been pooled. Data are shown as mean±SEM; n represents number of tissue specimens taken from different rats. *Statistically significant difference (P<0.05) compared with control rats.

**Involvement and Localization of Cyclooxygenase-1 and TP Receptors**

The assay strip (without endothelium) or the donor strip (with endothelium) were treated (or not) individually with either valeryl salicylate (3×10\textsuperscript{-3} mol/L) for 2 hours or S 18886 (5×10\textsuperscript{-9} mol/L) for 1 hour. After repeated rinses, the layered preparation was assembled and contractions to acetylcholine were recorded.

The contractions to acetylcholine (10\textsuperscript{-4} mol/L) were abolished when the donor tissue with endothelium had been treated with valeryl salicylate but remained unaffected when the bioassay strip had been exposed previously to the cyclooxygenase-1 inhibitor (Figure 3). Conversely, the contractile response to acetylcholine (10\textsuperscript{-4} mol/L) was nearly abolished when the bioassay strip was treated with S 18886, whereas the exposure of the donor strip with endothelium to the TP receptor antagonist produced only a moderate inhibition of the contractions (Figure 4). In both experimental protocols, acetylcholine produce no or minor contractions in the presence of a donor strip without endothelium.

In layered preparations, the addition of U 46619 (10\textsuperscript{-4} mol/L) produced a slowly developing contraction that was equivalent to the amplitude of the endothelium-dependent contraction to acetylcholine (10\textsuperscript{-4} mol/L) under the same conditions. The donor strip with endothelium or the bioassay strip were exposed, or not, to S 18886 (5×10\textsuperscript{-9} mol/L) before layering the preparation. The contractions to U 46619 (10\textsuperscript{-4} mol/L) were significantly decreased both in preparations in which the bioassay tissue or the donor strip were previously treated with S 18886 (ANOVA2, followed by Bonferroni post hoc test). However, the inhibition was significantly smaller when the donor strips were treated than when the bioassay strips were exposed to the TP receptor antagonist (Figure 5).
Figure 3. Inhibition of cyclooxygenase-1 by previous treatment with valeryl salicylate (VAS: 3×10⁻⁶ mol/L) and endothelium-dependent contractions to acetylcholine (10⁻⁶ mol/L) in layered preparations from SHR aorta in the presence of N⁷-nitro-L-arginine (10⁻⁴ mol/L) and tetrahydrobiopterin (10⁻⁴ mol/L). Valeryl salicylate produced significant inhibition of acetylcholine-induced contraction only when donor tissue had been treated, indicating that cyclooxygenase-1, involved in endothelium-dependent contractions, is located on endothelial cells. Data are shown as mean±SEM (n=7). *Statistically significant difference (P<0.05) when compared with untreated control.

Discussion

This study demonstrates the existence of a bioassayable EDCF in the SHR aorta. Furthermore, it demonstrates that acetylcholine-induced endothelium-dependent contractions involve stimulation of endothelial cyclooxygenase-1 that in turn produces a diffusible EDCF(s), capable of activation TP receptors on the vascular smooth muscle cells.

Figure 4. TP receptor antagonism by previous exposure to S 18886 (5×10⁻⁶ mol/L) and endothelium-dependent contraction to acetylcholine (10⁻⁶ mol/L) in layered preparations from SHR aorta in the presence of N⁷-nitro-L-arginine (10⁻⁴ mol/L) and tetrahydrobiopterin (10⁻⁴ mol/L). S 18886 produced significant inhibition of acetylcholine-induced contraction both when donor and assay tissue had been treated. However, treatment of assay tissue produced significantly larger inhibition than treatment of donor tissue. Inhibition of contraction to U46619 by donor tissue previously treated with S 18886 is best explained by dissociation of TP receptor antagonist from donor tissue and the subsequent “contamination” during equilibration period of assay tissue. Data are shown as mean±SEM (n=6). *Statistically significant difference (P<0.05) compared with untreated control rats. †Statistically significant difference (P<0.05) between treatment of donor strips and that of bioassay strips.

The current experiments were performed in the presence of N⁷-nitro-L-arginine, an analogue of L-arginine, and tetrahydrobiopterin, an essential cofactor for the activity of NO synthase, to amplify the endothelium-dependent contractions. Inhibitors of NO synthase augment endothelium-dependent contractions. In preliminary experiments, it has been shown that tetrahydrobiopterin selectivity augments the endothelium-dependent contractions to acetylcholine, only in SHR and only in the presence of N⁷-nitro-L-arginine. This potentiation, associated with the generation of superoxide anion by the autoxidation of tetrahydrobiopterin, does not alter the main characteristics of the endothelium-dependent contractions, for example, involvement of oxygen-derived free radicals, stimulation of cyclooxygenase-1, and the resulting activation of TP receptors.

In phenylephrine-contracted, layered preparations prepared with aortic strips of normotensive WKY rats, acetylcholine evoked relaxation when the donor strips contained endothelium, indicating that the present “sandwich”-like preparations can detect diffusible NO. Likewise, in the presence of N⁷-nitro-L-arginine and tetrahydrobiopterin, acetylcholine produced a contraction only when the donor strip of SHR aorta contained endothelium. Preliminary experiments, in which isometric tension was measured directly from the donor strip with endothelium alone, show that there was no contractile response recorded in response not only to acetylcholine but also to more potent stimuli such as KCl or phenylephrine. This was to be expected because of the 90° rotation of the donor strip in relation to the length axis of the transducer, which does not allow proper recording of contractile force from the mainly circularly running smooth muscle bundles in the aortic wall. Furthermore, the

Figure 5. Effect of previous treatment of layered preparations (donor strips with endothelium) from SHR aortas with S 18886 (5×10⁻⁶ mol/L) on contraction to U 46619 (10⁻⁶ mol/L) in the presence of N⁷-nitro-L-arginine (10⁻⁴ mol/L) and tetrahydrobiopterin (10⁻⁴ mol/L). Whole time course of contractions is shown. S 18886 produced significant inhibition of U46619-induced contraction both when donor and assay tissue were treated. However, treatment of assay tissue produced significantly larger inhibition than treatment of donor tissue. Inhibition of contraction to U46619 by donor tissue previously treated with S 18886 is best explained by dissociation of TP receptor antagonist from donor tissue and the subsequent “contamination” during equilibration period of assay tissue. Data are shown as mean±SEM (n=6). *Statistically significant difference (P<0.05) compared with untreated control rats. †Statistically significant difference (P<0.05) between treatment of donor strips and that of bioassay strips.
experiments performed with the bioassay preparation, destroyed by a prolonged incubation in distilled water, layer with an intact donor strip, did not respond either to acetylcholine, KCl or phenylephrine, demonstrating that even when stitched to the bioassay strip, the donor strip does not contribute to the development of tension as recorded by the transducer. Therefore, the contractions to acetylcholine, recorded in the used layered preparation was indeed due to a diffusible endothelium-derived contracting factor(s) and not to artifacts linked to the experimental procedure.

The involvement of oxygen-derived free radicals in endothelium-dependent contraction has been demonstrated in canine basilar arteries and in the SHR aorta. These earlier findings were confirmed in the current study since in the layered preparations, the contractions were reduced by the acute treatment with catalase or the combination of superoxide dismutase plus catalase. In earlier experiments involving the contraction of isolated intact aortic rings from SHR, superoxide dismutase plus catalase, at the same concentration as used in the current study, did not prevent endothelium-dependent contractions unless the animals were treated chronically in vivo with dimethylthiourea, a scavenger of hydroxyl radicals. These observations were interpreted by the poor ability of these enzymes to access the site where the oxygen-derived free radicals are generated and therefore to be able to scavenge enough of the reactive oxygen species. This interpretation is confirmed by the current study, showing nearly full blockade of the endothelium-dependent contractions to acetylcholine by catalase or by the combination of the superoxide dismutase plus catalase. This observation strongly suggests the presence of reactive oxygen species in the extracellular space. The lack of activity of superoxide dismutase alone indicates that superoxide anions per se are not sufficient enough to evoke the contractions, although they must be the source of the secondary reactive oxygen species, such as hydrogen peroxide and hydroxyl radicals. Since superoxide dismutase did not affect the contractions, the effect of catalase alone or the combination of superoxide dismutase plus catalase would suggest that hydrogen peroxide or its product, hydroxyl radicals, is mainly responsible for the endothelium-dependent response. Iron chelation by deferoxamine prevents the formation of the hydroxyl radical from H$_2$O$_2$ (Fenton reaction). The inhibition produced by deferoxamine is in favor of role for the hydroxyl radical. However, the absence of effect of mannitol, a scavenger of the hydroxyl radical, is somehow puzzling. Mannitol is a poorly cell-permeable agent, and the very short half life of the hydroxyl radical may have prevented mannitol from scavenging the hydroxyl radical under the current experimental conditions. Earlier studies have reported an improved endothelial function by deferoxamine and no significant effect of mannitol. Obviously, the respective role of H$_2$O$_2$ or/and the generation of hydroxyl radical in the production of endothelium-dependent contractions deserve further investigation.

Figure 6. Endothelium-dependent contractions to acetylcholine in SHR aorta. Unbroken lines: Current study demonstrates that in response to acetylcholine, reactive oxygen species (scavenged by superoxide dismutase, SOD, and catalase) generated by endothelial cells from an unknown source (X, which is not necessarily different from cyclooxygenase-1) activates the endothelial cyclooxygenase-1 pathway (COX-1, inhibited by valeryl salicylate, VAS). The later produces endoperoxide(s) (EP) such as prostaglandin H$_2$ (PGH$_2$) or isoprostane(s) to activate TP receptors on smooth muscle cells (inhibited by S 18886). Broken lines, Hypothosis to reconcile present data and earlier studies. Contraction obtained under bioassay conditions is only 50% of contraction observed in intact rings. Therefore, an additional pathway can be suggested. In intact rings, cyclooxygenase-1 situated on smooth muscle could, because of the closer proximity between endothelial and smooth muscle cells, also be activated by diffusing reactive oxygen species. Activation of smooth muscle cyclooxygenase-1 would contribute to acetylcholine-induced contraction However, at the present time, there is no experimental evidence for this hypothesis.

The reactive oxygen species are likely to activate cyclooxygenase(s). Endothelium-dependent contractions to acetylcholine are inhibited by either nonspecific inhibitors of cyclooxygenase or a preferential inhibitor of cyclooxygenase-1 but not by a preferential inhibitor of cyclooxygenase-2. The current study confirms these observations as valeryl salicylate, a irreversible inhibitor of cyclooxygenase-1, abolished the contractions. This inhibitor was chosen for its long duration of action and its specificity. These results demonstrated that under the present experimental conditions, endothelial cyclooxygenase plays a pivotal role in mediating endothelium-dependent contractions, as a previous study suggested.

The involvement of endogenous agonists of TP receptors, other than thromboxane A2, in endothelium-dependent contractions to acetylcholine in the SHR aorta is also very likely as TP receptor antagonists but not inhibitors of thromboxane synthase abolish these responses. The current study also confirms these earlier conclusions. However, to determine the exact localization of the TP receptor was more difficult than to demonstrate the endothelial nature of the cyclooxygenase involved. Indeed, the available specific TP receptor antagonists caused reversible inhibitions. The antagonist with the longer remnant duration of action was S 18886. However, if previous exposure of the bioassay tissue to S 18886 fully inhibited the contraction to acetylcholine that of the endothelium also produced a moderate but significant inhibition of the response. This could be interpreted by either a role for TP receptors on both endothelium and vascular smooth muscle or by the artificial contamination of the untreated strip by the TP receptor antagonist slowly dissociating from the previously treated strip. This latter interpretation is likely to be correct since the contractions to exogenous U 46619, a potent TP receptor agonist, were
inhibited when the donor tissue was previously treated with S 18886. As the donor tissue does not contribute to the contraction, the only explanation for this inhibition of the response to U 46619 is the dissociation of the antagonist from the binding sites on the donor strip which results to the diffusion and blockade of the receptors present on the untreated assay strip. Altogether, these results indicate that acetylcholine stimulates the endothelial production of reactive oxygen species, most likely hydrogen peroxide and possibly the hydroxyl radical, from a yet unknown source. These oxygen species activate endothelial cyclooxygenase-1 that releases a diffusible substance(s), possibly prostaglandin H₂ or an isoprostane, which in turn stimulates TP receptors on the vascular smooth muscle cells (Figure 6).

It seems unlikely that under the experimental conditions of the current study, the totality of the released EDCF was bioassayed. In intact aortic rings of SHR, in the presence of N⁵-nitro-arginine and tetrodroybiopetin, the amplitude of the maximal endothelium-dependent contraction to acetylcholine represents 90% of the reference response to KCl, whereas in the current study in the layered preparations, the maximal responses to acetylcholine averaged only 45% of the KCl contraction. This difference might be due to the experimental paradigm per se (endothelial degradation linked to the manipulation needed to prepare the sandwich-like preparations, for instance) or due to the increased inactivation of EDCCs (reactive oxygen species and endoperoxides) as a consequence of the increased distance between the endothelium and the vascular smooth muscle cells. A third hypothesis can also be proposed. In intact rings from SHR aorta, H₂O₂ can theoretically diffuse more easily from the endothelial cells to the vascular smooth muscle cells than in the sandwich preparation. Exogenous H₂O₂ produces (like the co-administration of xantheine plus xanthine oxidase) greater contractions in SHR than in WKY in both control and endothelium-denuded aortic segments. These contractions share the same characteristics as the endothelium-dependent contractions produced by acetylcholine because they are sensitive to catalase, cyclooxygenase inhibitors, and TP receptor antagonists.

Therefore, in intact rings, considering that the expression of cyclooxygenase-1 is larger in aortic smooth muscle of SHR when compared with WKY, the additional activation of cyclooxygenase-1 in the vascular smooth muscle by endothelium-derived reactive oxygen species cannot be excluded (Figure 6).

Perspectives

The existence of a diffusible EDCF and the possibility to bioassay this endothelial factor should provide new means for its characterization and identification. Only when this is achieved will the relevance of this, a factor in the pathophysiology of hypertension, be properly assessed.

References

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