Aula – Endotélio e Função Vascular

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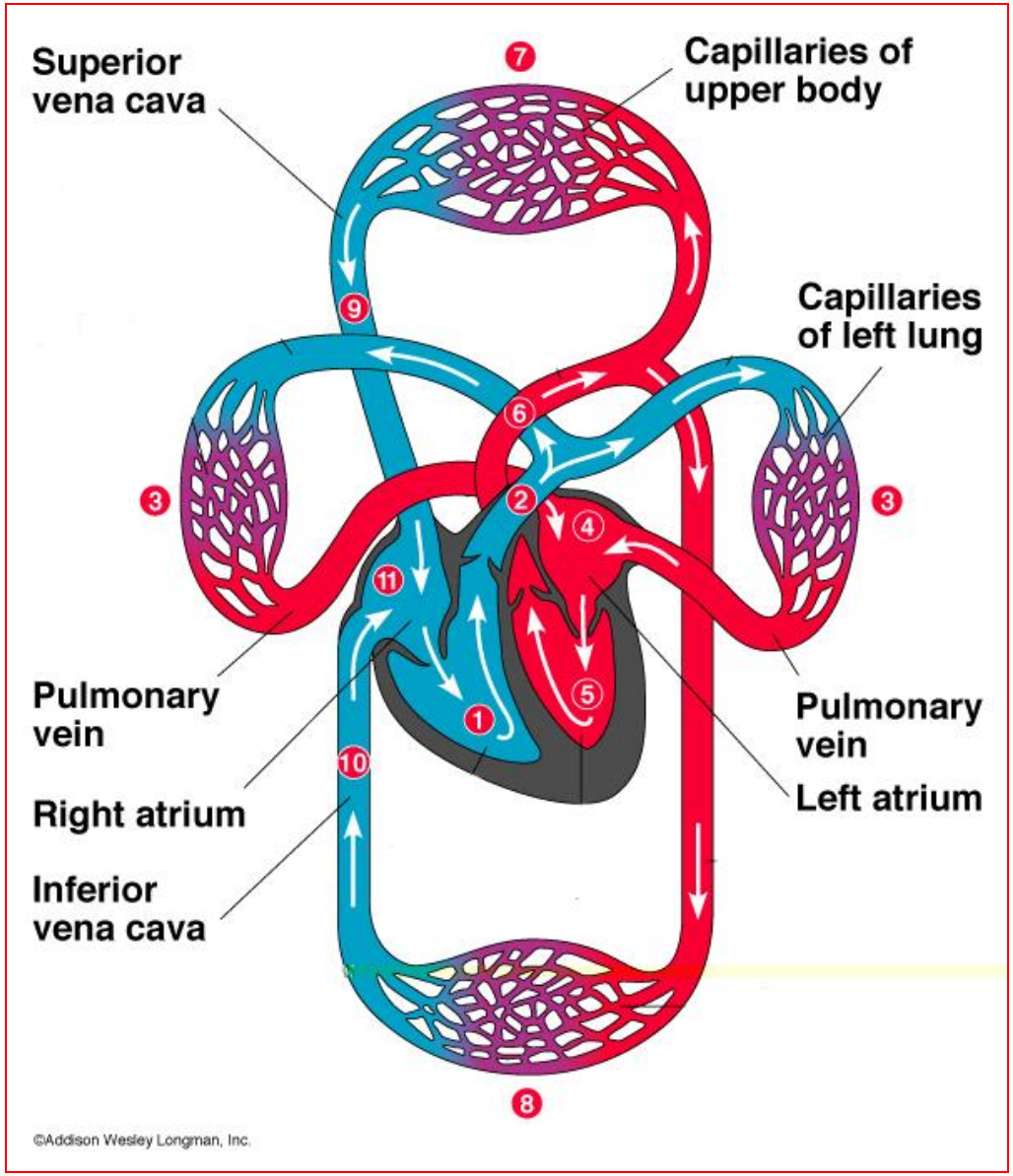
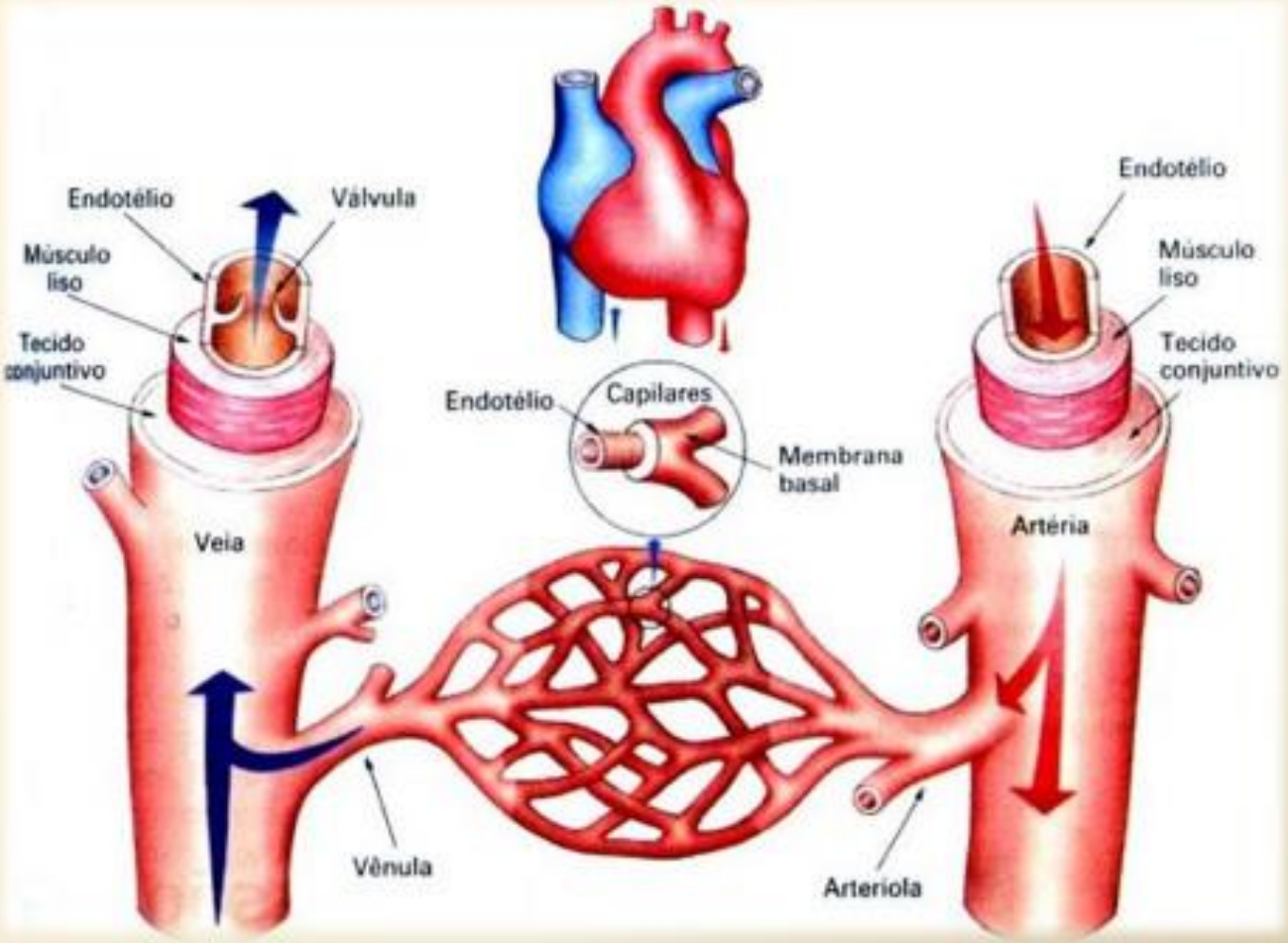
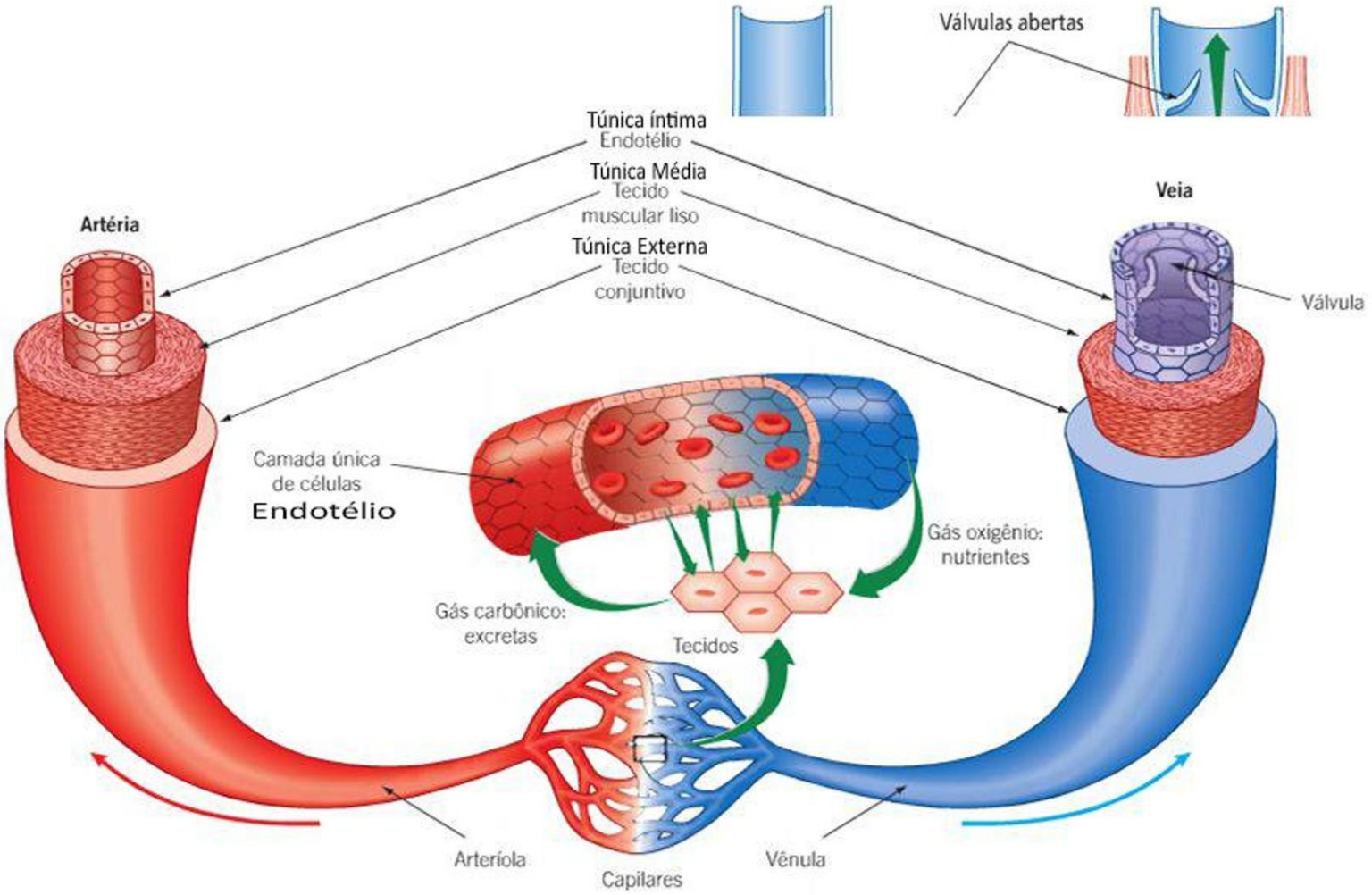
Ciências Fisiológicas

SISTEMA CARDIOVASCULAR

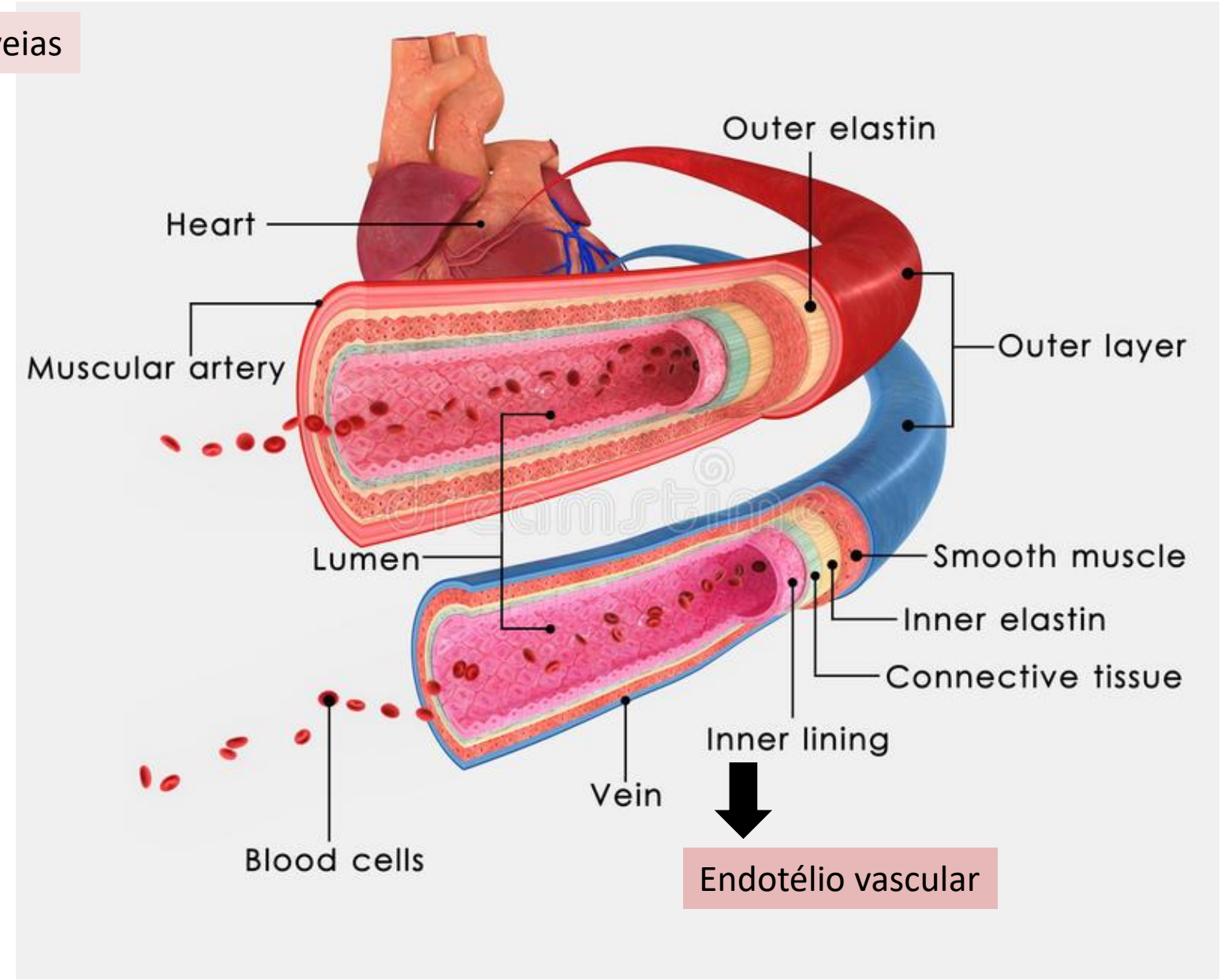


VASOS SANGUÍNEOS



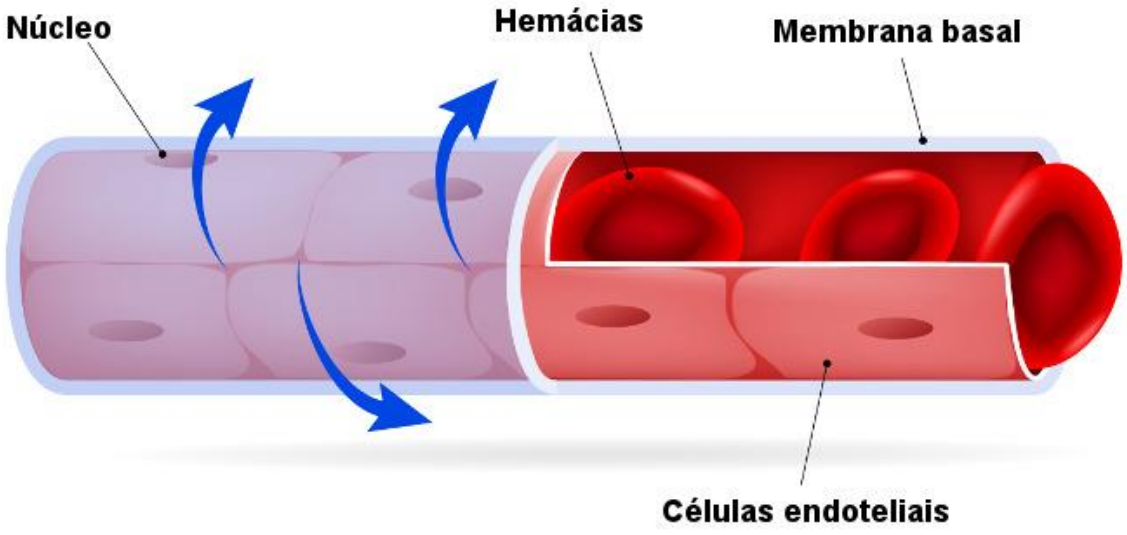

Válvulas abertas
Túnica íntima
Endotélio
Túnica Média
Tecido
muscular liso
Túnica Externa
Tecido
conjuntivo
Artéria
Veia
Válvula
Camada única
de células
Endotélio
Gás oxigênio:
nutrientes
Gás carbônico:
excretas
Tecidos
Arteríola
Capilares
Vênula

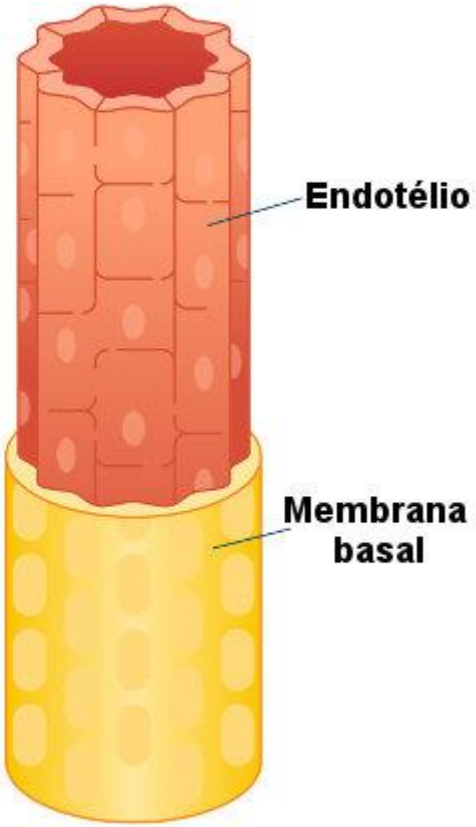
Artérias e veias



Endotélio vascular

capilar



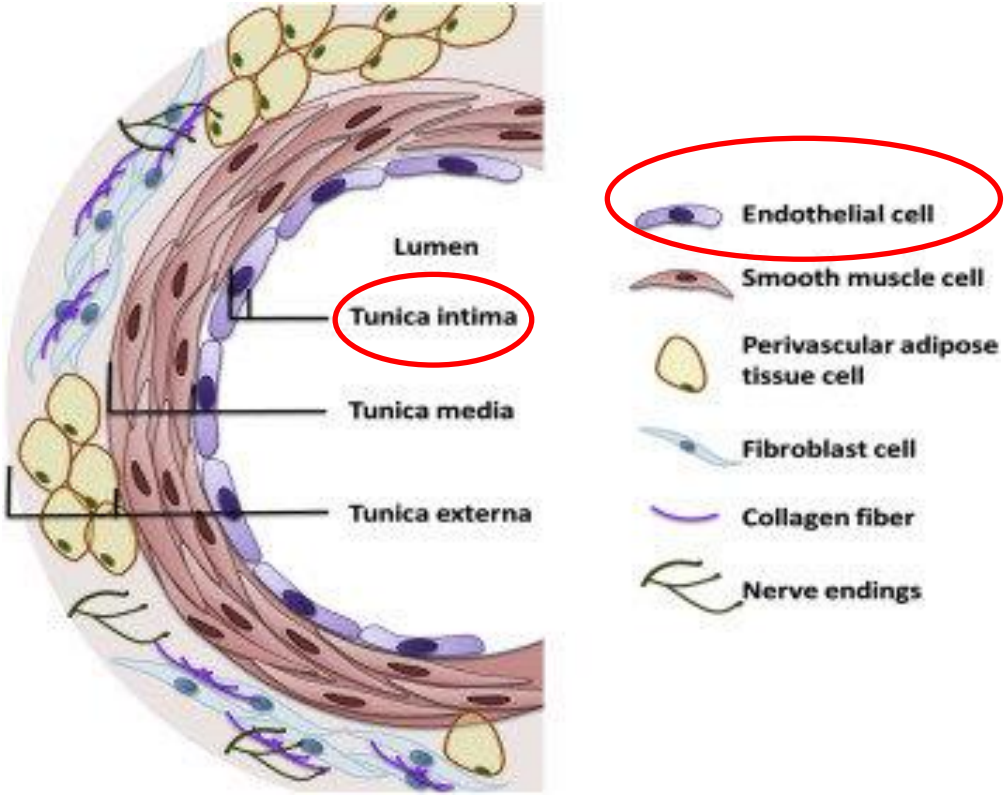


A espessura da parede do capilar é de cerca de 0,5 µm e seu diâmetro fica em torno de 7 µm a 9 µm

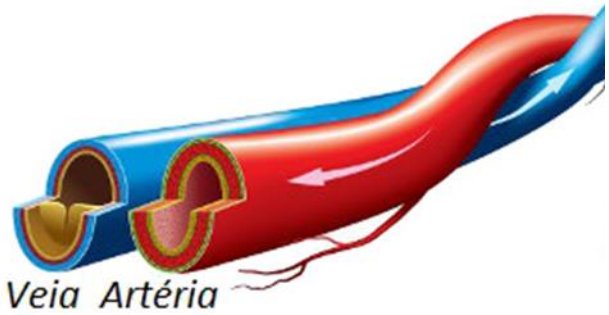
A extensão de um capilar normalmente fica em torno de 0,5 mm a 1 mm, entretanto, esses vasos formam uma rede complexa que, em sua totalidade, chega à quantidade surpreendente de 96.000 km.

Transporte

Trocas
Distribuição-redistribuição

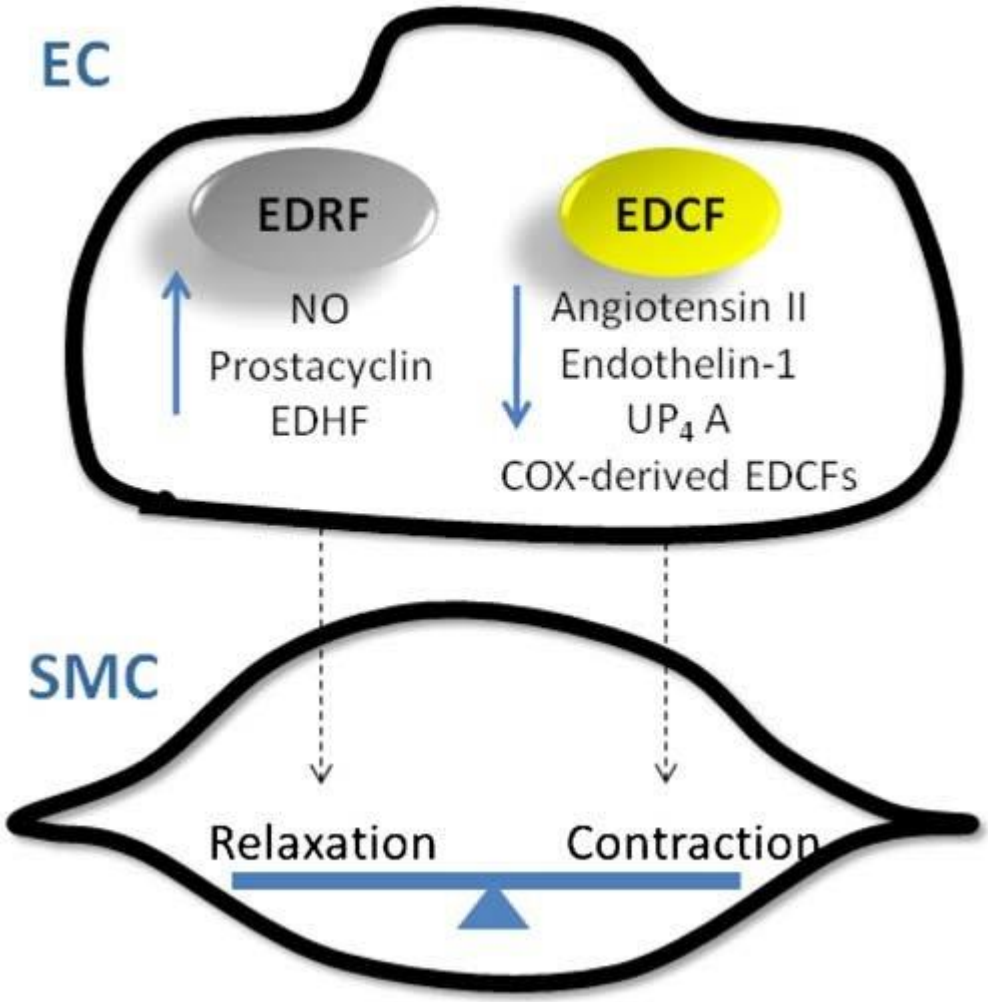


Endotélio - REGULAÇÃO DO TONUS VASCULAR



PA= DC x RVP (tônus vascular

⬆ PA= DC x RVP ⬆



The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine

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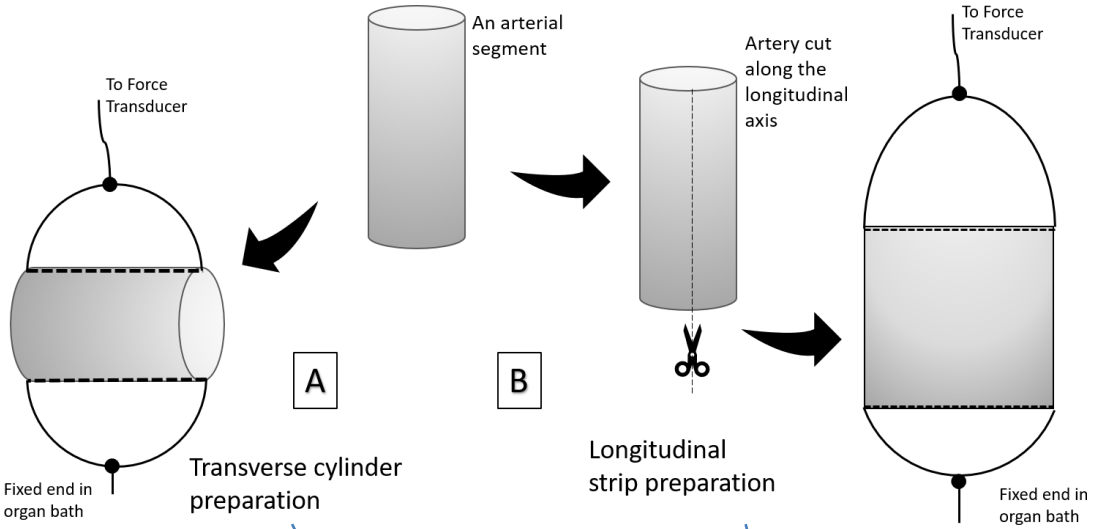
By the middle of 1951, my favorite in vitro smooth muscle preparation had shifted from the rabbit duodenum to the ***rabbit thoracic aorta****.* ***I had found that the helical (spiral) strip of that vessel, properly cut and mounted in organ chambers for isotonic recording, gave very reproducible contractions to epinephrine and norepinephrine after equilibration in oxygenated Krebs bicarbonate solution****. I had at first planned to study the effects of disturbances in energy-metabolism on these contractions, but I* ***became much more interested in using the aortic strip for studies on drug-receptor interactions.***

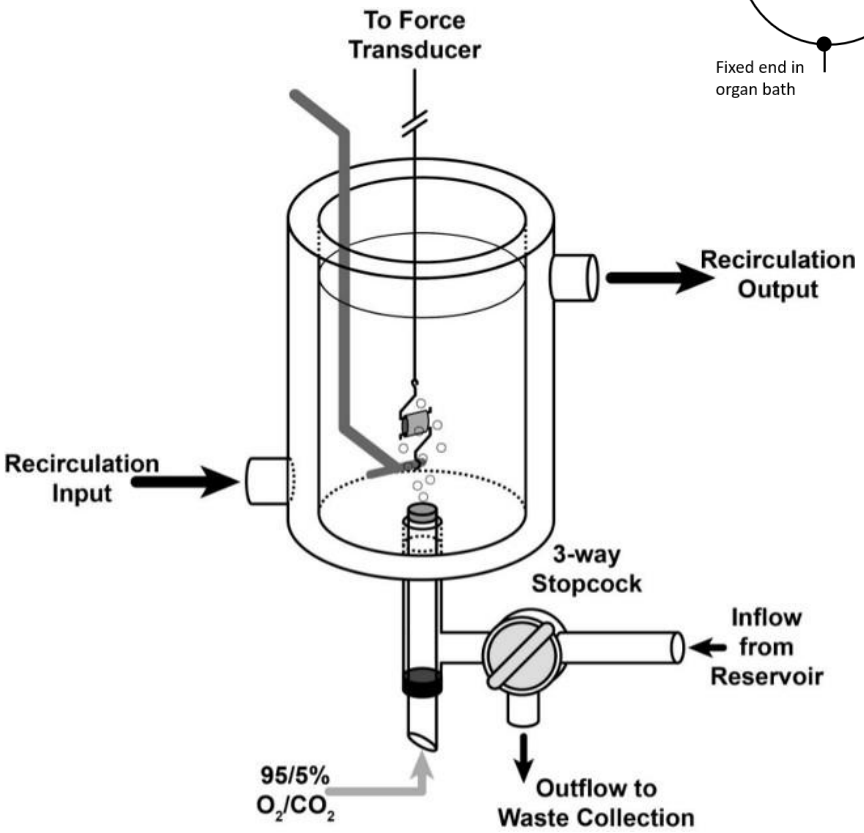
By ***1953, I had published a paper entitled "Reactions of strips of rabbit aorta to epinephrine, isoproterenol, sodium nitrite and other drugs****". Among the other drugs was* ***acetylcholine****.* ***I found that it only produced contractions****, whether it was added to resting strips or strips precontracted with some other agent. That was a paradoxical response* ***since acetylcholine was known to be a very potent vasodilator in vivo.*** *Little did I suspect then what* ***I was able to show many years later*** *– namely, that relaxation of arteries by acetylcholine is strictly endothelium-dependent, and that my method of preparing the strips inadvertently resulted in the mechanical removal of all the endothelial cells.*

Robert F. Furchgott
Biographical
The Nobel Prize in Physiology or Medicine 1998



Ring = anel

Strip = tira



Aorta torácica de coelhos

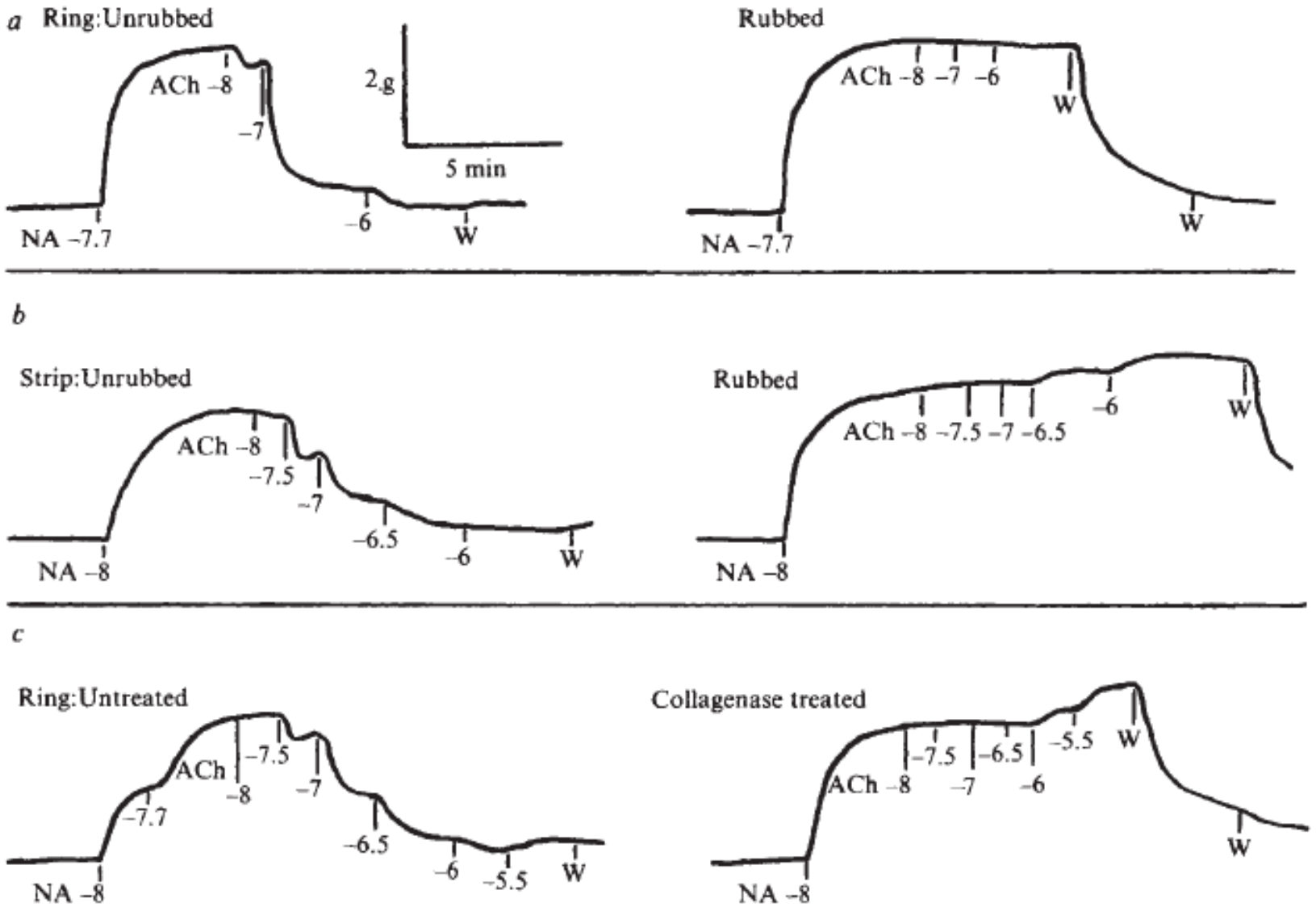
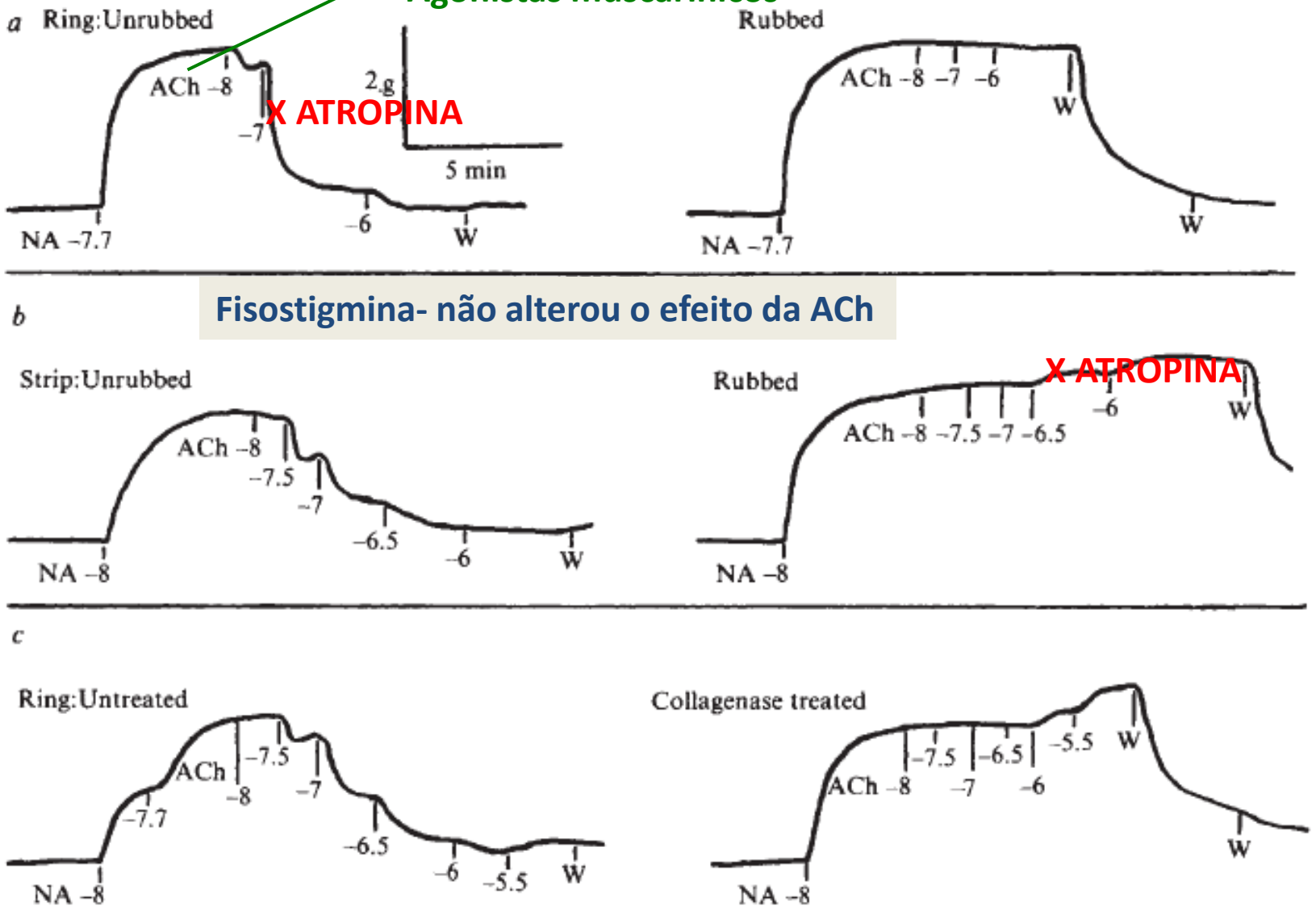


Figure 1

Qual o receptor envolvido?

= Metacolina
= Carbacol
Agonistas muscarínicos

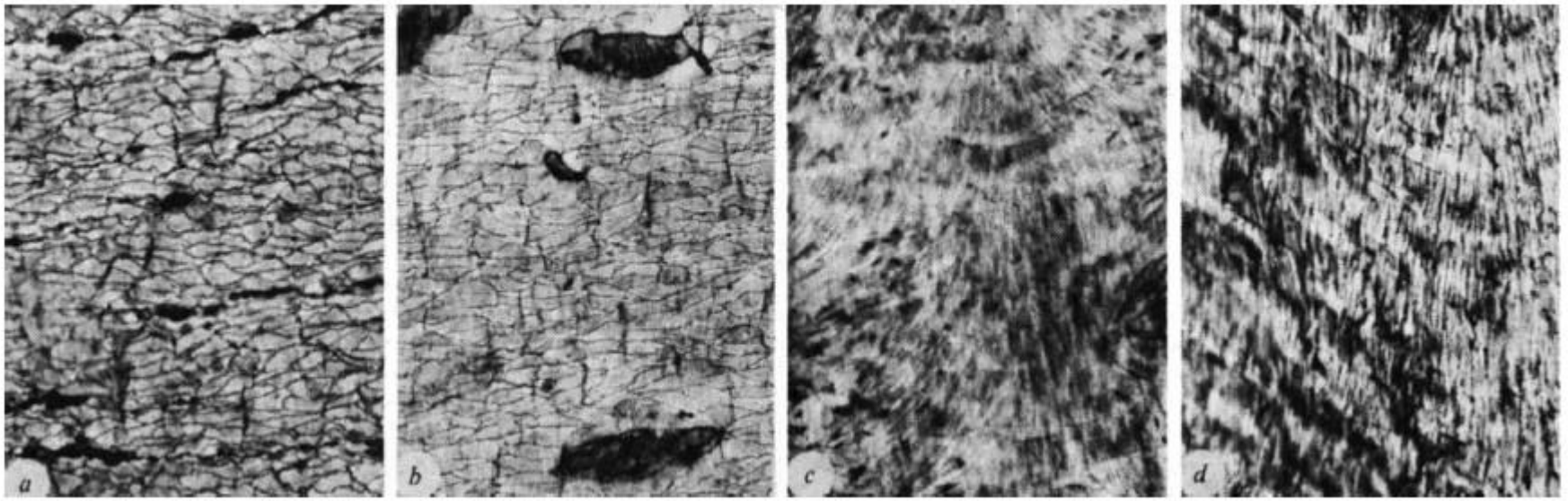
Atropina = antagonista muscarínico



Fisostigmina- não alterou o efeito da ACh

Figure 1

Qual a participação das células endoteliais?



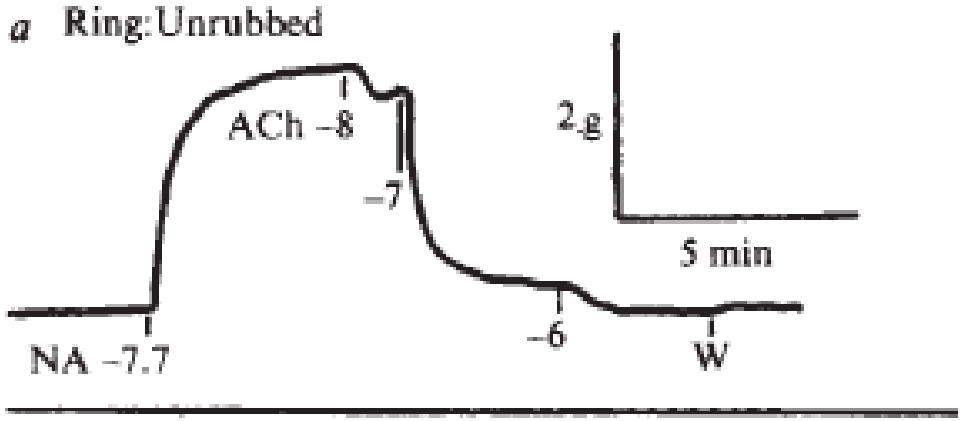
a, Strip that was stained immediately after being cut from freshly excised and trimmed aorta.

b, Strip made from an unrubbed ring at the end of an experiment on drug testing.

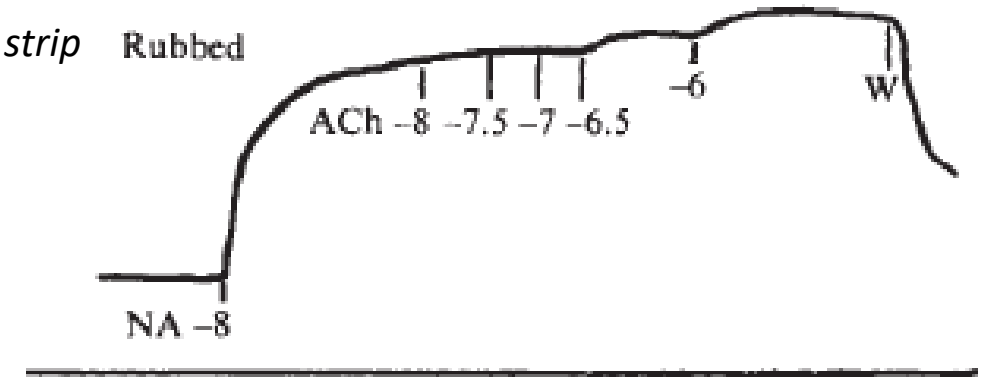
c, Strip that had had its intimal surface rubbed on filter paper for 1 min before being used in an experiment in which it gave no relaxation in response to ACh

d, Strip made from a ring from a segment of aorta that had been exposed intraluminally to 0.2% collagenase

Figure 2

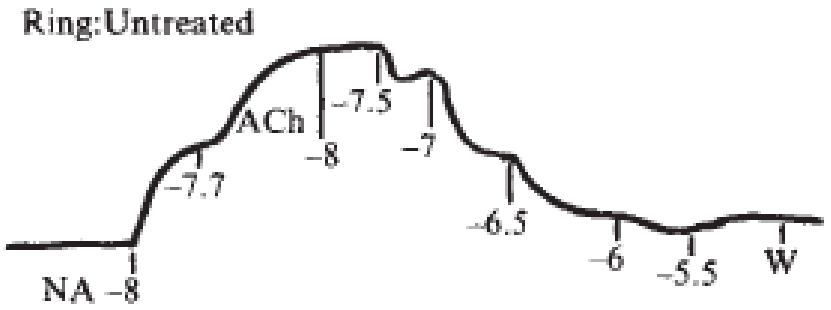

a Ring: Unrubbed
ACh –8
–7
2 g
5 min
NA –7.7
–6
W


b

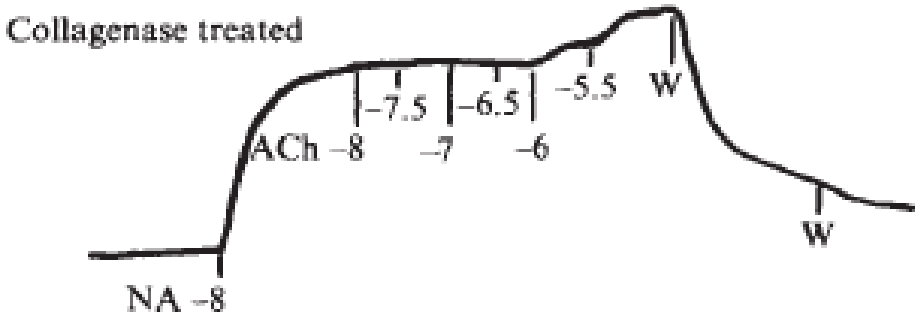

strip Rubbed
ACh –8 –7.5 –7 –6.5
–6
W
NA –8


c

c

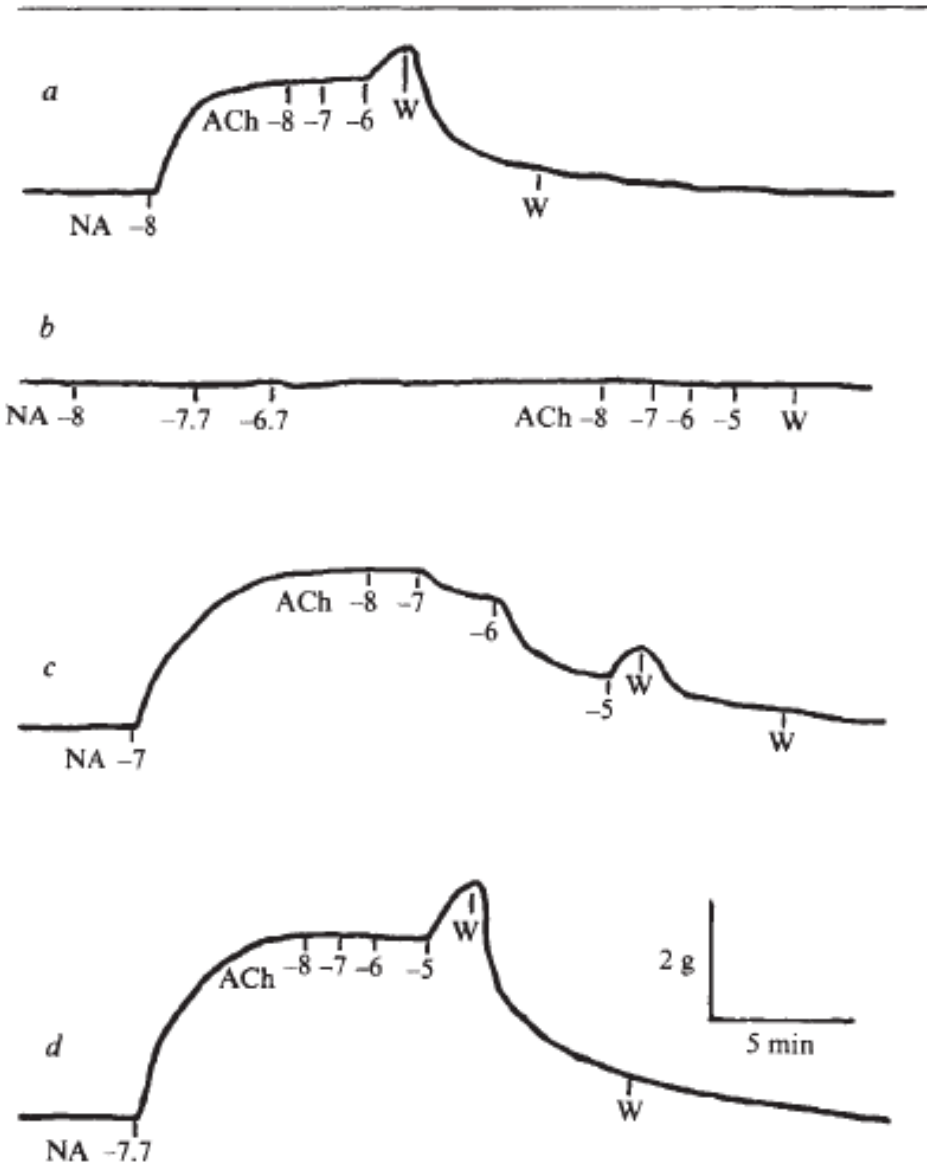

Ring:Untreated
ACh
-7.5
-8
-7
-7.7
-6.5
-6
-5.5
W
NA -8


b


Collagenase treated
-7.5
-6.5
-5.5
W
ACh -8
-7
-6
W
NA -8


d

A substância “relaxante” é liberada das células endoteliais?



strip transversal sem endotélio (registro)

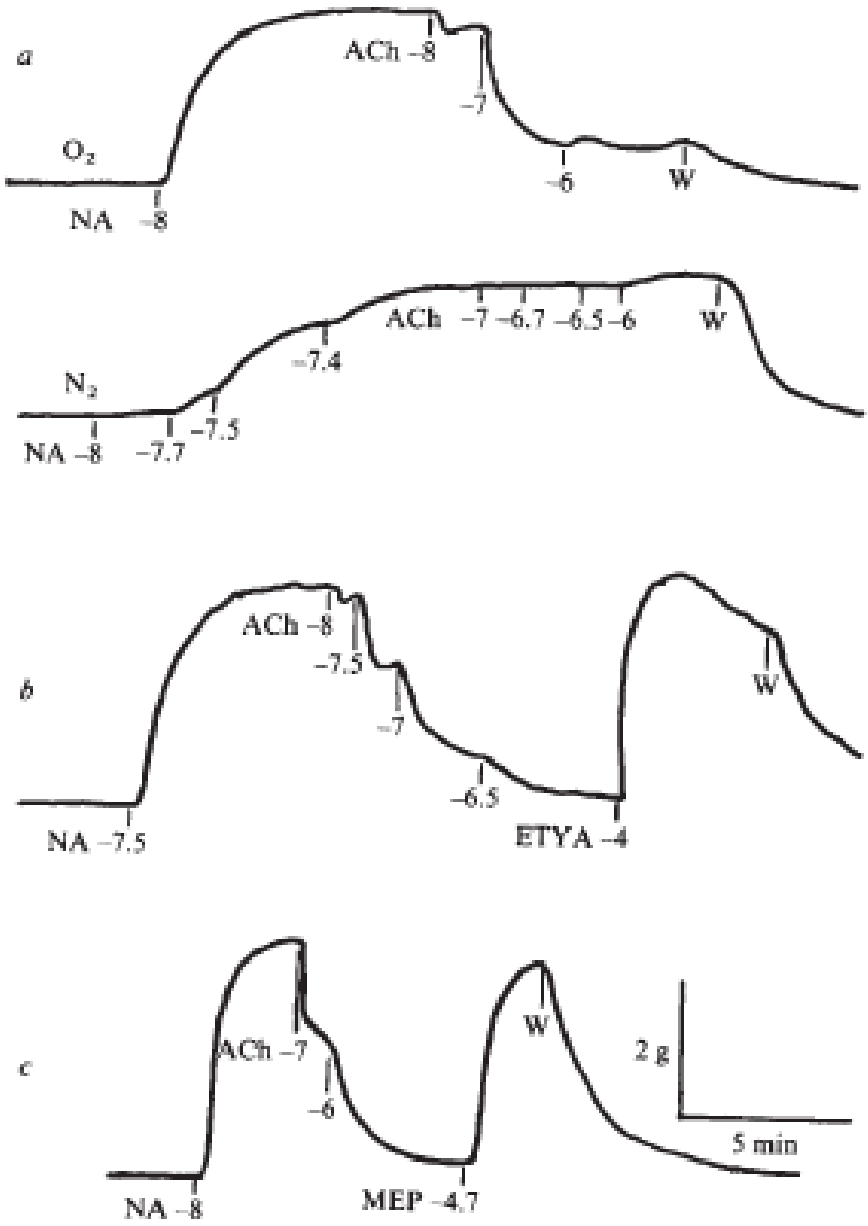
strip longitudinal com endotélio, mas sem tensão,

strip transversal sem endotélio (registro), montado em sandwich com strip longitudinal com enddotélio

strip transversal sem endotélio (registro) sem o strip longitudinal

Figure 3

Qual é a sustância?



Rulled out
Bradicinina, AMPc e GMPc, adenosina, 5-AMP < efeito que a ACh
Prostraciclina e outros produtos da COX, pois a aspirina e indometacina sem efeito

anel com endotélio, a anoxia (N2) reduz a resposta contrátil, maiores concentrações de NA foram necessárias para produzir a mesma resposta contrátil (-7,4) e as respostas da ACh foram totalmente abolidas

Nesta condição, nitrito de sódio, gliceril trinitrito e isoprenalina ainda produzem um bom relaxamento

ETYA, inibidor de LOX e COX e Mepacrine inibidor da liberação de ácido aracdônico (inibidor PLA2), inibiram o relaxamento induzido pela ACh

These results

suggest that ACh acting on the muscarinic receptor of the endothelial cells somehow activates a reaction sequence in which arachidonic (or some other unsaturated fatty acid) is liberated and then oxidized by lipoxygenase to a product that is responsible for the relaxation of the smooth muscle cells. The involvement of lipoxygenase is attractive despite negative results with the lipoxygenase inhibitor BW755C (ref. 13).

Figure 4

Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor

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Qual é a sustância?

Celula endoteliais de aorta de porco

Celula musculo liso vascular ~~X~~ aorta de porco

Celula ~~X~~ 3T3

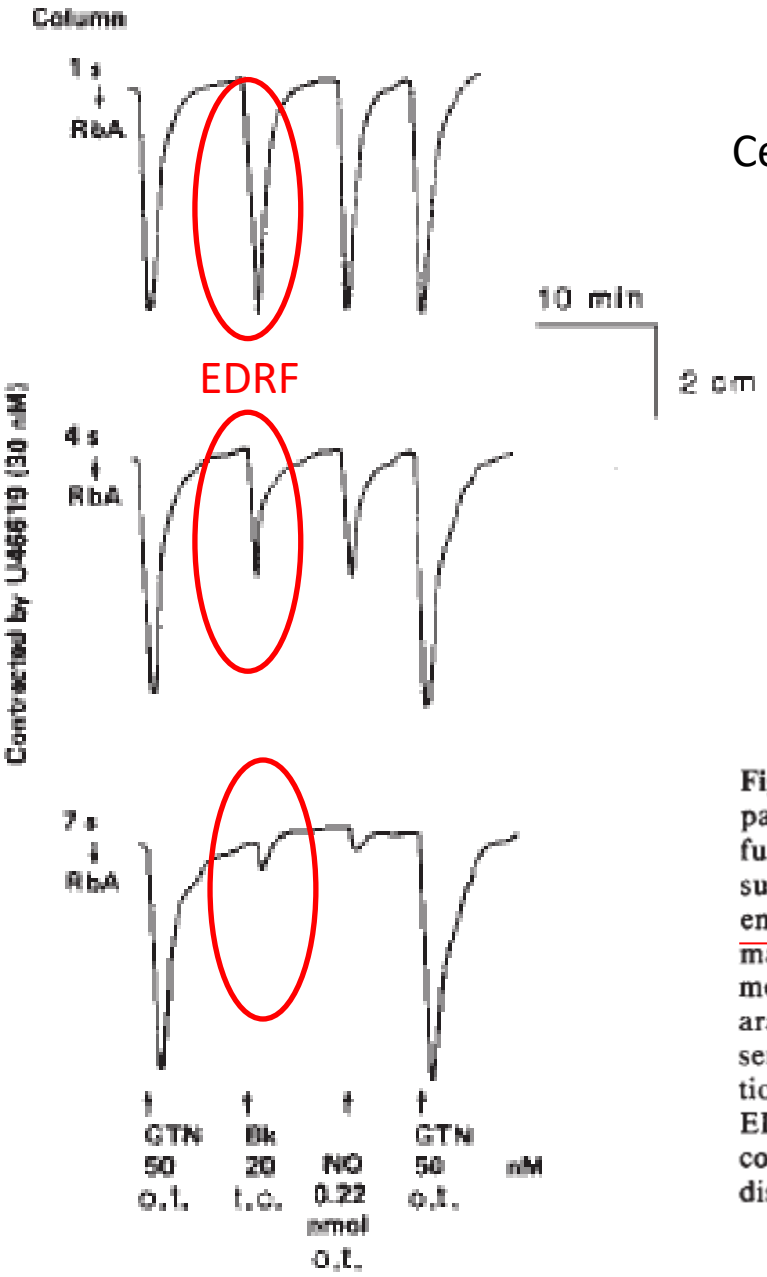
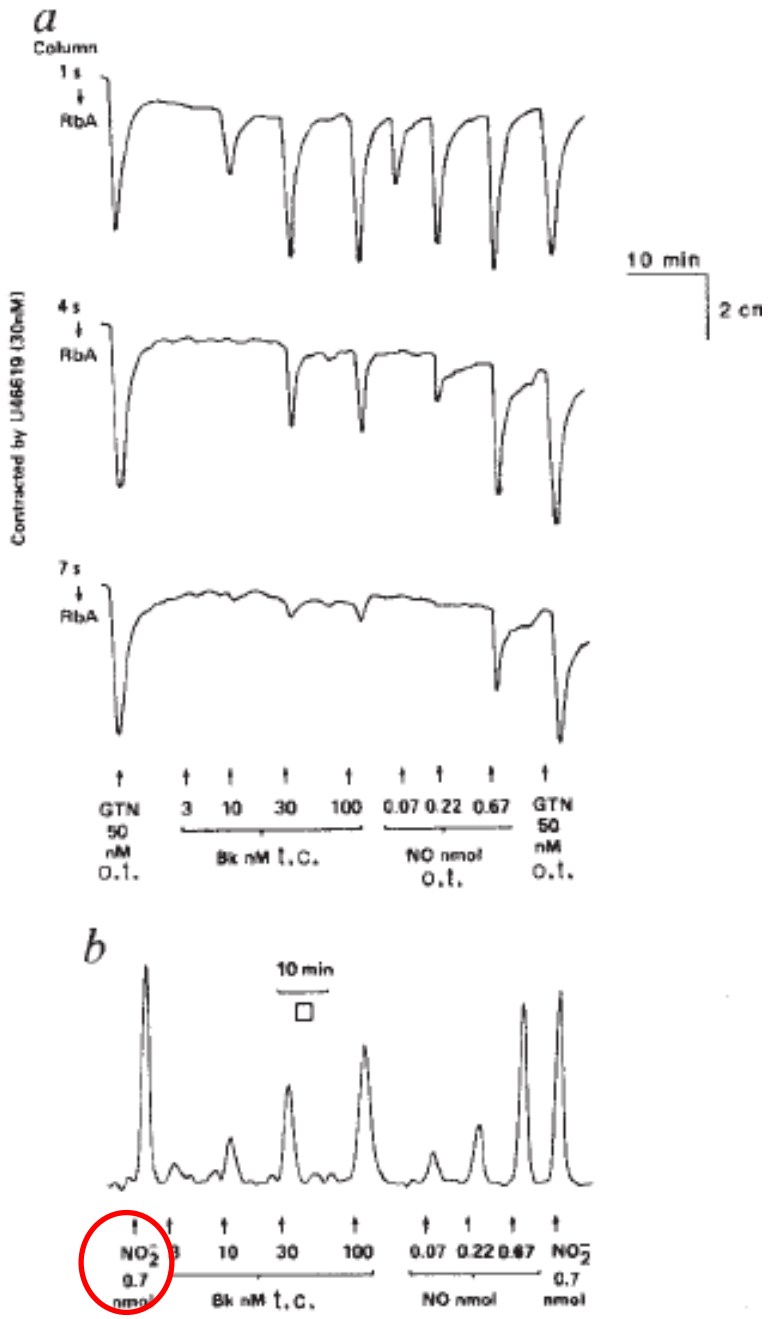


Fig. 1 Relaxation of rabbit aortae by EDRF and NO. A column packed with endothelial cells cultured on microcarriers was perfused with Krebs' buffer (5 ml min^{-1}). The effluent was used to superfuse three spiral strips of rabbit aorta (RbA), denuded of endothelium, in a cascade. The tissues were contracted submaximally by a continuous infusion of 9,11-dideoxy-9α, 11α-methano epoxy-prostaglandin $F_{2\alpha}$ (U46619; 30 nM) and were separated from the cells by delays of 1, 4 and 7 s, respectively. The sensitivity of the bioassay tissues was standardized by administration of glyceryl trinitrate (GTN; 50 nM) over the tissues (o.t.). EDRF was released from the cells by a 1 min infusion through the column (t.c.) of bradykinin (Bk, 20 nM). NO (0.22 nmol) was dissolved in He-deoxygenated H_2O and administered as a 1 min infusion.



* NO reacts readily with O_2 to produce NO_2, which then forms NO_2^- and NO_3^- in neutral aqueous solution according to the following reactions:

$$2NO + O_2 \rightarrow 2NO_2 \quad (1)$$

$$2NO_2 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+ \quad (2)$$

Fig. 2 *a*, Bioassay. Relaxation of rabbit aortae by EDRF and NO. The bioassay tissues were relaxed in a concentration-dependent manner by EDRF released from the cells by bradykinin (Bk; 3–100 nM t.c.) and by NO (0.07–0.67 nmol, o.t.) as in Fig. 1. *b*, Chemiluminescence. Release of NO by bradykinin (Bk) from a replicate column of the cells used in the bioassay. The amounts of NO (administered as 1 min infusion into the column effluent) which relaxed the bioassay tissues were also detectable by chemiluminescence. Effluent from the column, or Krebs' buffer into which authentic NO was injected, was passed continuously (5 ml min^{-1}) into a reaction vessel containing 75 ml 1.0% sodium iodide in glacial acetic acid under reflux. NO was removed from the refluxing mixture under reduced pressure in a stream of N_2, mixed with ozone and the chemiluminescent product measured with a photomultiplier. The amounts of NO detected were quantified after correcting for baseline drift using a polynomial fit and reducing electrical noise, by Fourier transformation and application of a Gaussian function. The areas under the peaks were converted to nmol of NO by reference to a NO_2^- standard curve. Similar results were obtained in two other experiments. □, Area equivalent to 0.22 nmol NO.

Hemoglobina
se liga ao NO e

liga ao EDRF

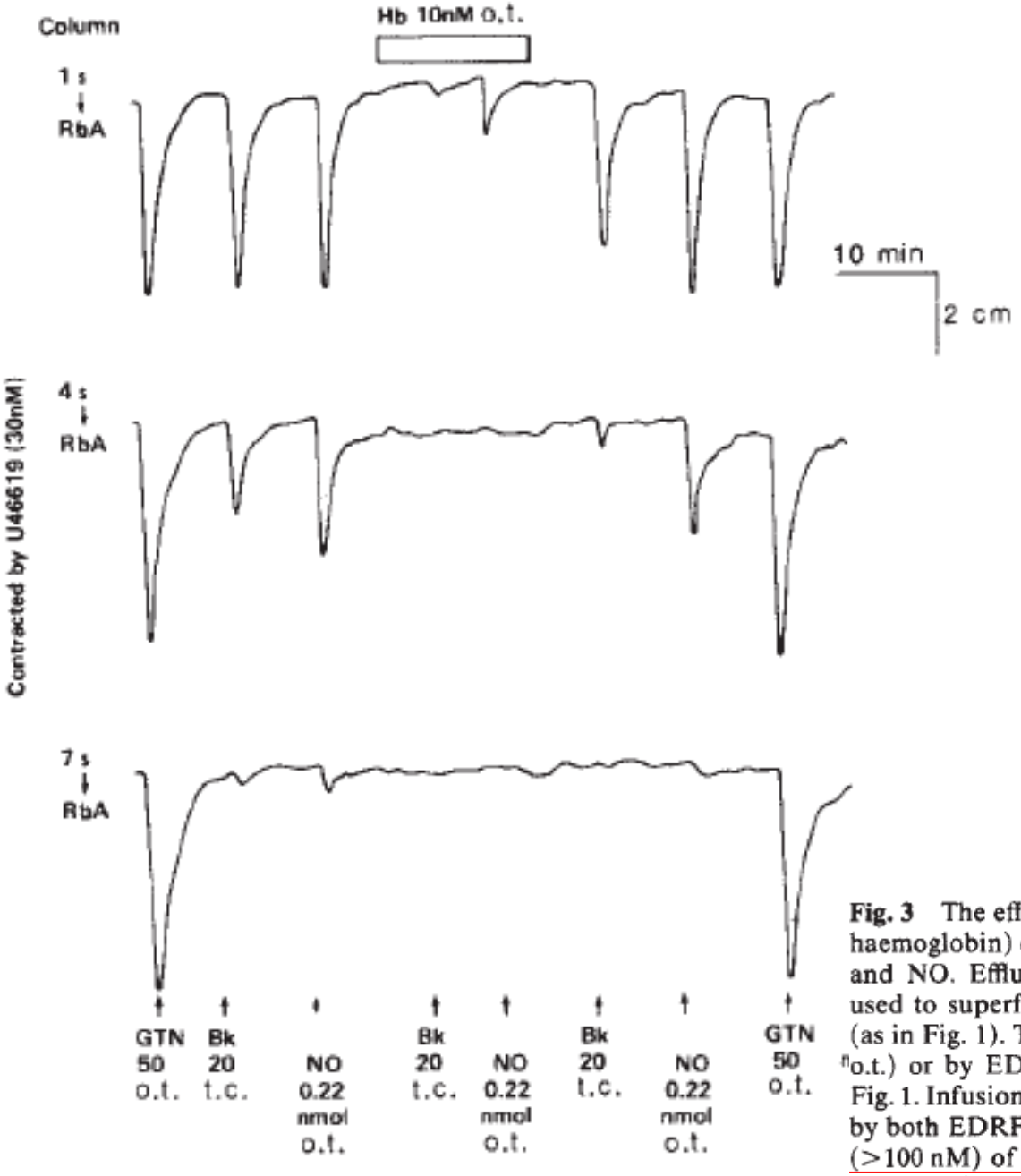


Fig. 3 The effect of purified human haemoglobin (Hb; <3% Met haemoglobin) on the relaxation of rabbit aortae induced by EDRF and NO. Effluent of a column containing endothelial cells was used to superfuse three spiral strips of rabbit aorta in a cascade (as in Fig. 1). The bioassay tissues were relaxed by NO (0.22 nmol o.t.) or by EDRF released by bradykinin (Bk, 20 nM t.c.) as in Fig. 1. Infusion of Hb (10 nM o.t.) inhibited the relaxations induced by both EDRF and NO to a similar extent. Larger concentrations (>100 nM) of Hb were needed to inhibit the relaxations induced by GTN (data not shown) confirming a previous report[7]. Similar results were obtained in two other experiments.

Superoxide dismutase (SOD) reduces the inactivation of EDRF[9,10]. Infusions of SOD (15 U ml^{-1}) over the tissues reduced the decay of both EDRF and authentic NO to a similar extent (Fig. 4), indicating that O_2^- contributes to the inactivation of both compounds.

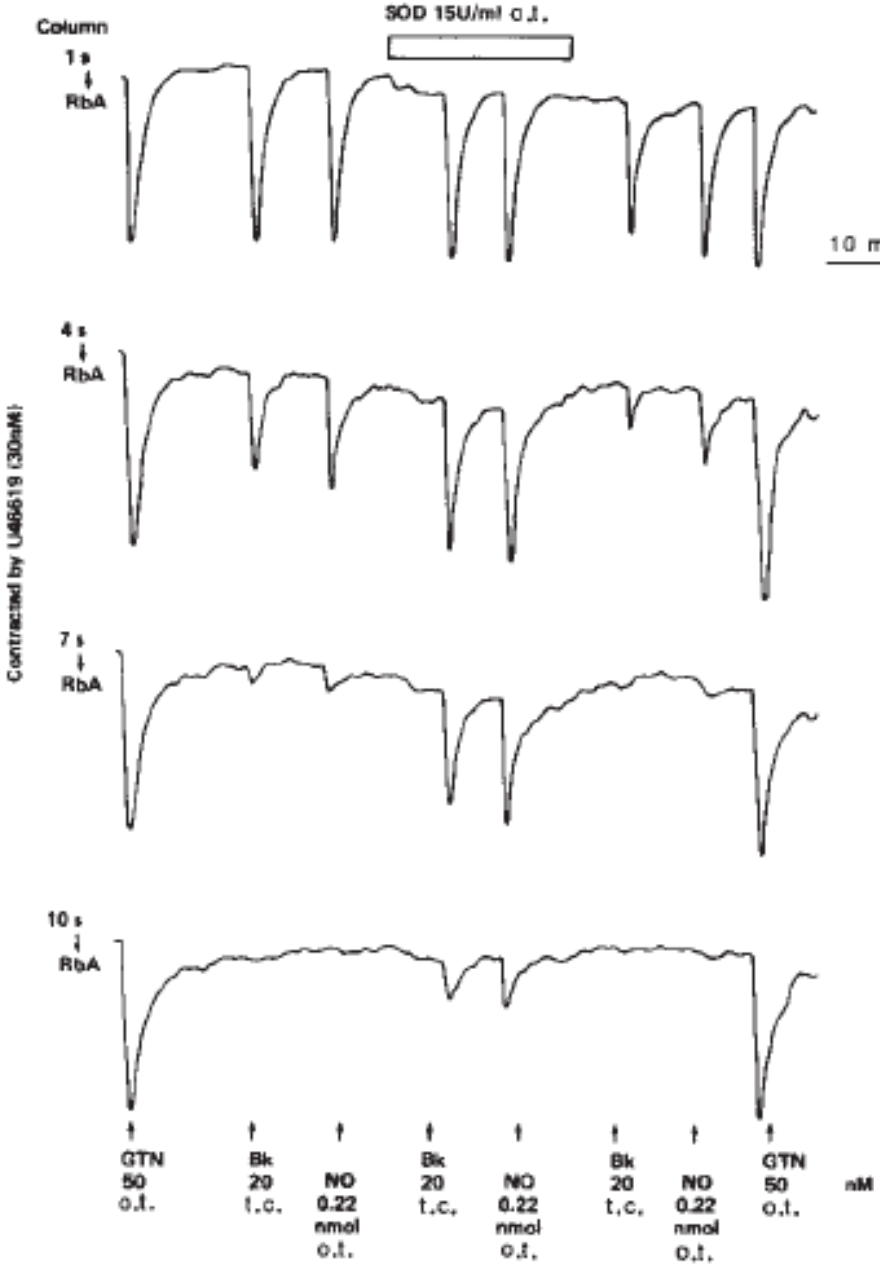
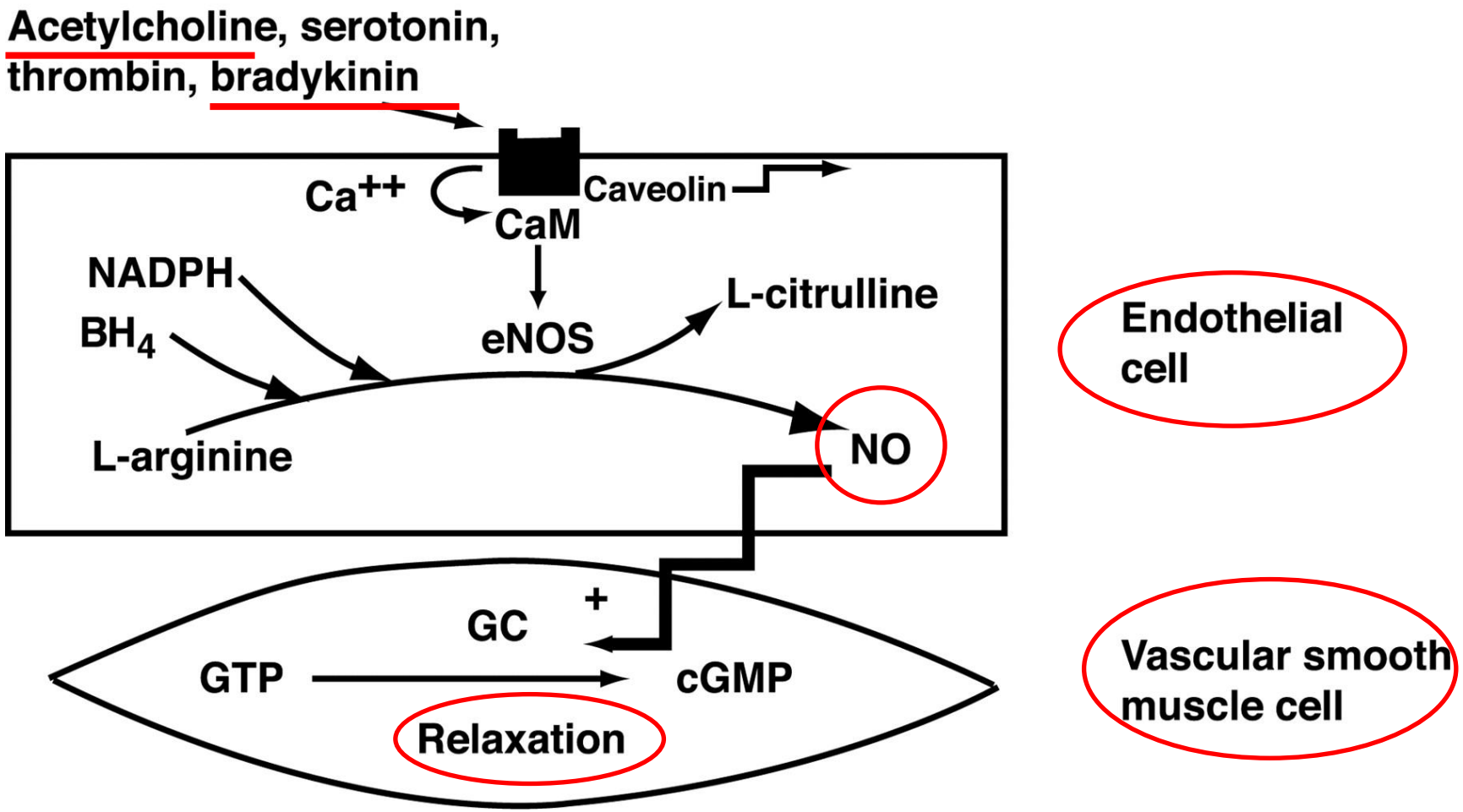


Fig. 4 The effect of SOD on the relaxation of rabbit aortae induced by EDRF and NO. Effluent of a column of endothelial cells was used to superfuse four strips of rabbit aorta in a cascade (as in Fig. 1). The bioassay tissues were relaxed by NO (0.22 nmol, o.t.) or by EDRF released by bradykinin (20 nM Bk t.c.). Infusion of SOD (15 U ml^{-1} o.t.) increased the stability of EDRF and NO to a similar extent, so that the fourth tissue was relaxed by both compounds. Similar results were obtained in two other experiments.

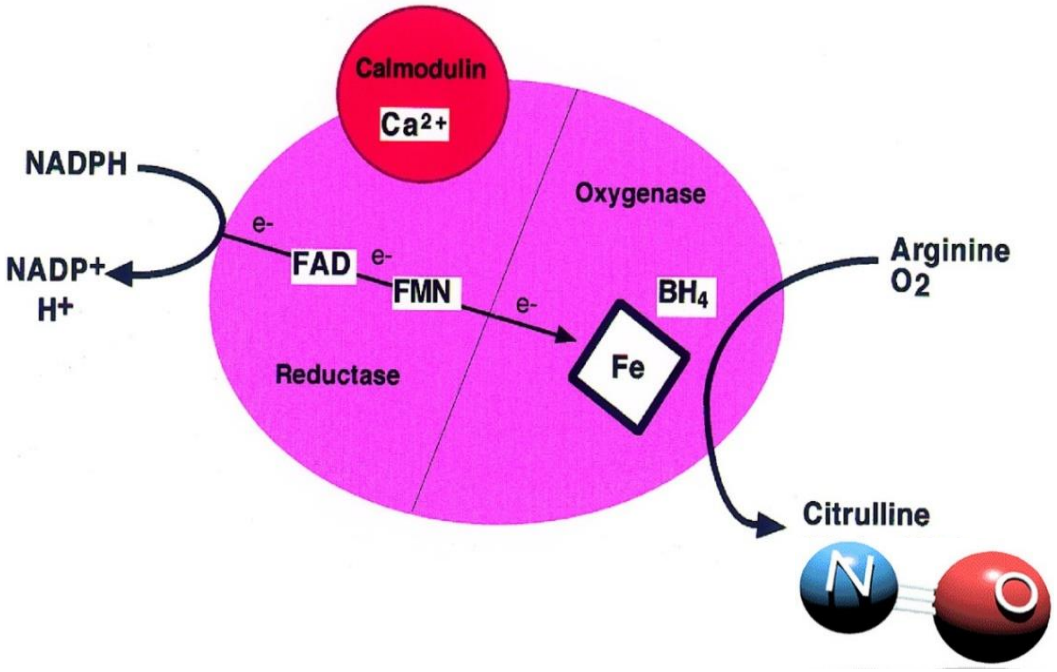
Whether NO is released from vascular endothelial cells as NO itself, or as a precursor which gives rise to NO following release, deserves further investigation.

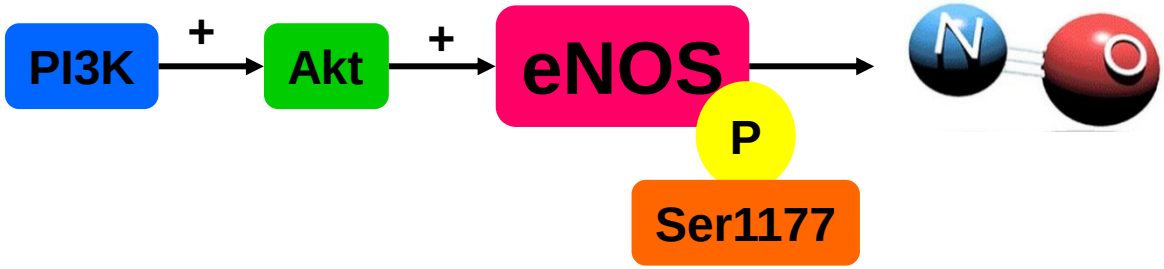
Palmer et al., 1987

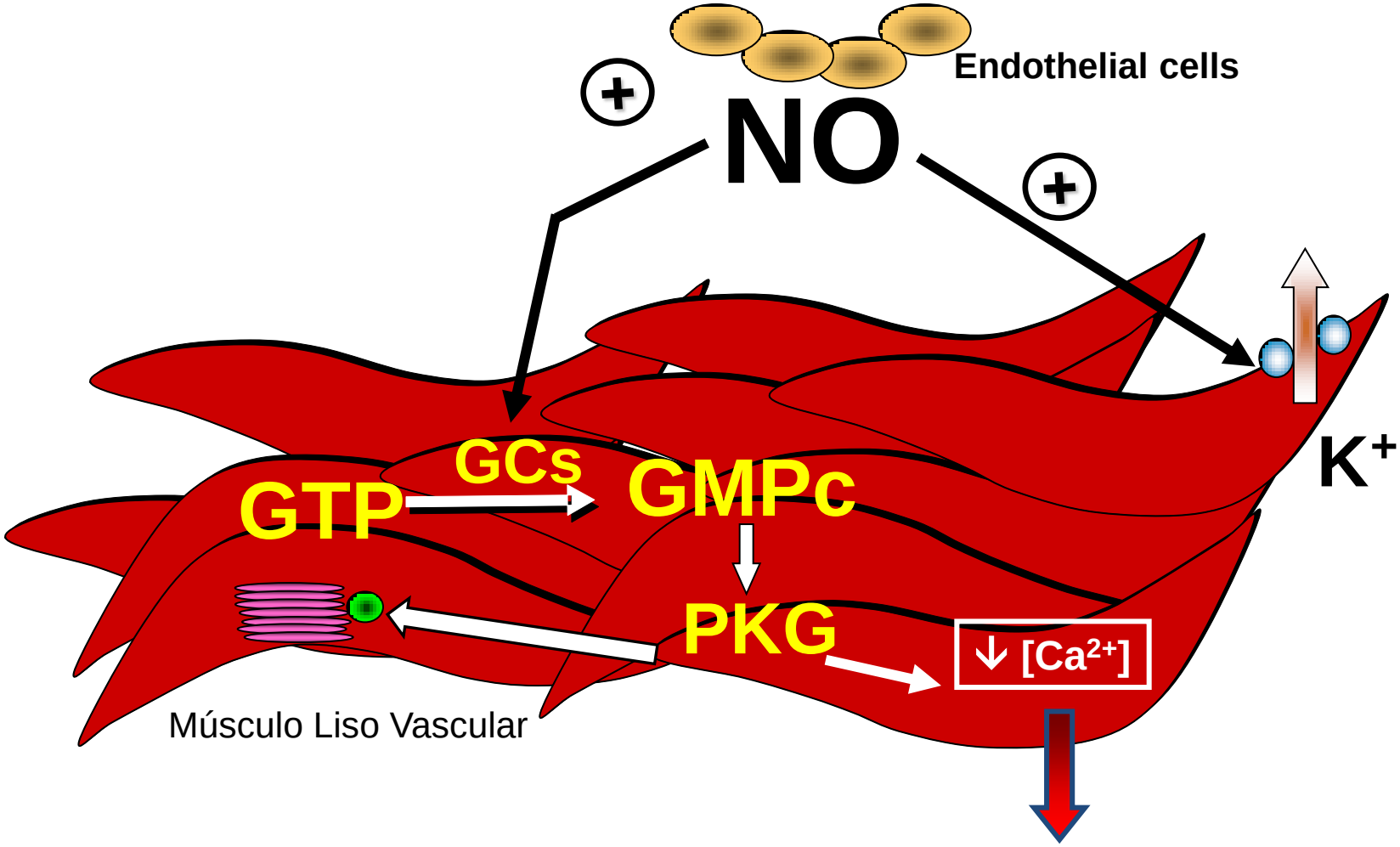

Acetylcholine, serotonin,
thrombin, bradykinin
Ca++
CaM
Caveolin
NADPH
BH4
eNOS
L-citrulline
L-arginine
NO
Endothelial cell
GC
+
GTP
cGMP
Relaxation
Vascular smooth muscle cell

4 isoformas de NOS:

- nNOS
- iNOS
- **eNOS**
- mtNOS

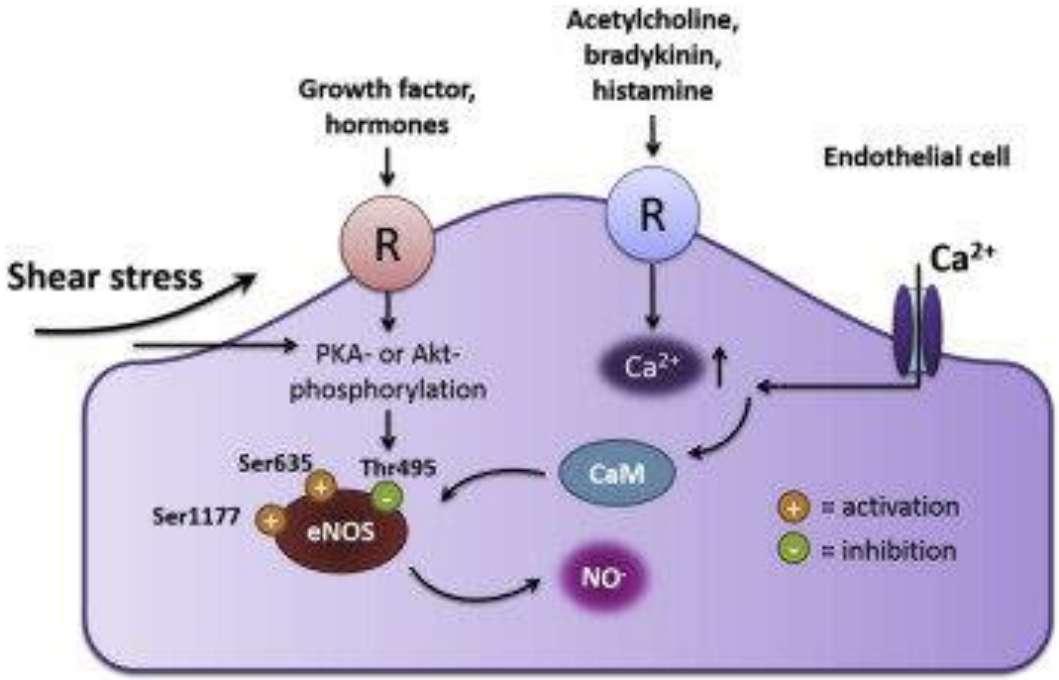






Cálcio ATPase do reticulo sarcoplasmático

Relaxamento Vascular



Endothelial nitric oxide synthase (eNOS) can be activated in calcium-dependent or- independent ways. On the one hand, agonists, such as acetylcholine, bradykinin and histamine, act on specific receptors (R) on the endothelial cell membrane to increase the intracellular concentration of calcium, which binds to calmodulin (CaM) and leads to the activation of calmodulin-binding domain of eNOS to produce nitric oxide (NO). On the other hand, phosphorylation of eNOS independently of the calcium concentration is also important for the activation of the enzyme. Thr495 is an inhibitory site but Ser635 and Ser1179 are activation sites. The responses to hemodynamic shear stress and hormones are mediated mainly through this calcium-independent pathway.

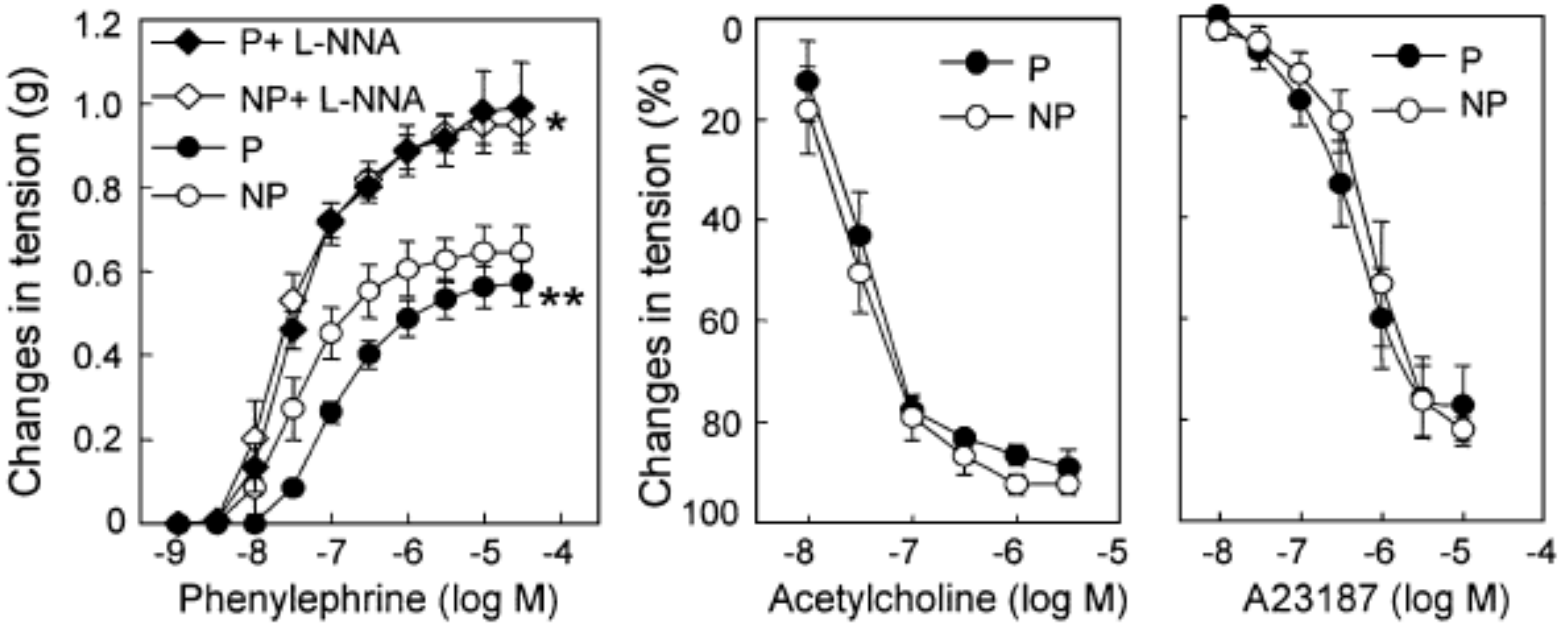


Fig. 1. Concentration-effect curves to phenylephrine, acetylcholine, and A23187 of aortic rings from late pregnant (P) and nonpregnant (NP) rats in the absence or in the presence of the NOS inhibitor N^{ω}-nitro-L-arginine (L-NNA, 100 μM). Values are mean ± SEM (n = 6–7). *P < 0.001 compared to NP and P curves. **P < 0.01 compared to NP curve.

Ballejo et al., 2002

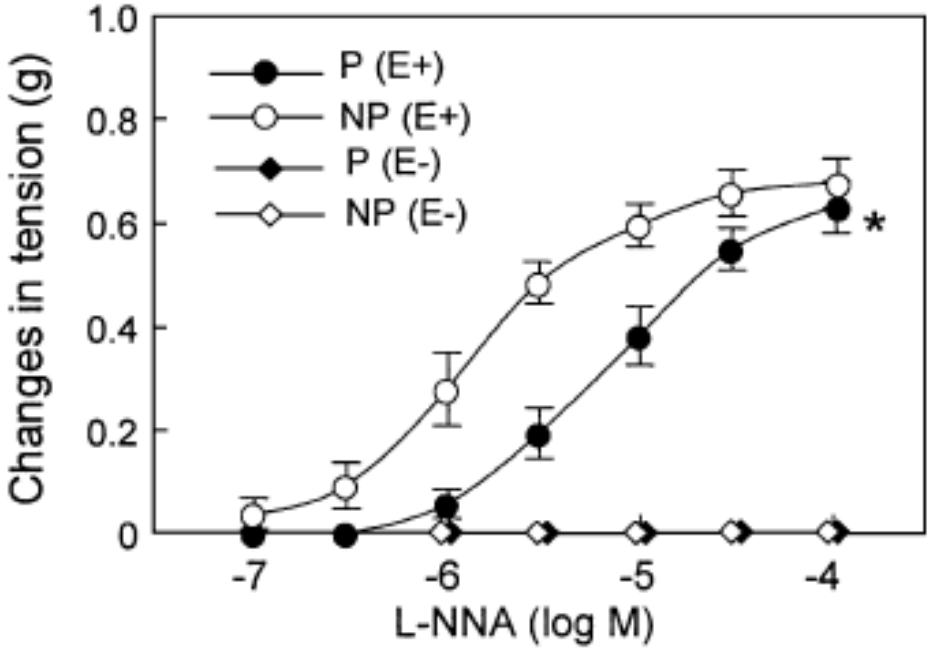
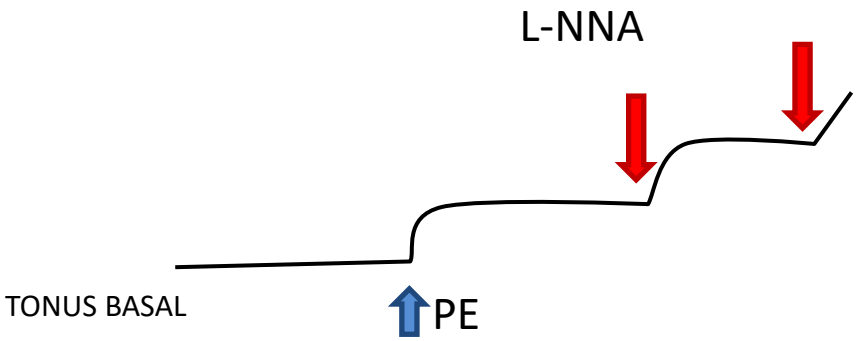
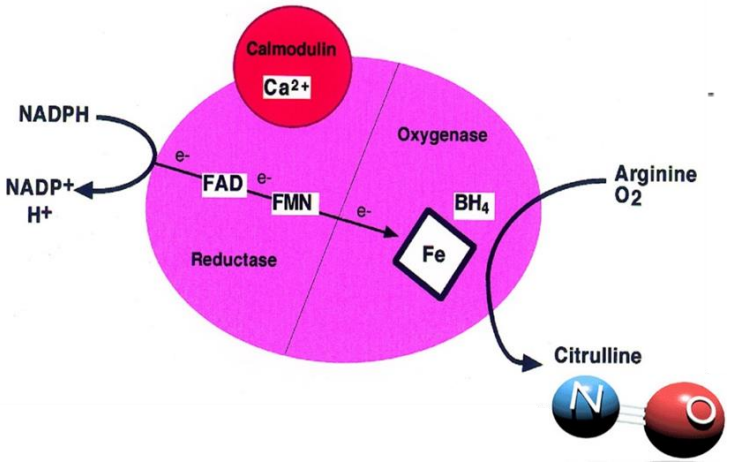


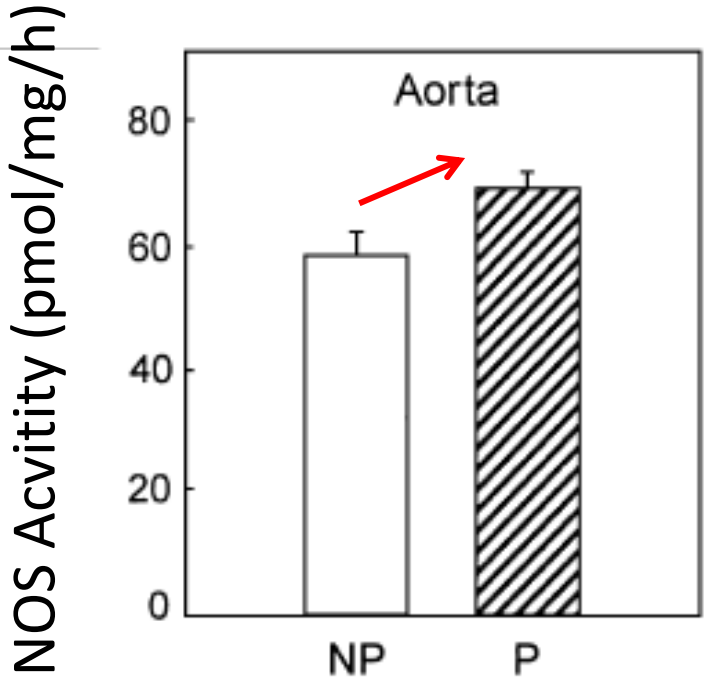
Fig. 2. Concentration-effect curves to N^{ω}-nitro-L-arginine (L-NNA) of aortic rings precontracted with phenylephrine in preparations with endothelium (E+; n = 9) or after endothelium removal (E−; n = 5) from late pregnant (P) and nonpregnant (NP) rats. Values are mean ± SEM. *P < 0.001 compared to NP.

Ballejo et al., 2002



NOS activity was measured by determining the conversion of L–[^{3}H]-arginine to L–[^{3}H]-citrulline based on a modification of the method described by Salter et al [25]. Thoracic aorta





Adaptada de Ballejo et al., 2002

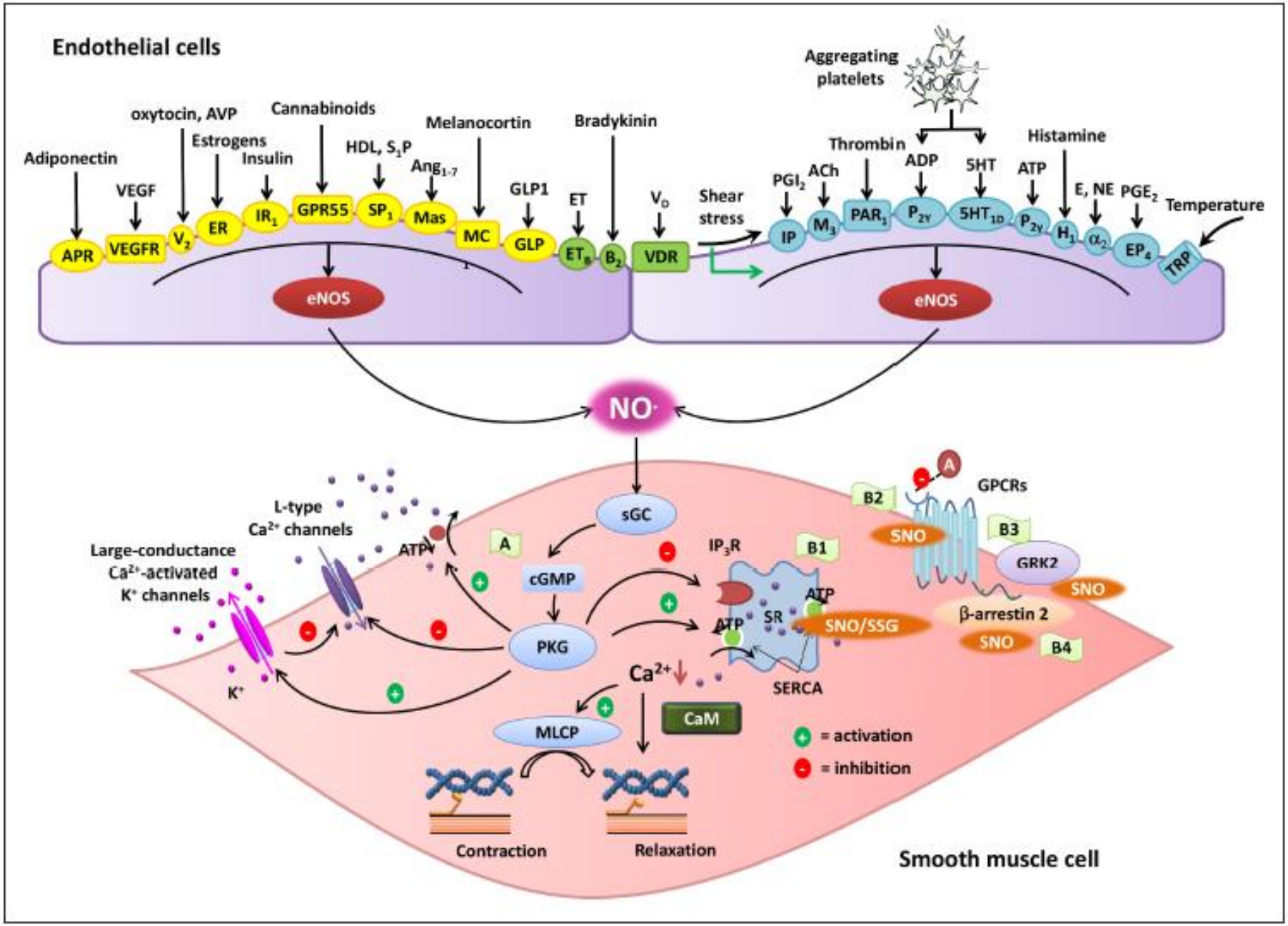
Review

Thirty Years of Saying NO

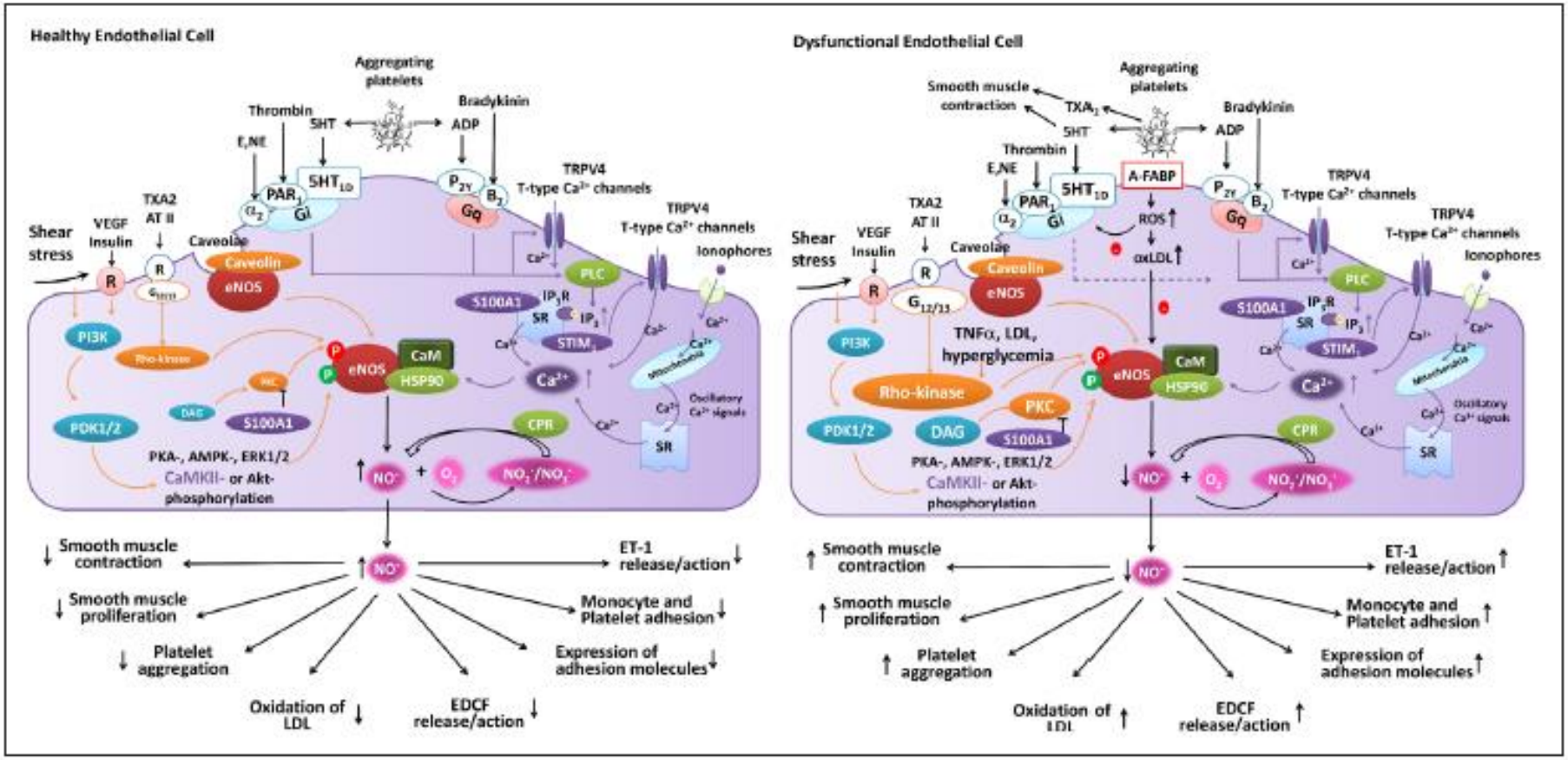
Sources, Fate, Actions, and Misfortunes of the Endothelium-Derived Vasodilator Mediator

Paul M. Vanhoutte, Yingzi Zhao, Aimin Xu, Susan W.S. Leung

(*Circ Res*. 2016;119:375-396. DOI: 10.1161/CIRCRESAHA.116.306531.)



Vanhoutte et al., 2016



Vanhoutte et al., 2016

Table 2. Summary of the Multiple and Diverse Impacts of Aging and Major Pathological Situations on the Different Steps of the eNOS–NO–sGC Pathway Responsible for Endothelium-Dependent, NO-Mediated Vasodilatations

Condition	Receptor Coupling	Arginase Activity	BH_4 Levels	ADMA Levels	eNOS Expression* and Presence	eNOS Dimerization	eNOS Coupling	eNOS Activity	NO Disposition†	NO Vasodilator Effect
Aging	…	↑	…	…	↓	↓	↓	↓	↑	↓
Postmenopause	…	…	↓	↑	↓	…	…	↓	↑	↓
Hyperlipidemia	↓	…	…	…	…	↓	…	…	…	…
Obesity	…	…	…	…	…	…	↓	↓	↑	
Diabetes mellitus (type II)	…	↑	↓	↑	↓	…	↓	↓	↑	↓
Hypertension‡	…	↑	↓	↑	↓	…	↓	↓	↑	↓
Heart failure	…	…	…	…	…	…	↓	↓	↑	↓
Ischemia/ reperfusion, angioplasty	↓	…	…	…	…	…	↓	↓	↑	
Chronic inflammation	…	↑	↓	↑	…	…	↓	↓	↑	

For description of the molecular events underlying the changes shown see text and Vanhoutte et al.[15] ↓ indicates reduction; ↑, acceleration; ADMA, asymmetrical dimethyl arginine; BH_4, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; and NO, nitric oxide.

*Due mainly to induction of nuclear factor κB.

†Due mainly to increased reactive oxygen species production.

‡In particular, if accompanied by increased levels of angiotensin II and/or aldosterone.

Vanhoutte et al., 2016

SHR (spontaneously hypertensive rats)

- HA sem etiologia específica
- ↑ RVP
- ↑ atividade simpática
- ↑ níveis de angiotensina II
- Disfunção endotelial e vascular

Development of a Strain of Spontaneously Hypertensive Rats*

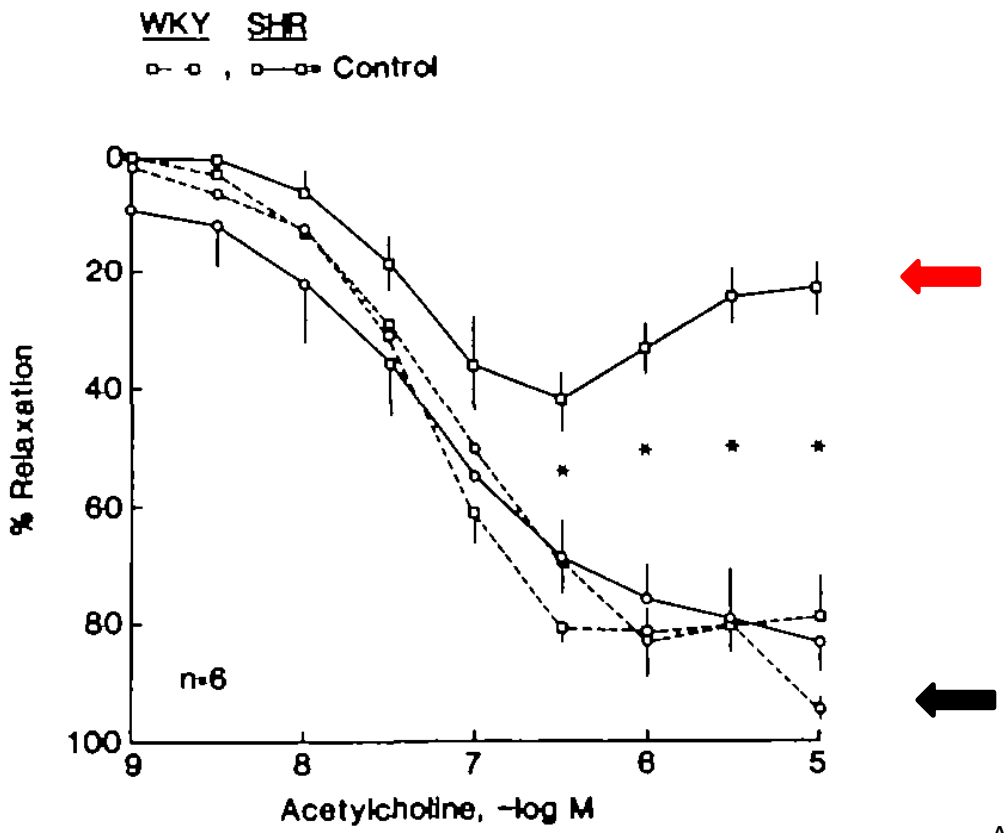
KOZO OKAMOTO and KYUZO AOKI

Department of Pathology, Kyoto University School of Medicine, Kyoto.
(Director: Prof. K. Okamoto)

(Received for Publication, January, 11, 1963)

PINTO; PAUL; GANTEN, 1998; TANASE, 1979; TRIPPODO; FROHLICH, 1981; LUNDIN; RICKSTEIN; THORÉN, 1984; OKAMOTO; AOKI, 1963; CATT *et al.,* 1969; BOLTERMAN *et al.,* 2005

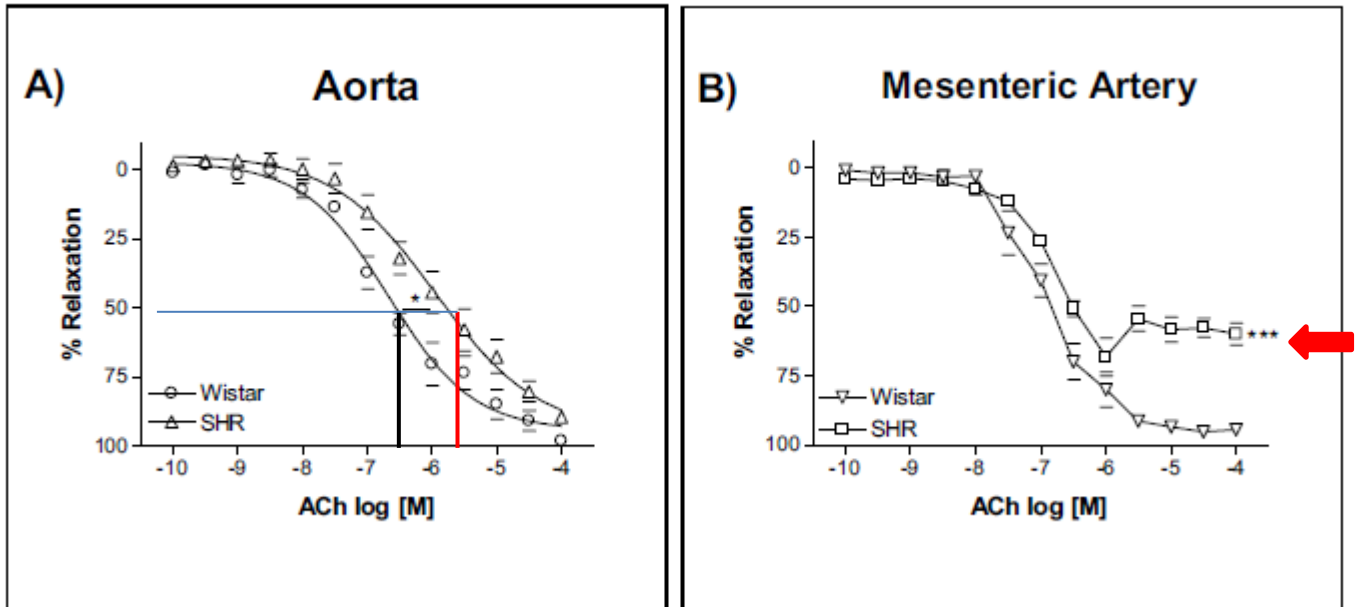
Avaliação funcional de **disfunção endotelial** em anéis de aorta torácica de ratos



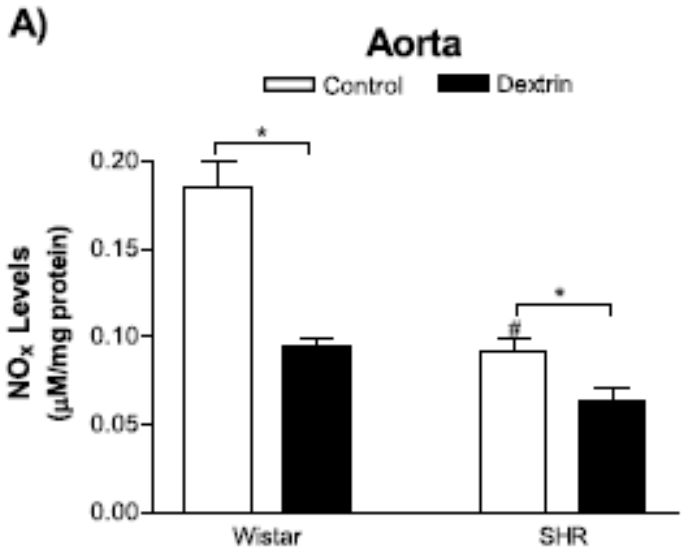
Adaptada de Lüscher & Vanhoutte, 1986

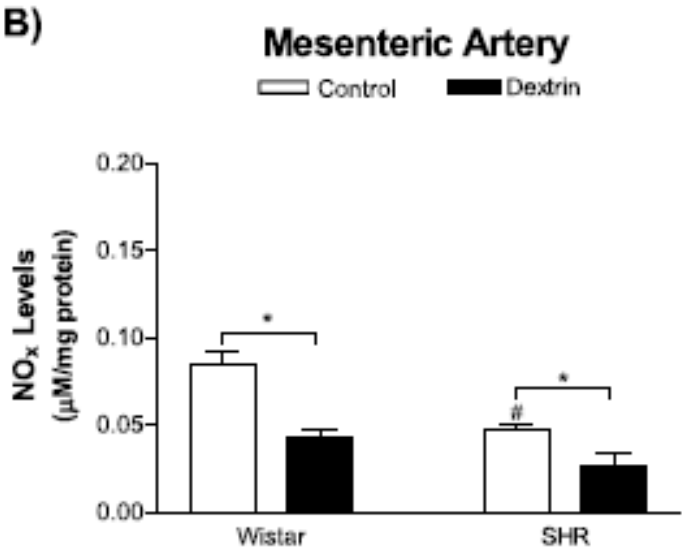
Avaliação funcional de disfunção endotelial em anéis de aorta torácica e artéria de resistência de ratos

The second or the third branch of the mesenteric arteries (internal diameter = 200–300 µm)



Adaptada de *Potje et al., 2019*





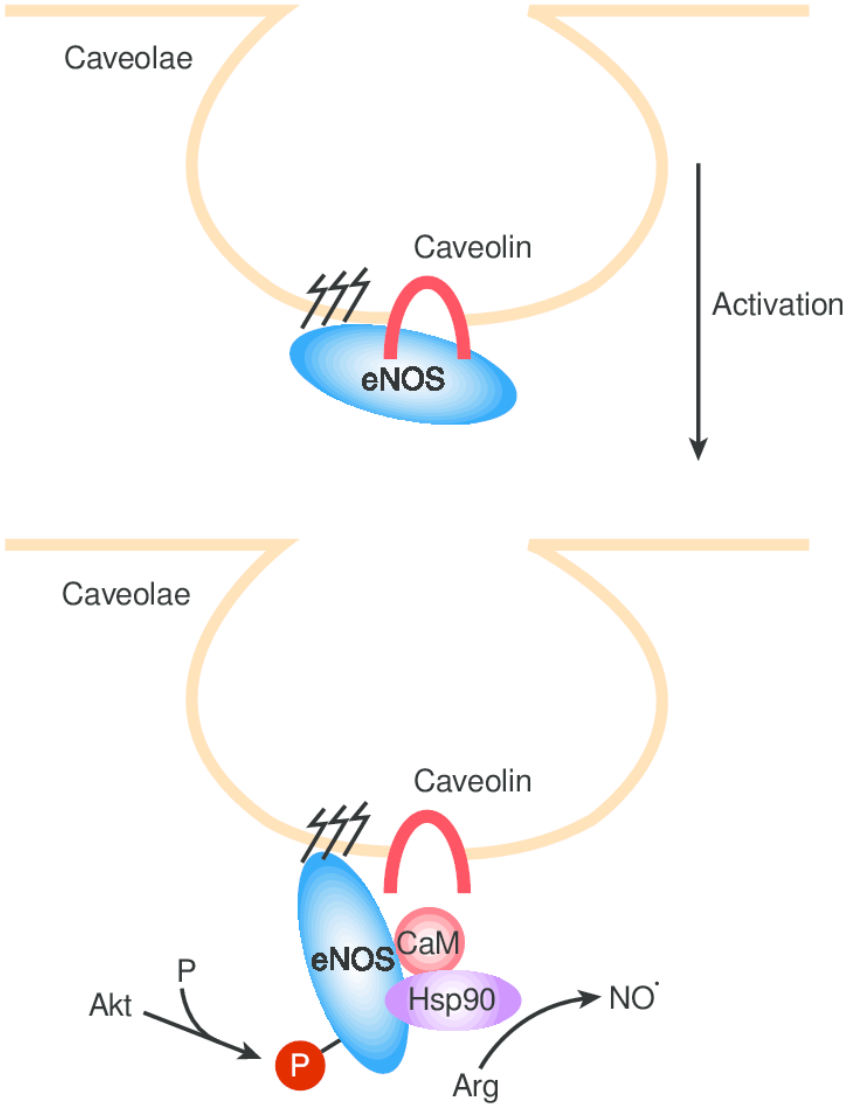
Colorimetric Griess reaction. The thoracic aorta and mesenteric artery rings of normotensive rats and SHRs were incubated in a bath chamber containing Krebs-Henseleit solution (95% O_2 and 5% CO_2; pH 7.4) at 37 °C. The rings were treated with dextrin (10 mM) for 60 min or not (control). Some rings were incubated in the presence of L-NAME (1 mM, 30 min) or BH_4 (100 µM, 30 min). Then, each sample was stimulated with PE (10 µM) followed by ACh (10 µM). Next, 50 µL of the bath solution of each sample was collected and added to 50 µL of Griess reagent (a 1:1 dilution of N-(1-Naphthyl)ethylenediamine dihydrochloride 1% in distilled water and sulfanilamide 1% in phosphoric acid 5%) in a 96-well plate. Sodium nitrite was used as standard. The standard curve ranged from 3 µM to 200 µM for nitrite. The absorbance was read at 540 nm. The results were normalized to total protein.

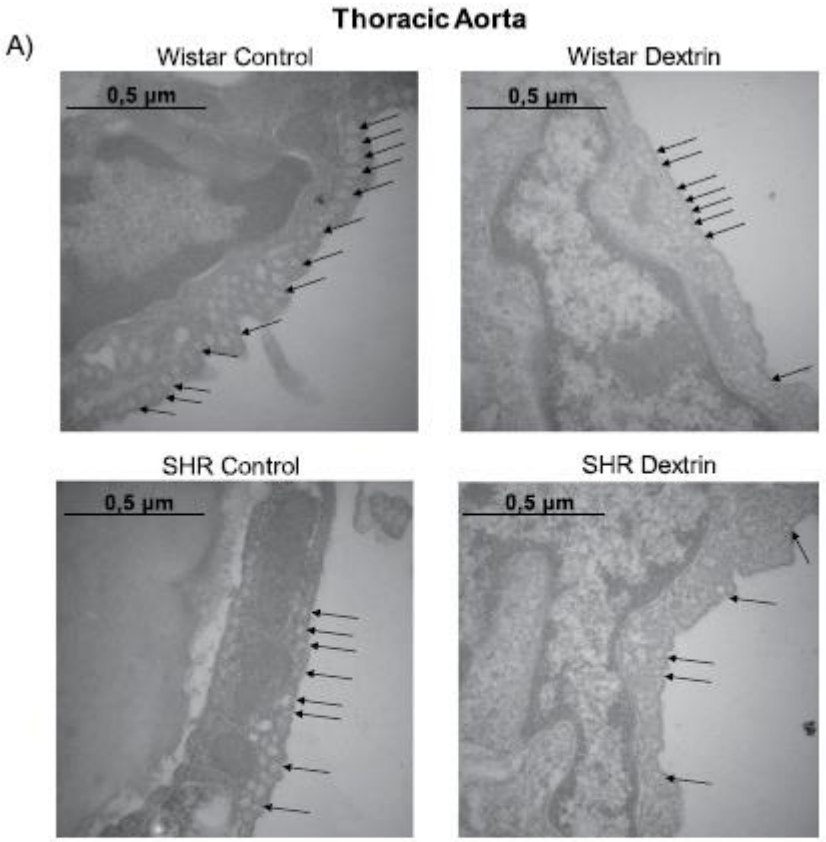
Adaptada de Potje et al., 2019

NO reacts readily with O_2 to produce NO_2, which then forms NO_2^- and NO_3^- in neutral aqueous solution according to the following reactions:

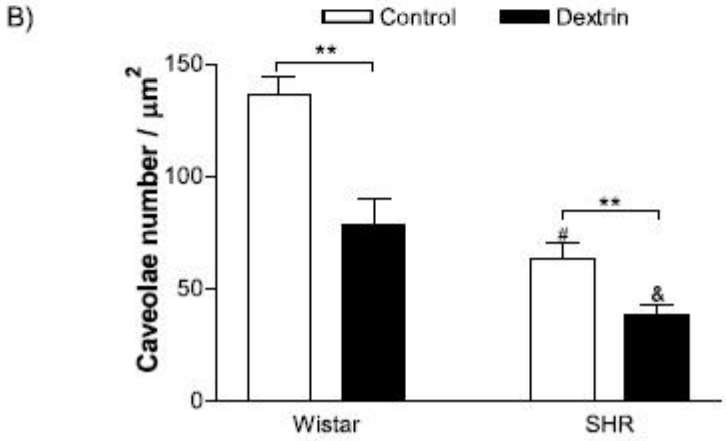
$$2NO + O_2 \rightarrow 2NO_2 \quad (1)$$

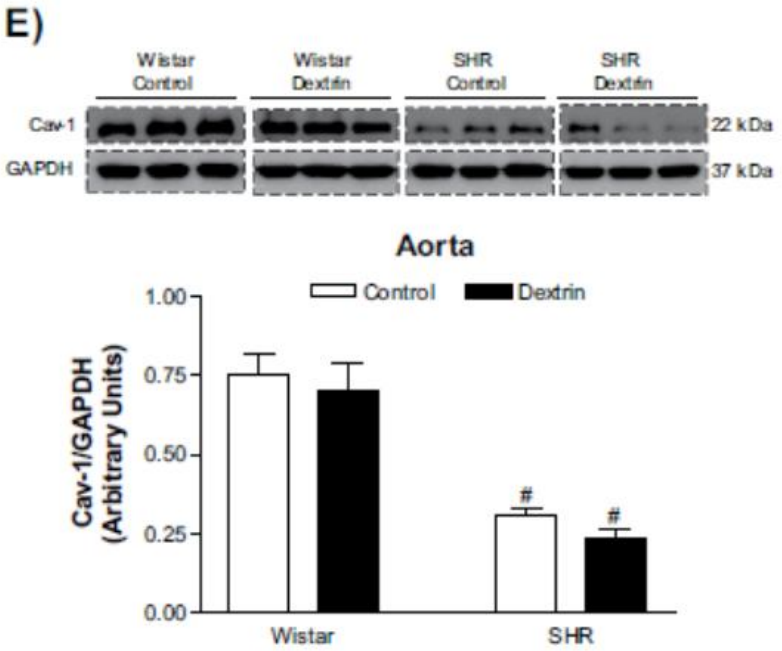
$$2NO_2 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+ \quad (2)$$


Caveolae
Caveolin
eNOS
Activation
Caveolae
Caveolin
eNOS
CaM
Hsp90
P
Akt
P
NO·
Arg

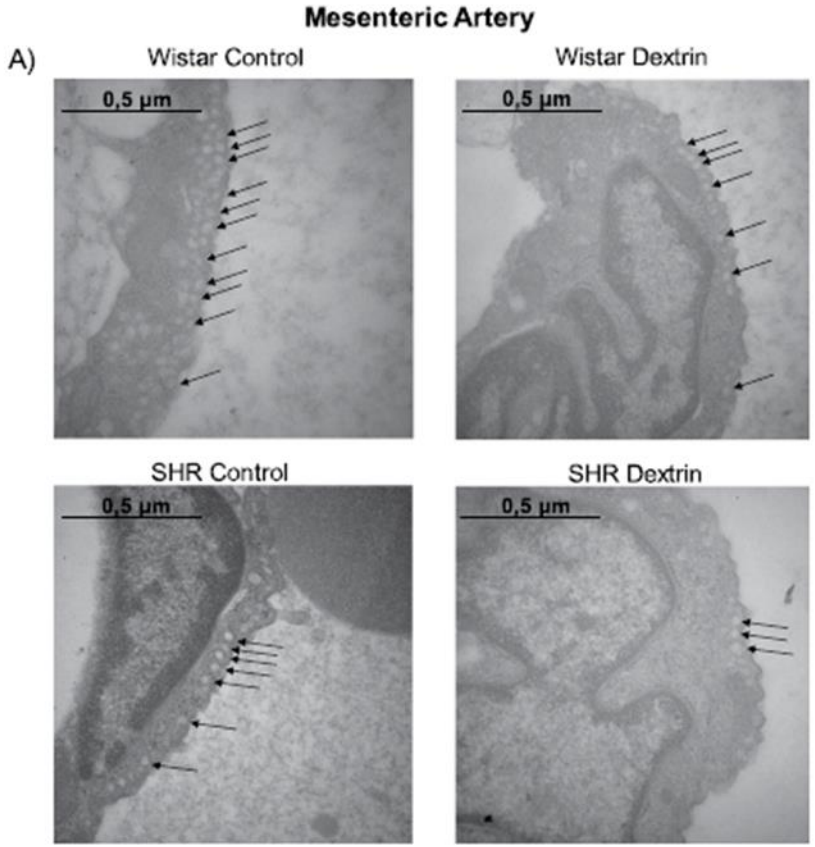


(A) Representative electron micrographs of caveolae-like structures Original magnification of 50,000× and 0.5-µm scale bar in each case.

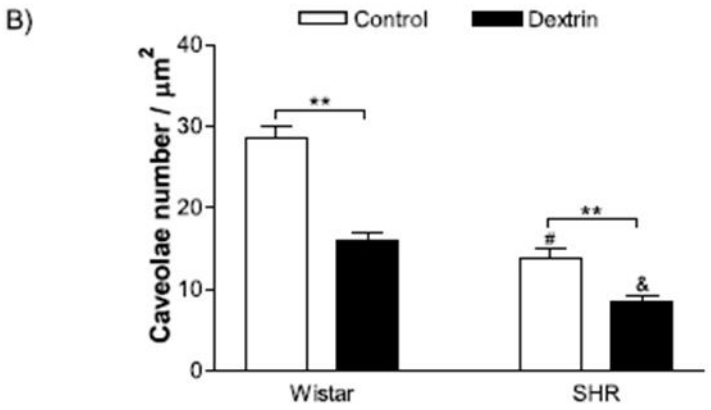


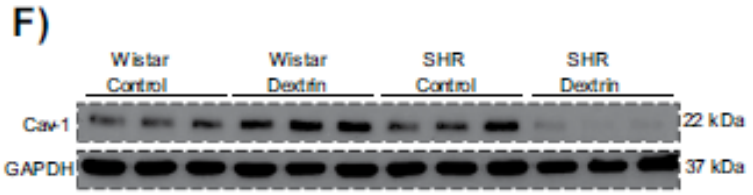


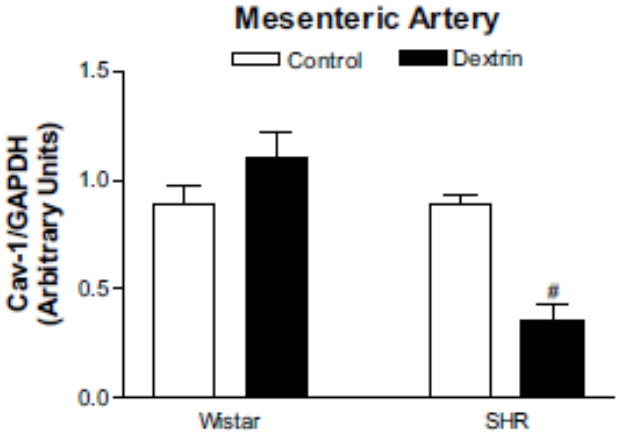
Adaptada de Potje et al., 2019



(A) Representative electron micrographs of caveolae-like structures Original magnification of 50,000× and 0.5-µm scale bar in each case.







Adaptada de Potje et al., 2019

É possível reverter farmacologicamente a disfunção endotelial?

Vascular Pharmacology 87 (2016) 38–48

Contents lists available at ScienceDirect

Vascular Pharmacology

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Apocynin reduces blood pressure and restores the proper function of vascular endothelium in SHR

Ligia A. Perassa [a,b,1], Murilo E. Graton [a,b,1], Simone R. Potje [a,b,1], Jéssica A. Troiano [a,b,1], Mariana S. Lima [b], Gabriel T. Vale [c], Ariana A.F. Pereira [a,b], Ana Claúdia M.S. Nakamune [a,b], Doris H. Sumida [a,b], Carlos R. Tirapelli [c], Lusiane M. Bendhack [d], Cristina Antoniali [a,b,*]

Free Radical Biology and Medicine 134 (2019) 53–63

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original article

Apocynin alters redox signaling in conductance and resistance vessels of spontaneously hypertensive rats

Murilo E. Graton[a,b], Simone R. Potje[c], Jéssica A. Troiano[a,b], Gabriel T. Vale[d], Ligia A. Perassa[a,b], Ana Cláudia M.S. Nakamune[a,b], Carlos R. Tirapelli[d], Lusiane M. Bendhack[c], Cristina Antoniali[a,b,*]

MATERIAL E MÉTODOS

Animais e Tratamento

Aprovação pelo Comitê de Ética no Uso de Animais
Processo CEUA FOA 2015-0450

Wistar
n = 20

SHR
n = 20

SHR Tratado
n = 20

Condições controladas
12 horas claro/escuro, 22 ± 2 °C,
água e ração *ad libitum*

Semanas de vida

0 — **4ª** — **10ª**

Amamentação | **Tratamento**

Não-tratado: água.

Tratado: apocinina, 30 mg/Kg,
diluída na água de beber.

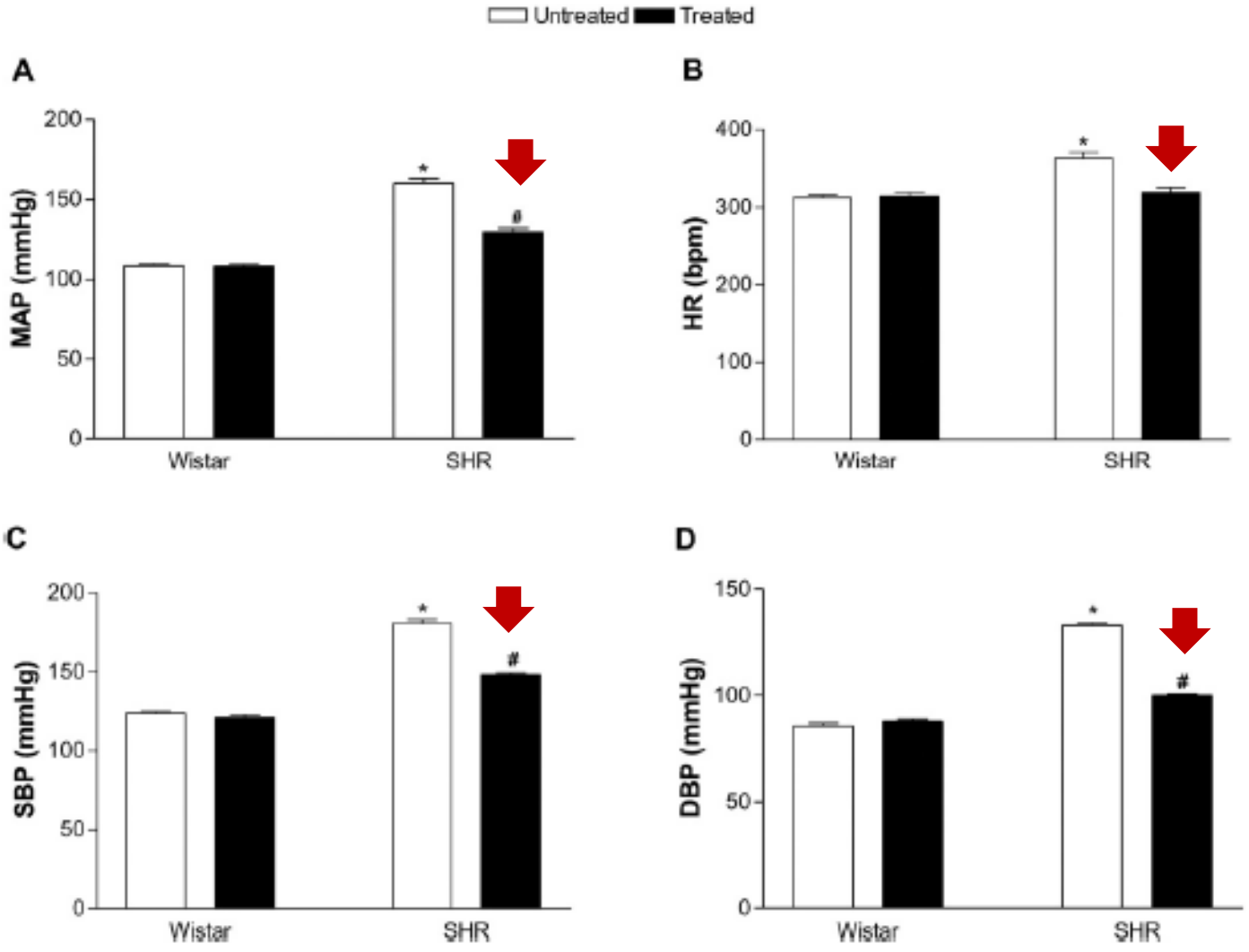


Fig. 3. Values, in mmHg or bpm, of (A) Mean Arterial Pressure, (B) Heart Rate, (C) Systolic Blood Pressure and (D) Diastolic Blood Pressure of Wistar and SHR untreated (white bars) and treated (black bars) with apocynin (30 mg/Kg). Values represent the mean ± SEM of the results, n = 7–9. *p < 0.05 untreated SHR versus other groups; #p < 0.05 treated SHR versus other groups (ANOVA).

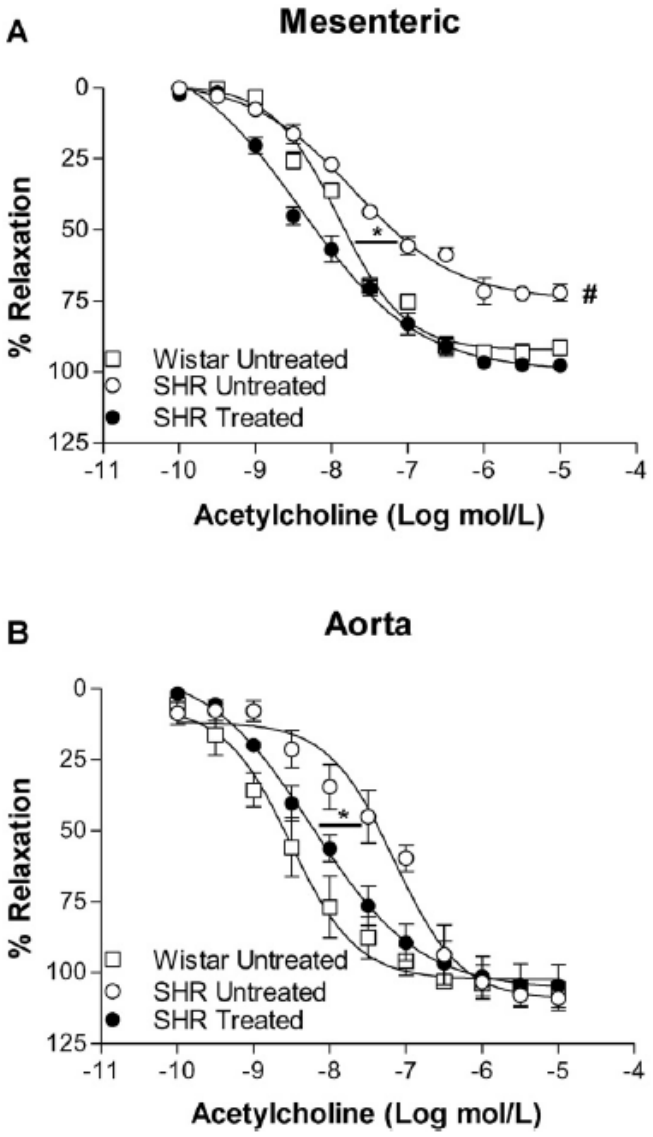


Fig. 5. Relaxation effect, in percentage, of Acetylcholine (ACh, 0.1 nmol/L–10 μmol/L) in rings from (A) the second or the third branches of mesenteric artery and (B) aorta of Wistar rats (white square indicates untreated Wistar rats) and (B) SHR (white circle indicates untreated SHR and black circle indicates treated SHR). Values represent the mean ± SEM of the results, n = 5. *$p < 0.05$ for pD_2 values in untreated SHR *versus* other groups; #$p < 0.05$ for Emax values in untreated SHR *versus* other groups (ANOVA).

Perassa et al., 2016

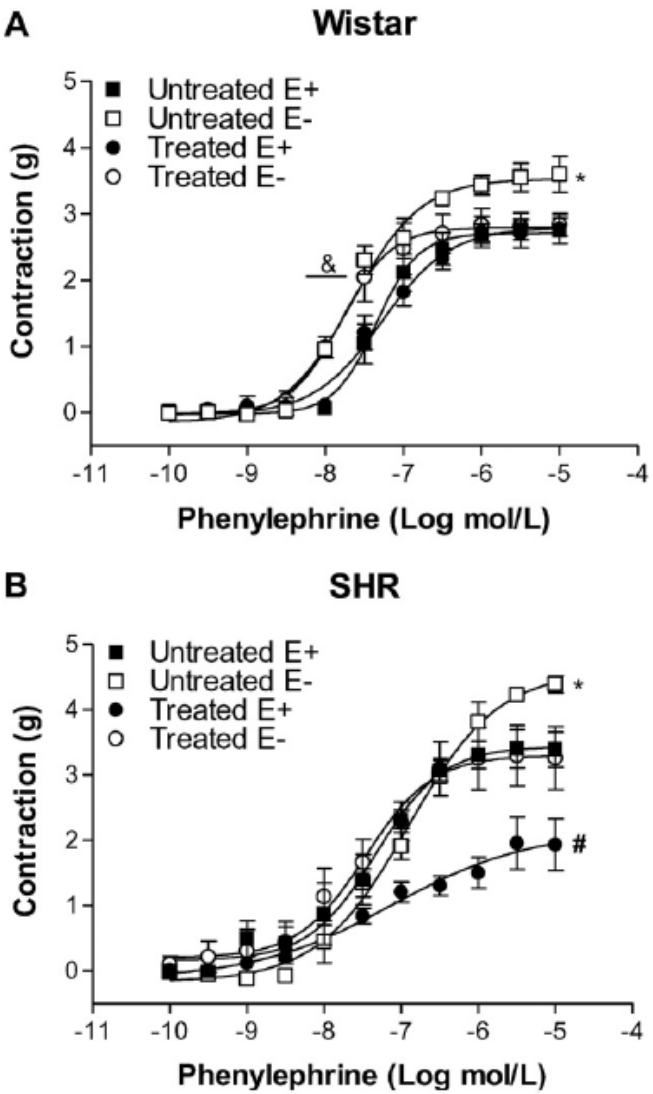


Fig. 7. Constrictor effect, in grams, of Phenylephrine (PE, 0.1 nmol/L–10 μmol/L) in denuded (E−) and intact (E+) endothelium aortic rings (black square indicates intact endothelium rings of untreated rats; white square indicates denuded rings of untreated rats; black circle indicates intact rings of treated rats; and, white circle indicates denuded rings of treated rats of (A) Wistar rats and (B) SHR. Values represent the mean ± SEM of the results, n = 4–6. $^{*}p < 0.05$ for Emax values of untreated E− *versus* other groups; $^{\&}p < 0.05$ for pD2 values of untreated and treated Wistar rats E− *versus* untreated and treated Wistar rat E+; $^{\#}p < 0.05$ for Emax values of treated SHR E+ *versus* other groups (ANOVA).

Perassa et al., 2016

Citometria de fluxo
Células endoteliais

[Ca++]

[NO]

[O2-]

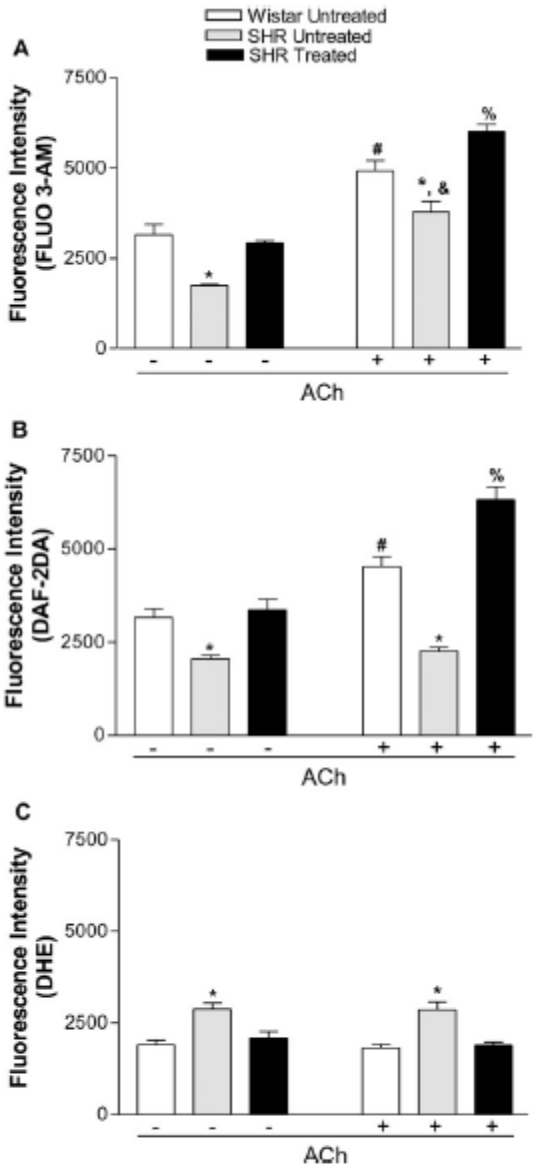
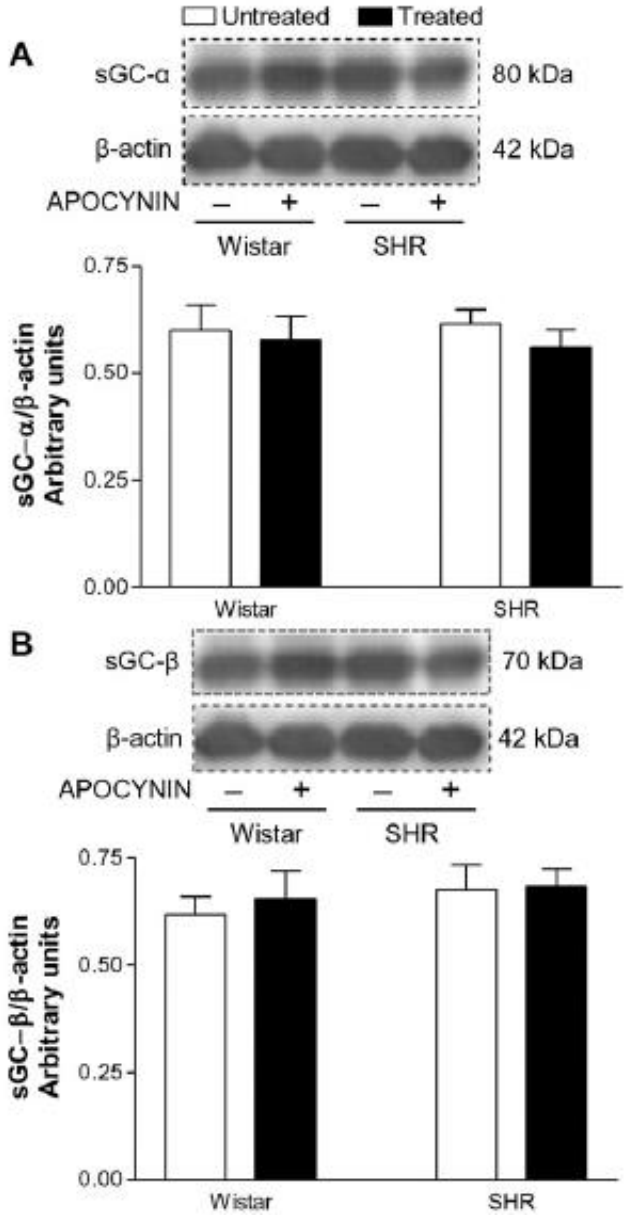


Fig. 8. Fluorescence intensity, in arbitrary units, of (A) FLUO 3-AM, (B) 4,5-diaminofluorescein diacetate (DAF 2-DA), and (C) dihydroethidium (DHE) in aortic endothelial cells of untreated Wistar rats (white bars), untreated SHR (gray bars), and treated SHR (black bars) in basal conditions and stimulated with acetylcholine (ACh, 1 μmol/L). Values represent the mean ± SEM of the results, n = 5. *p < 0.05 untreated SHR *versus* other groups; #p < 0.05 ACh stimulation *versus* basal in untreated Wistar rats; &p < 0.05 ACh stimulation *versus* basal in untreated SHR; %p < 0.05 ACh stimulation *versus* basal in treated SHR (ANOVA).

Perassa et al., 2016



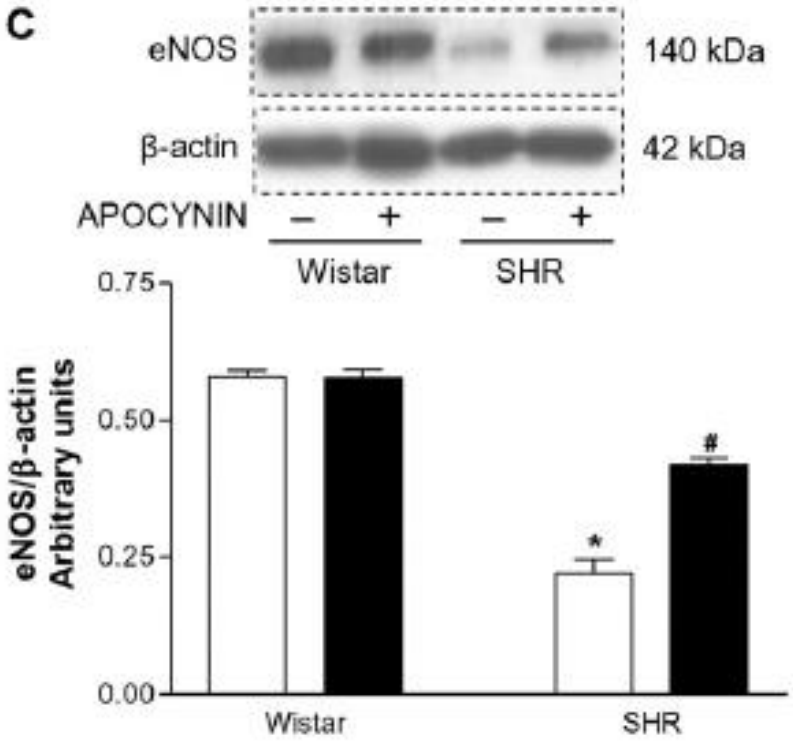


Fig. 9. Typical blots and protein expression, in arbitrary units, of (A) soluble guanylate cyclase alfa subunit (sGC-α), (B) soluble guanylate cyclase beta subunit (sGC-β), and (C) endothelial nitric oxide synthase (eNOS) in aortic homogenates of untreated (white bars) and treated (black bars) Wistar rats and SHR. Values represent the mean ± SEM of the results, n = 5. *p < 0.05 untreated SHR *versus* other groups; #p < 0.05 treated SHR *versus* other groups (ANOVA).

Perassa et al., 2016

Homogenato de aorta

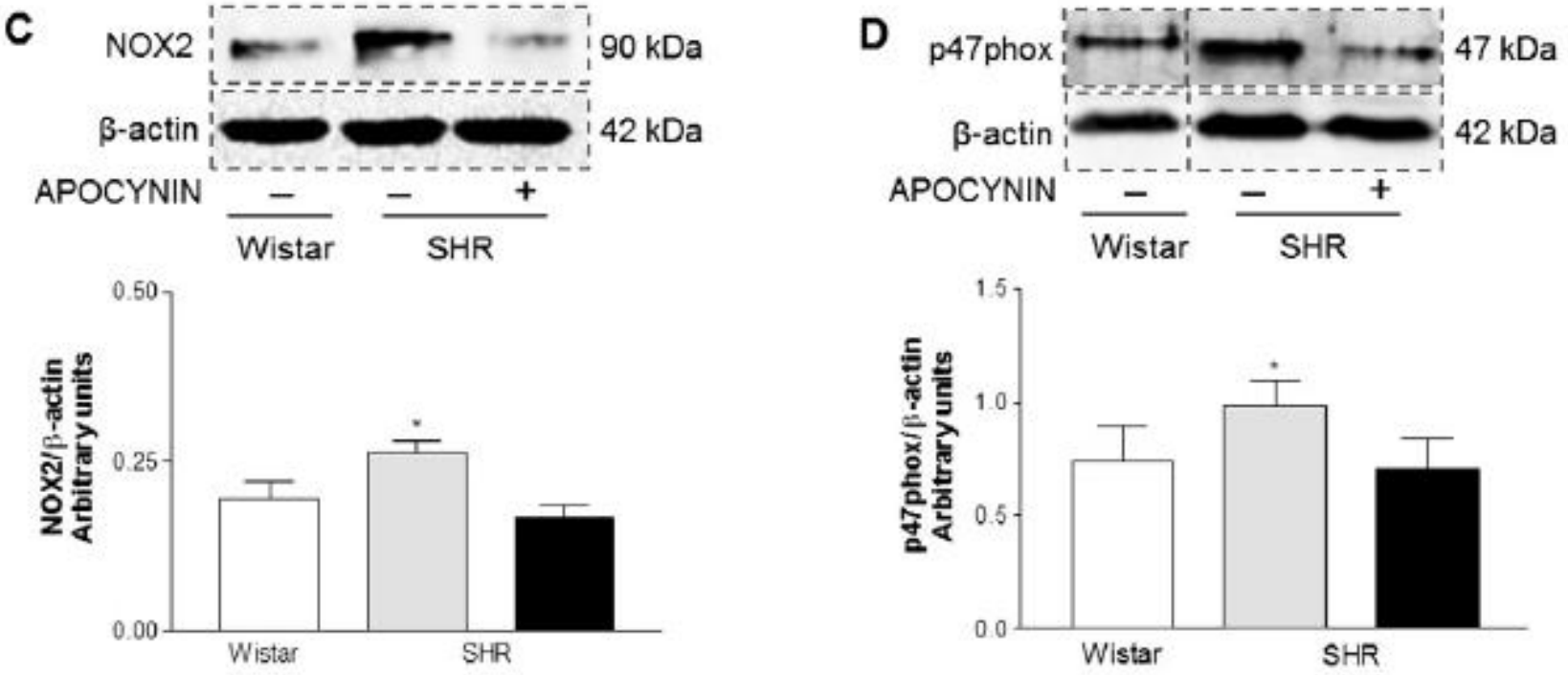


Fig. 10. Typical blots and protein expression, in arbitrary units, of (A) NAD(P)H oxidase isoform 1 (NOX1), (B) NOX organizer 1 (NOXO-1), (C) NAD(P)H oxidase isoform 2 (NOX2), (D) NOX organizer 2 (p47phox), and (E) NAD(P)H oxidase isoform 4 (NOX4) in aortic homogenates of Wistar rats (white bars), untreated SHR (gray bars), and treated SHR (black bars). Values represent the mean ± SEM of the results. *$p < 0.05$ untreated SHR *versus* other groups (ANOVA).

Adaptada de Perassa et al., 2016

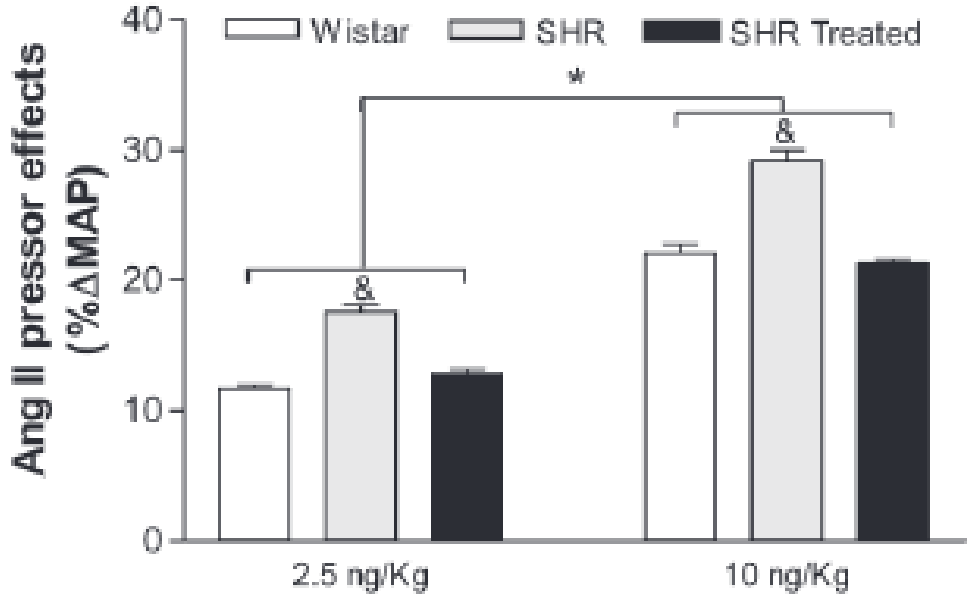


Fig. 2. Percentage of variation of Mean Arterial Pressure (%ΔMAP) of Wistar rats (white bars), SHR (gray bars), and SHR treated with apocynin (30 mg/kg/day) (black bars) after *in bolus* administration of angiotensin (Ang) II at doses of 2.5 and 10 ng/kg. Values represent the mean ± SEM of the results, n = 4–5. $^{\&}$ $p < 0.05$ SHR *versus* other groups, $^{*}p < 0.05$ both tested doses.

Graton et al., 2019

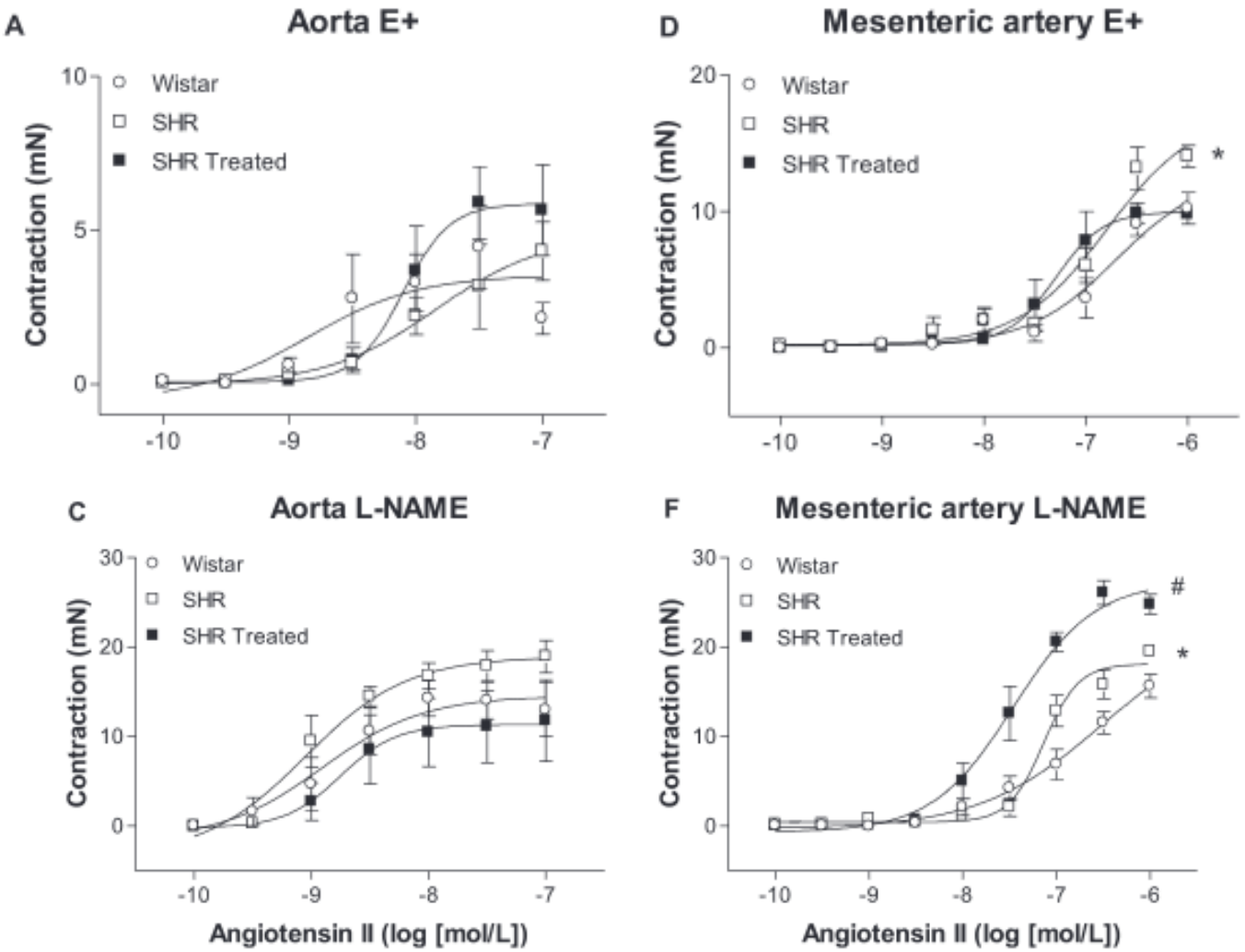


Fig. 9. Concentration-response curves to angiotensin II (0.1 nmol/L – 0.1 or 1 μmol/L) in aortic (A, B, and C) and mesenteric rings (D, E and F) of Wistar rats (white circles), SHR (white squares), and SHR treated with apocynin (30 mg/Kg/day) (black squares), in the presence (E +) or absence (E-) of endothelium or in the presence of L-NAME 100 μmol/L. Values represent the mean ± SEM of the results, n = 4–5. *p < 0.05 Emax values of SHR *versus* other groups, #p < 0.05 Emax values of SHR Treated *versus* other groups, &p < 0.05 Emax values of SHR and SHR Treated *versus* Wistar rats.

Adaptada de Graton et al., 2019

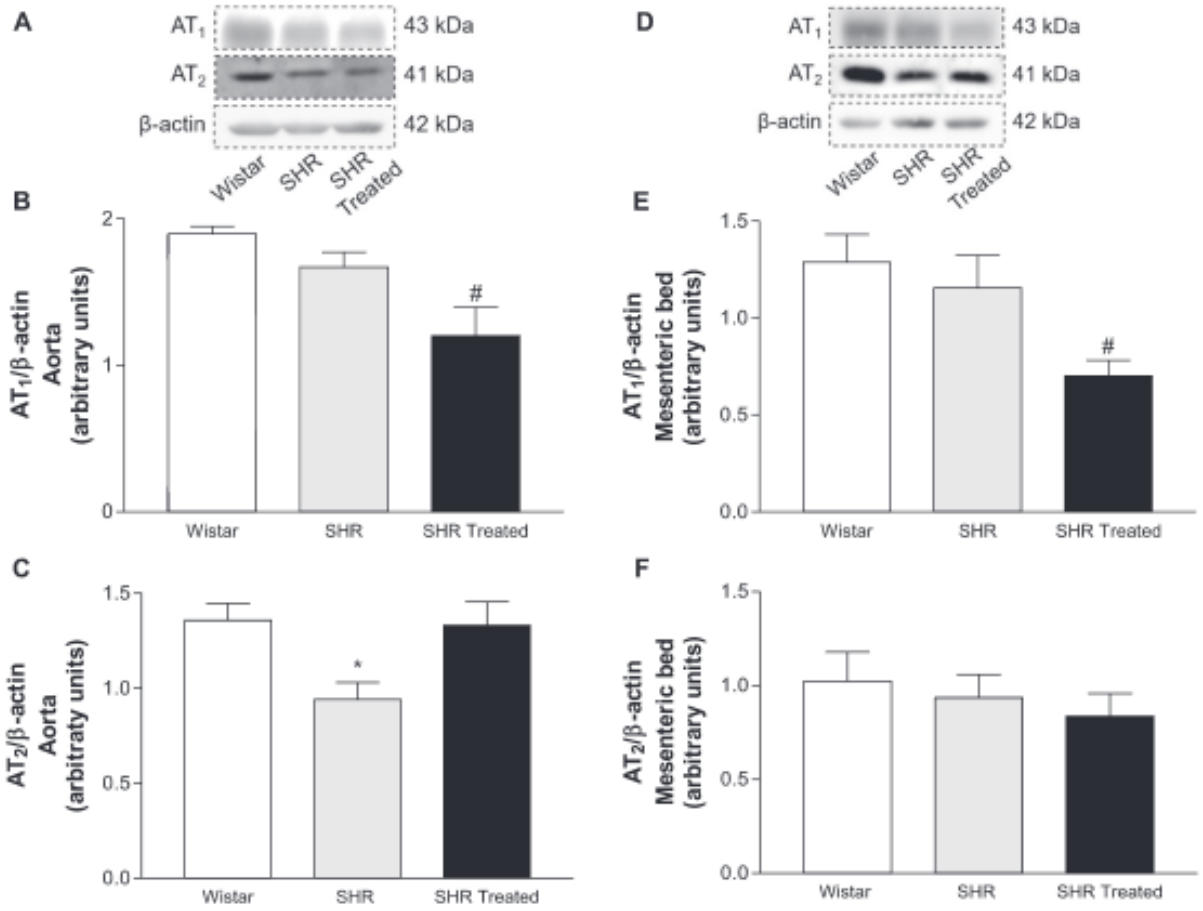


Fig. 8. Typical blots and protein expression, in arbitrary units, of angiotensin II type 1 (AT_1) and type 2 (AT_2) receptors in aortic (A, B and C) and mesenteric bed (D, E and F) of Wistar rats (white bars), SHR (gray bars) and SHR treated with apocynin (30 mg/Kg/day) (black bars). Values represent the mean ± SEM of the results, n = 5–6. $^{\#}p < 0.05$ SHR Treated *versus* Wistar rats, $^{*}p < 0.05$ SHR *versus* other groups.

Graton et al., 2019

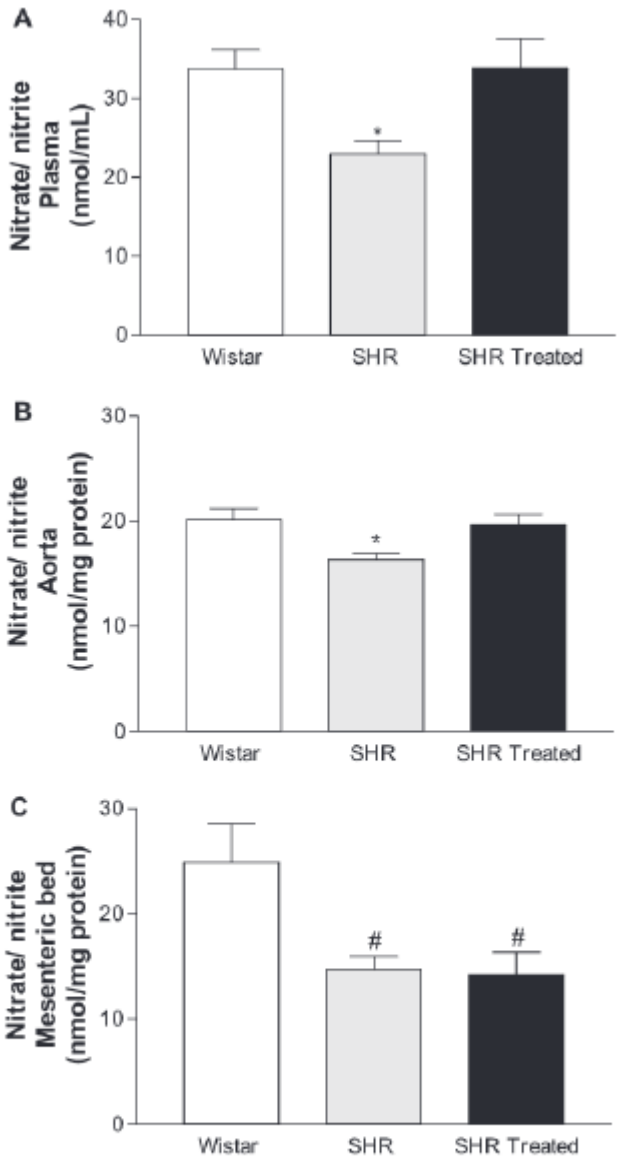


Fig. 6. Nitrate/nitrite levels in plasma (A), in nmol/ mL, aortic (B), and mesenteric bed (C) homogenates, in nmol/mg protein, of Wistar rats (white bars), SHR (gray bars), and SHR Treated (black bars). Values represent the mean ± SEM of the results, n = 8–10. *p < 0.05 SHR *versus* other groups, #p < 0.05 SHR or SHR Treated *versus* Wistar rats.

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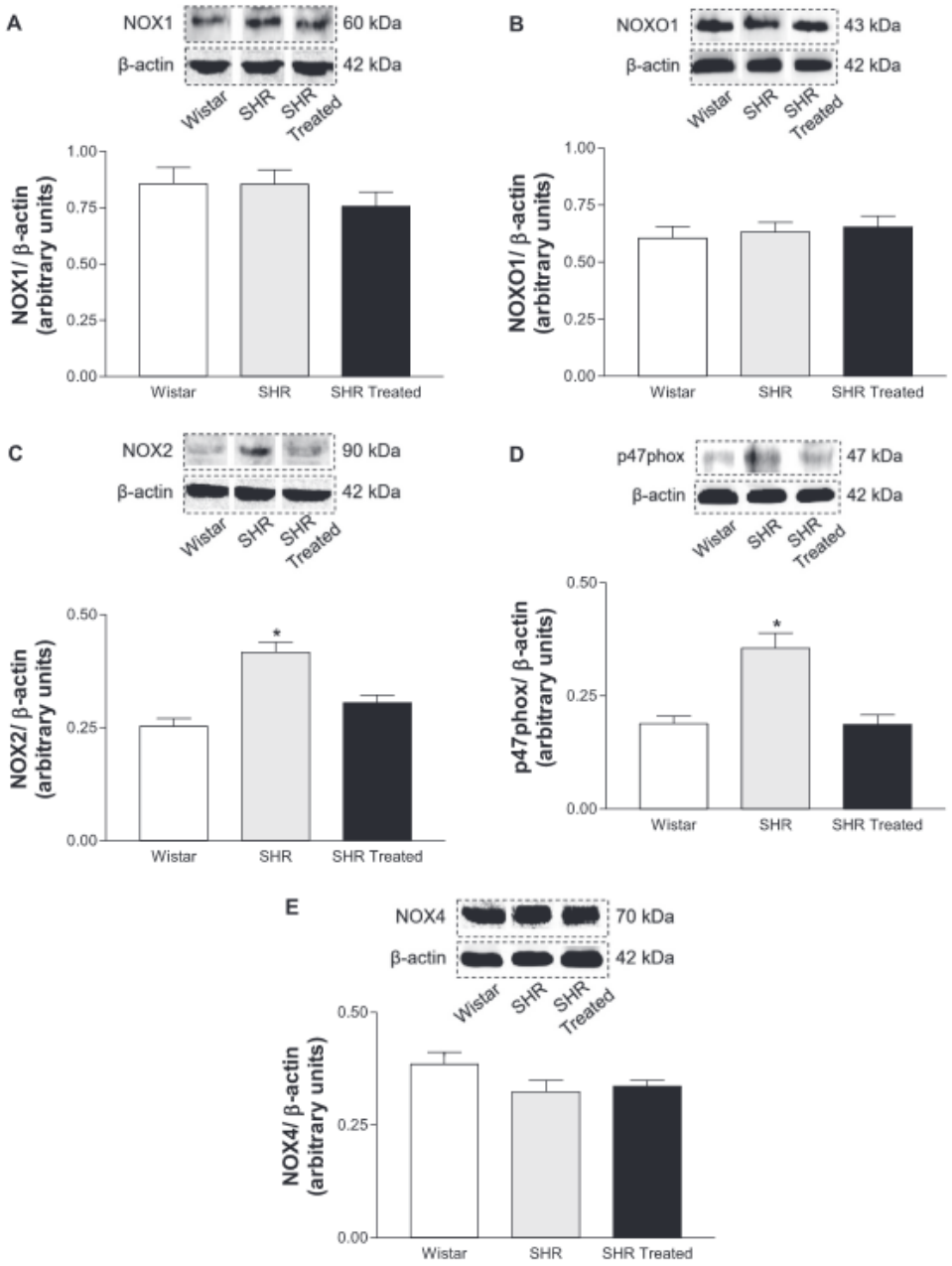

Homogenato de leito mesenterico

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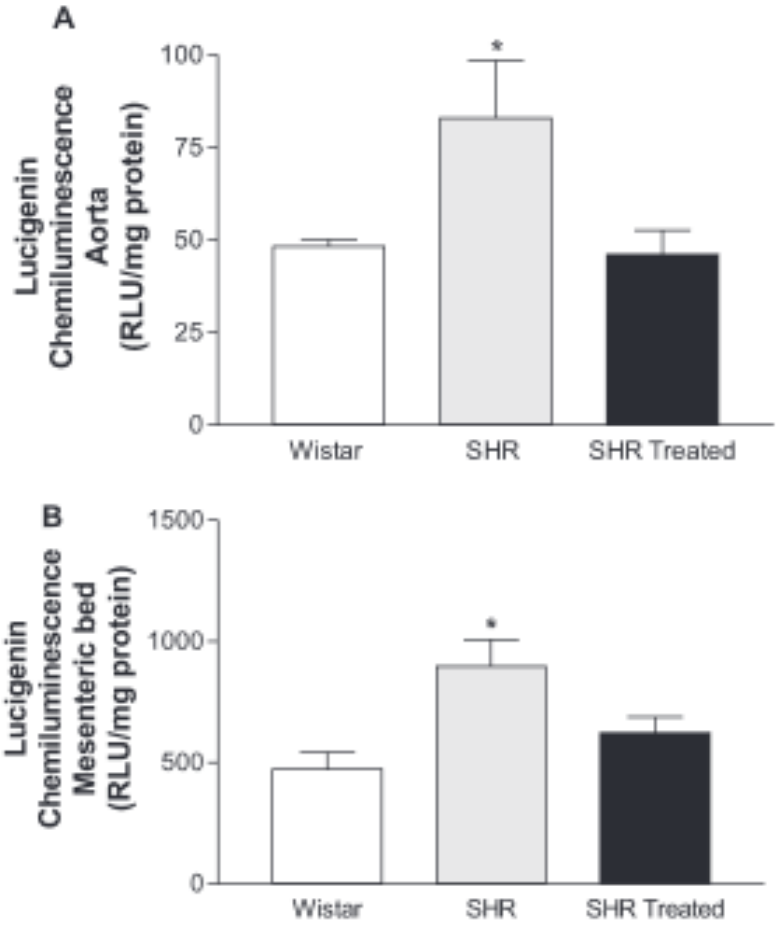

Fig. 4. NOX-dependent oxidants production detected by lucigenin chemiluminescence, in RLU/mg of protein, in aortic (A) and mesenteric bed (B) homogenates of Wistar rats (white bars), SHR (gray bars), and SHR treated with apocynin (30 mg/Kg/day) (black bars). Values represent the mean ± SEM of the results, n = 5–7. *p $<$ 0.05 SHR *versus* other groups.

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