Moduladores da Inflamação

Celsius, sec I D.C.

Rubor et tumor cum calore et dolore

Primeira documentação dos sinais típicos da inflamação

Importância da observação clínica
em vez de medicina baseada em filosofia

Virchow, 1871

The inflamatory reaction is consequence of an excessive intake by intersticial cells, of blood...filtering through the vessel wall

Inflamação é decorrente de um processo de proliferação patológica das células devido ao extravasamento de nutrientes dos vasos.

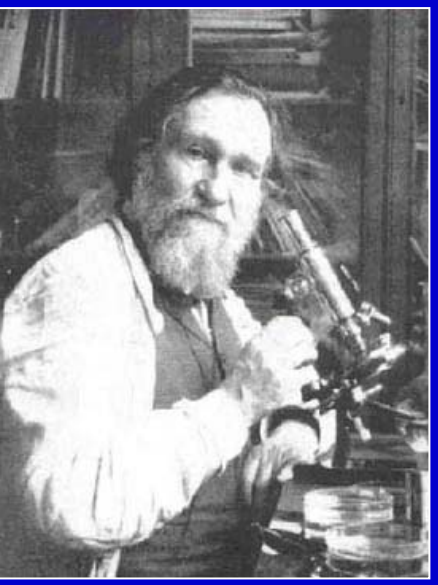
Envolvimento das células

Cohnheim, 1873

Finally...there lies outside the vessel ...a colourless blood corpuscle

Corpúsculos sanguíneos eram vistos como um mecanismo patológico pelo qual a infecção se espraiava como conseqüência da injúria vascular.

Descrição pioneira da diapedese



Metchnikoff, 1908

The primum movens of the inflammatory reaction is a digestive action...toward the noxious agent

Inflamação como mecanismo celular de defesa, direcionada pelos vasos em vez de um aspecto da patologia por si só.

Fagócitos são protetores e não patogênicos

Inflammation as a triplice response to injury

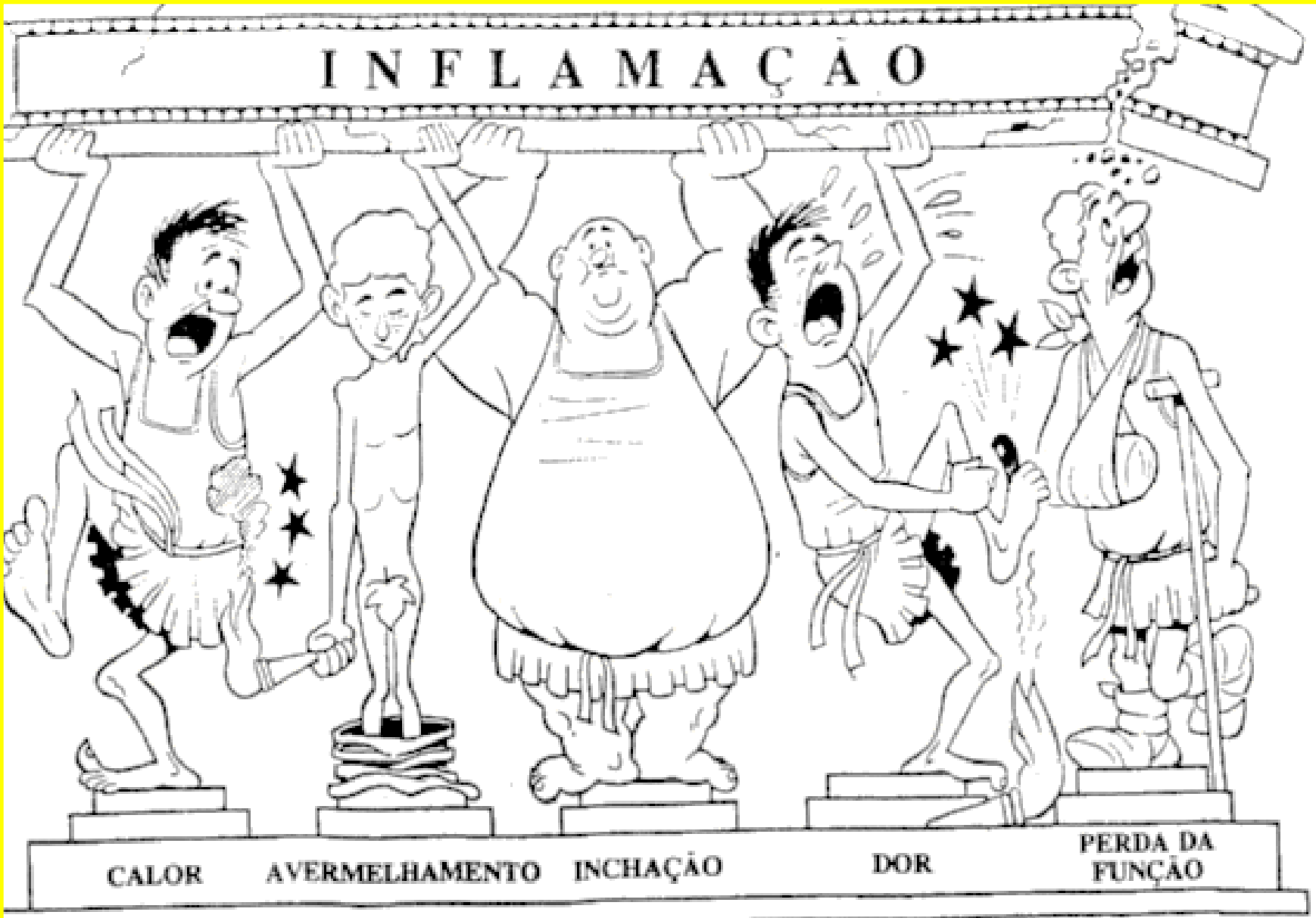
Lewis, 1927

Inflamação se caracteriza por eventos vasculares; mediada por agentes químicos e por axônios

Reconhecimento da inflamação neurogênica

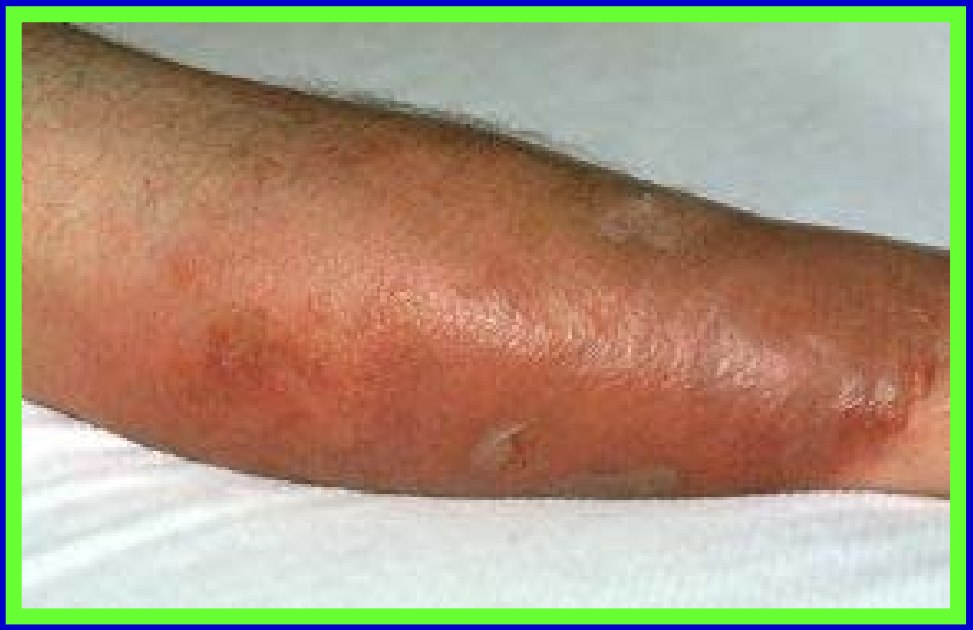
Rocha e Silva, 1974

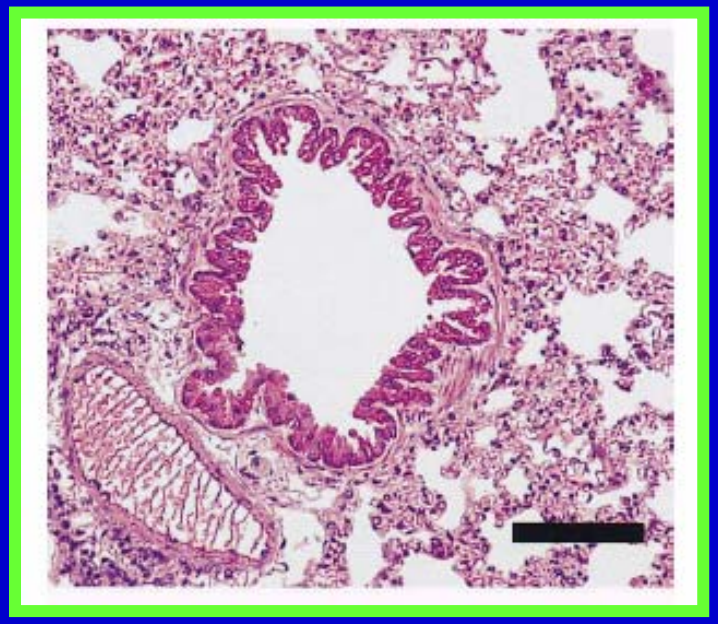
Inflammation as a multimedated phenomenon, of a pattern type in which all mediators would come and g at the appropriate moment....increasing vascular permeability, attracting leucocytes, producing pain, local edema and necrosis.


INFLAMAÇÃO
CALOR
AVERMELHAMENTO
INCHAÇÃO
DOR
PERDA DA FUNÇÃO

INFLAMAÇÃO

Estereotipia
Mobilização
Substâncias endógenas





INFLAMAÇÃO
Aspectos positivos

Diluição da toxina

Acesso de anticorpos

Transporte de fármacos

Formação de fibrina

Aporte de nutrientes

Estimulação da resposta imune

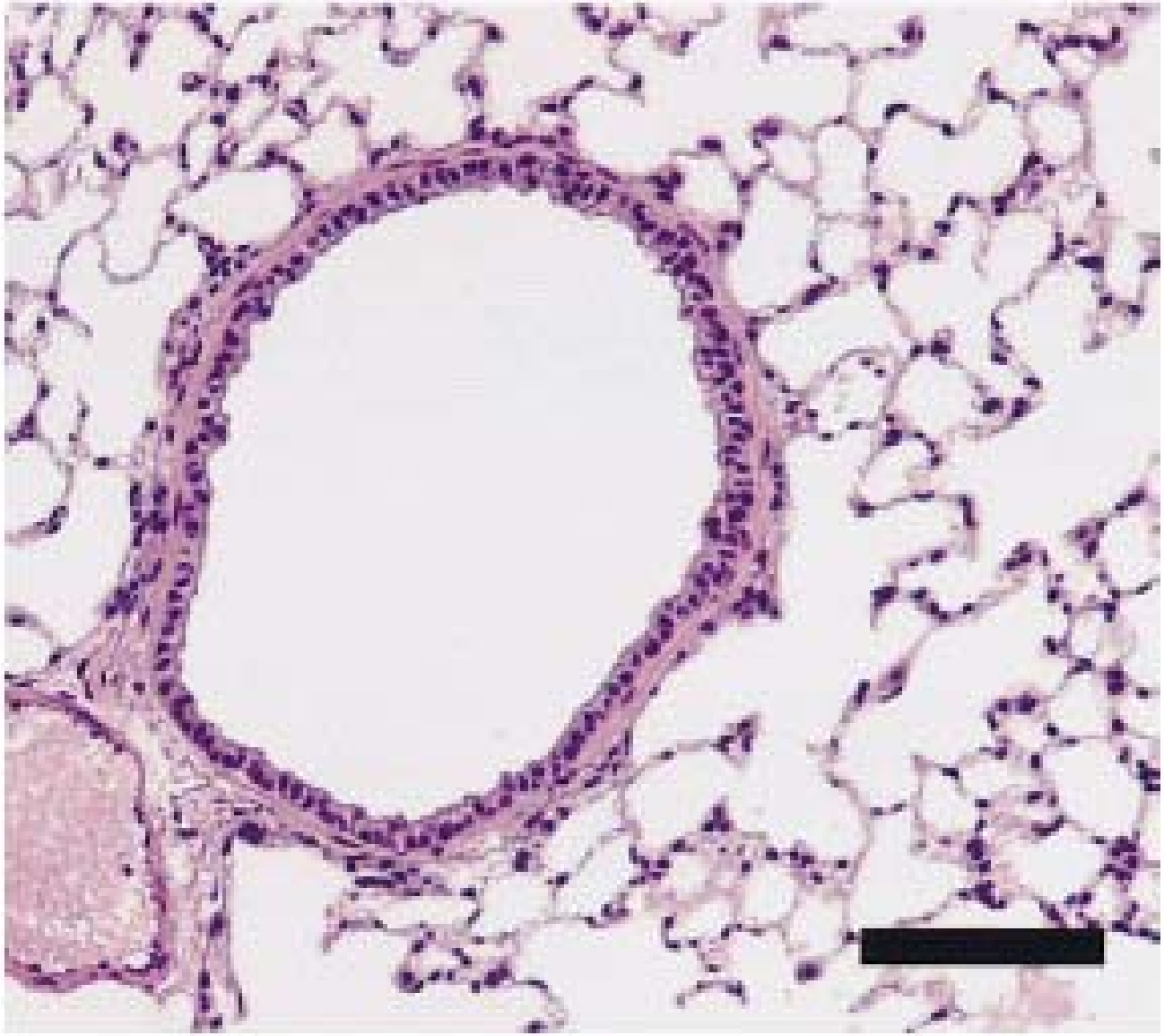
INFLAMAÇÃO
Aspectos negativos

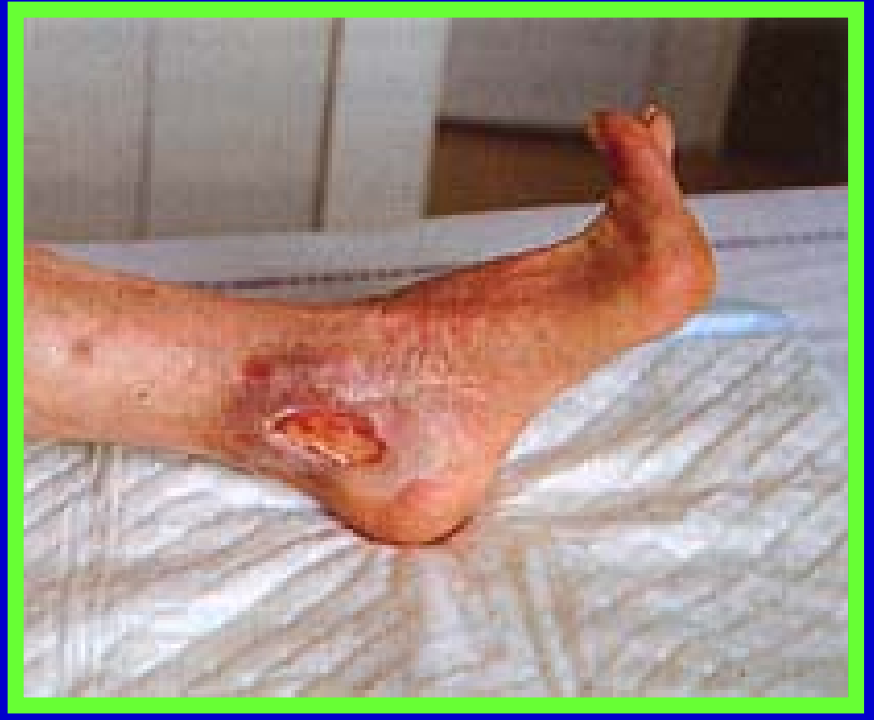
Lise e morte de tecidos sadios

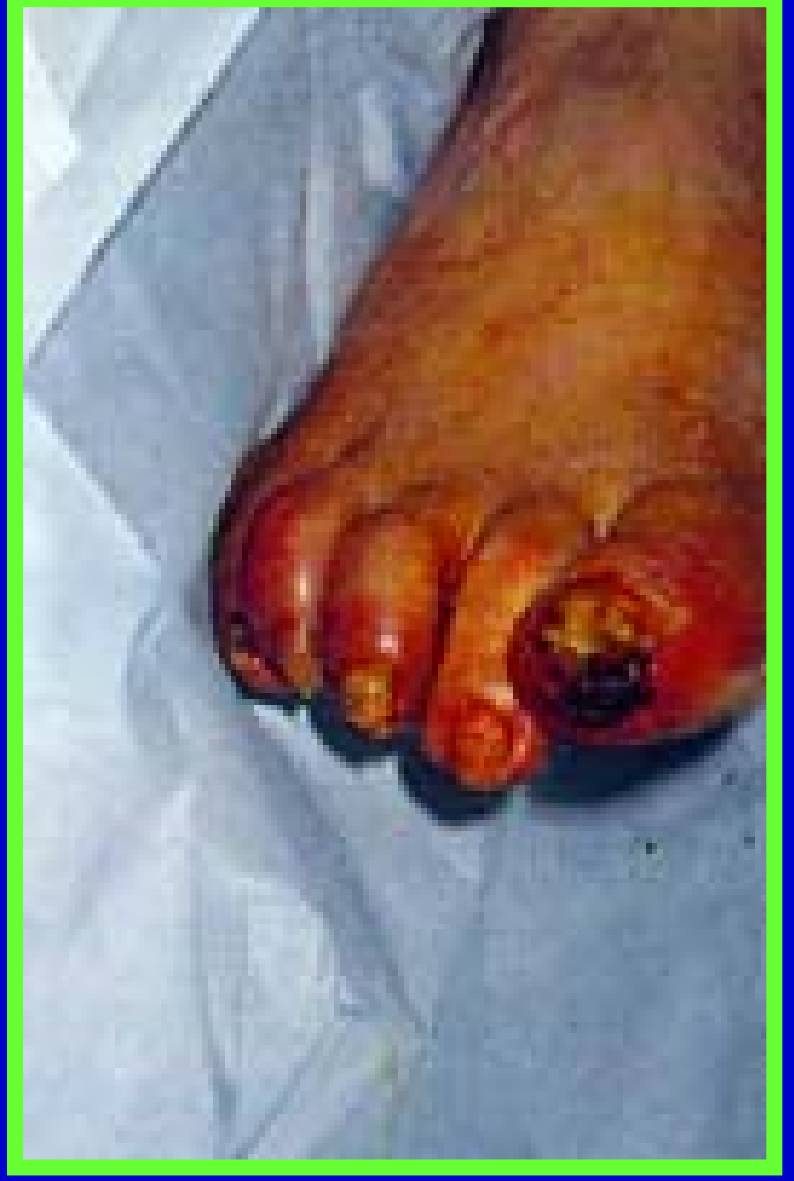
Edema (aumento de pressão)

Resposta inaproriada

Hipersensibilidade







Estímulo

↓

Molecular (Regulação de moléculas sinalizadoras e de enzimas)

Celular

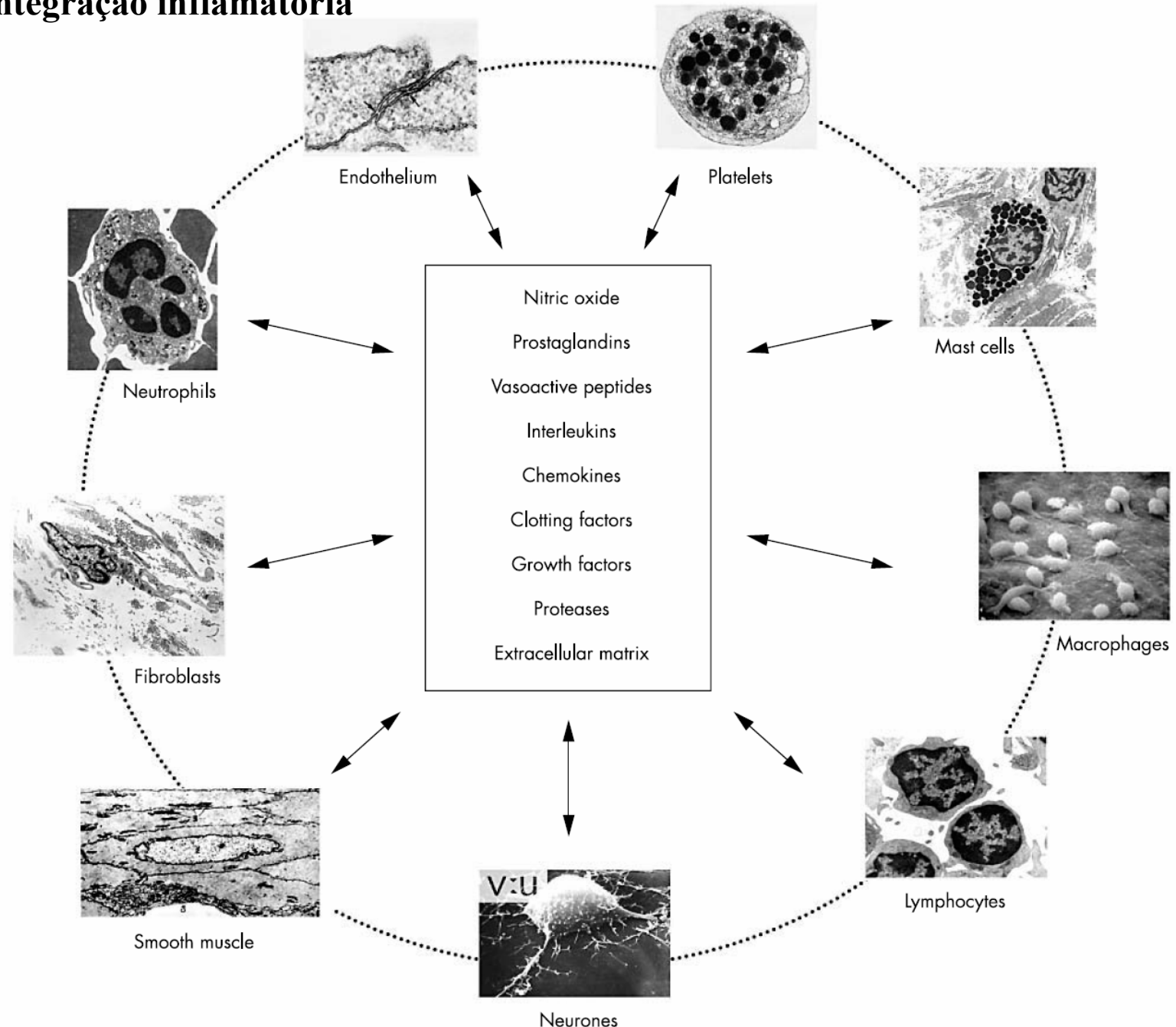
↓

Fisiológico

- Coagulação
- Metabolismo
- Vasodilatação
- Alteração de matriz extracelular
- Angiogênese
- Vasopermeação
- Atividade neuronal
- Espasmo muscular

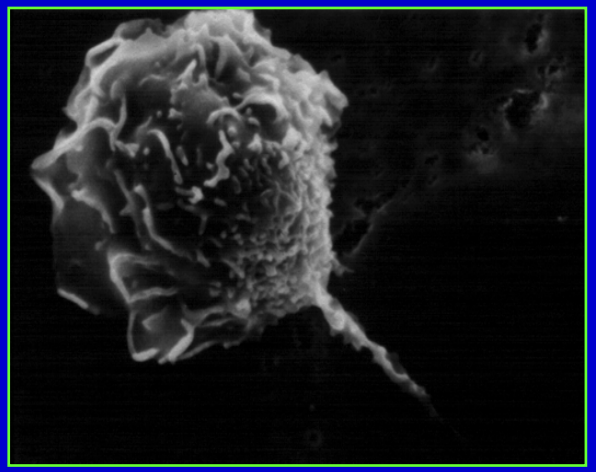
Dor – Calor - Rubor - Tumor

Integração inflamatória



In vivo rolling can be modeled ex vivo

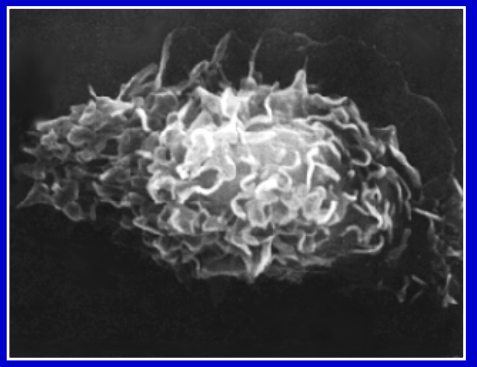
Inflammation starts with activated endothelium



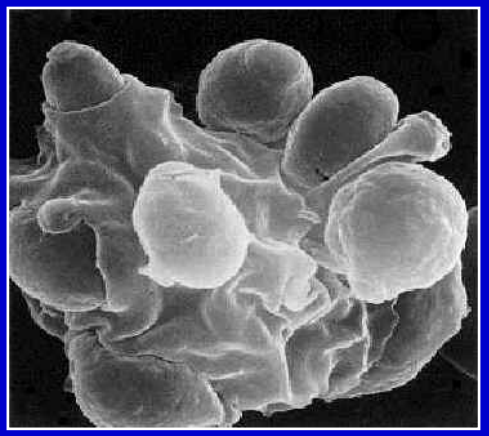
Control **Inflamed EC**

Zimmerman, McIntyre, Prescott. JCI 1985; 2235

Mastócito



Macrófago



Neutrófilo

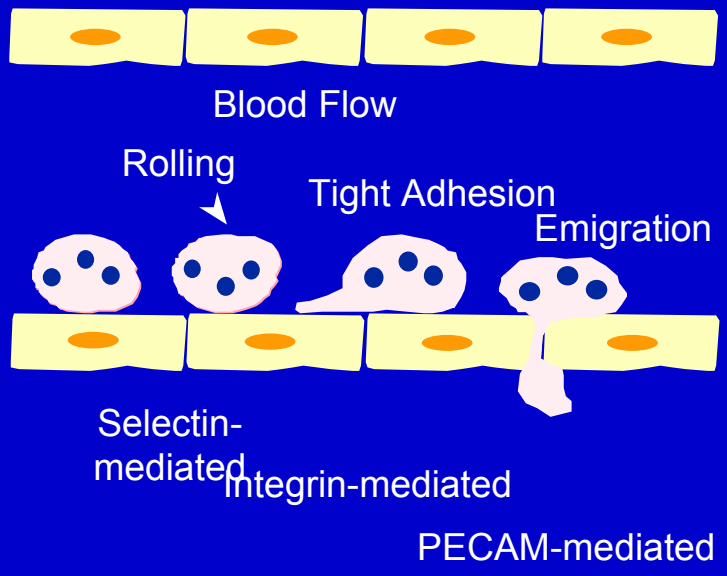
Leukocytes roll in vivo

Yang et al, J Exp Med
1999 Dec
20;190(12):1769-82

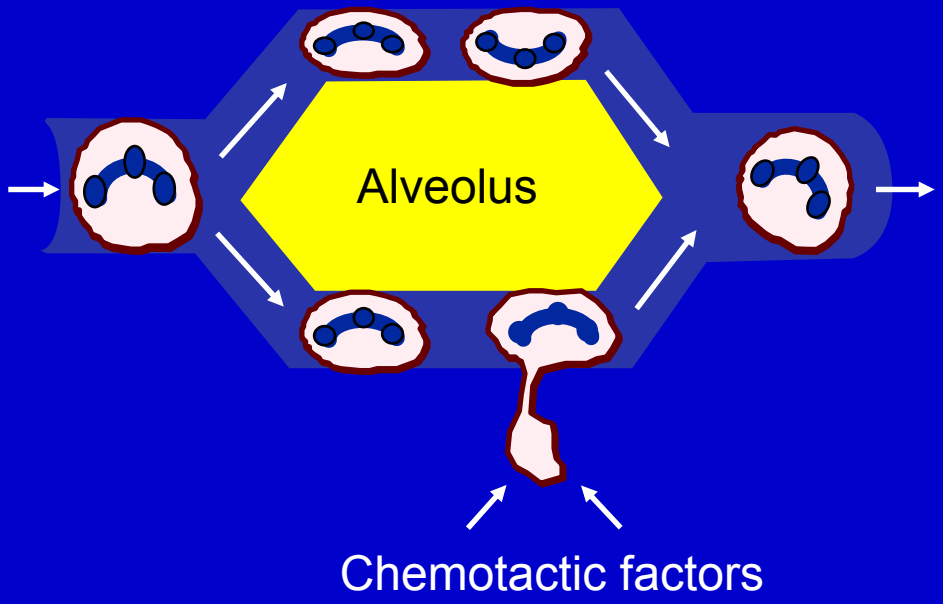
Intravital microscopy of cremaster muscle venules within 30 min of surgery

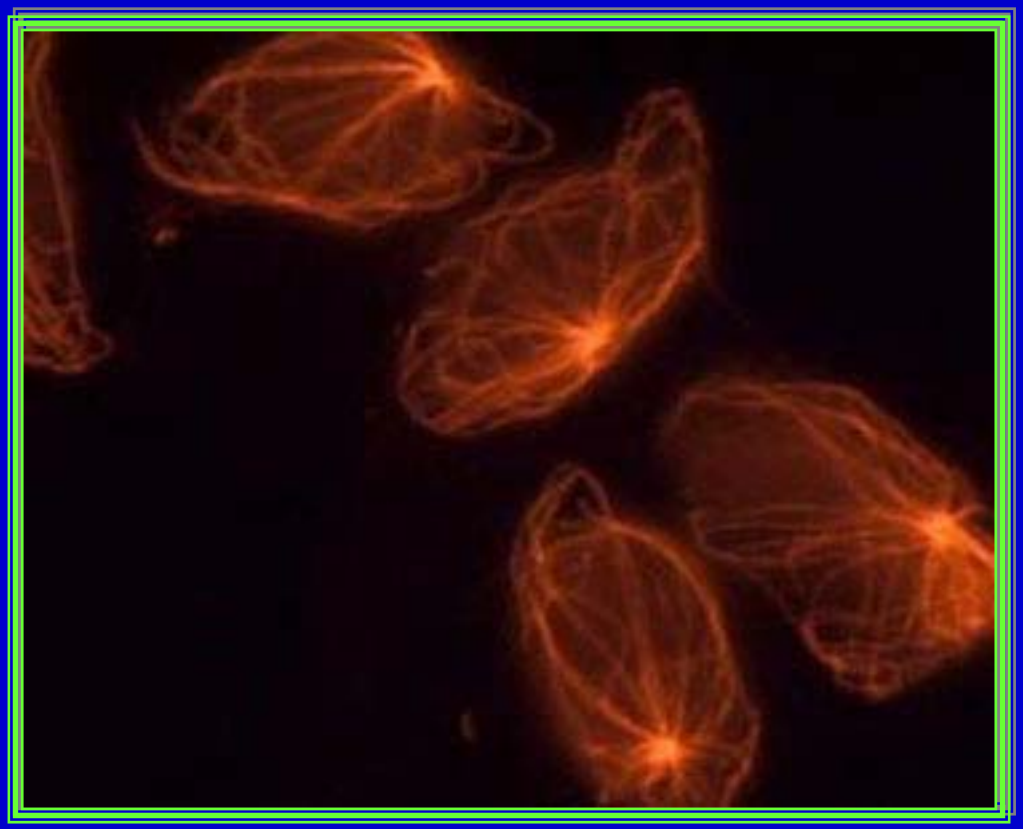
PMN Targeting in Acute Inflammation: Variations in Different Vascular Beds

Systemic Venule



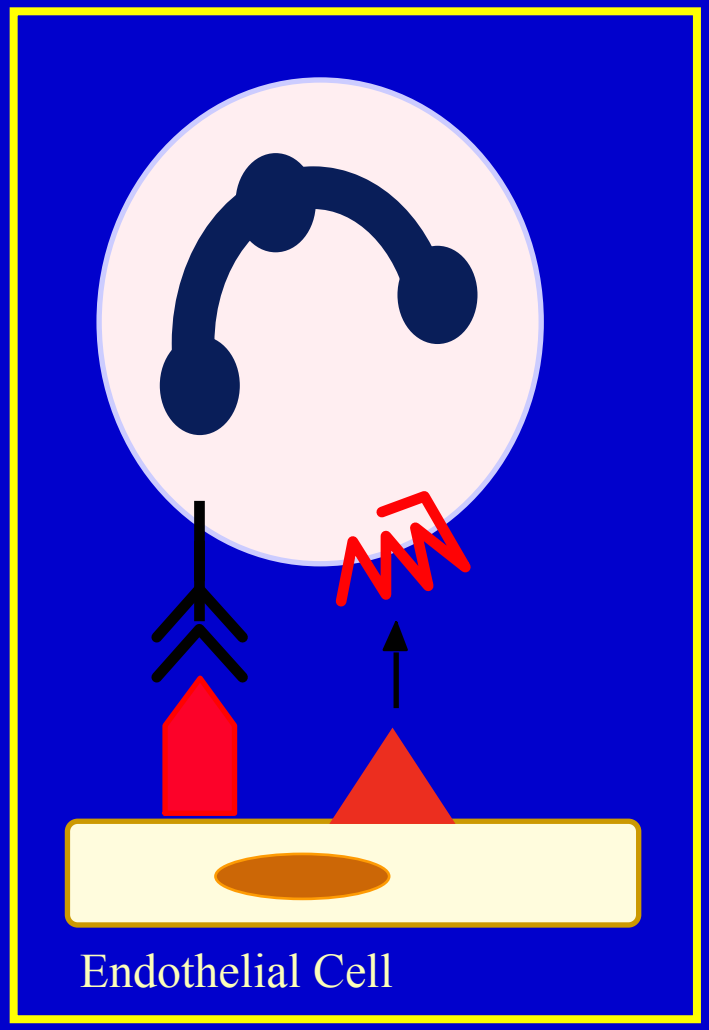
Pulmonary Capillary Bed





Inicio da quimiotaxia

Interação leucócito – endotélio



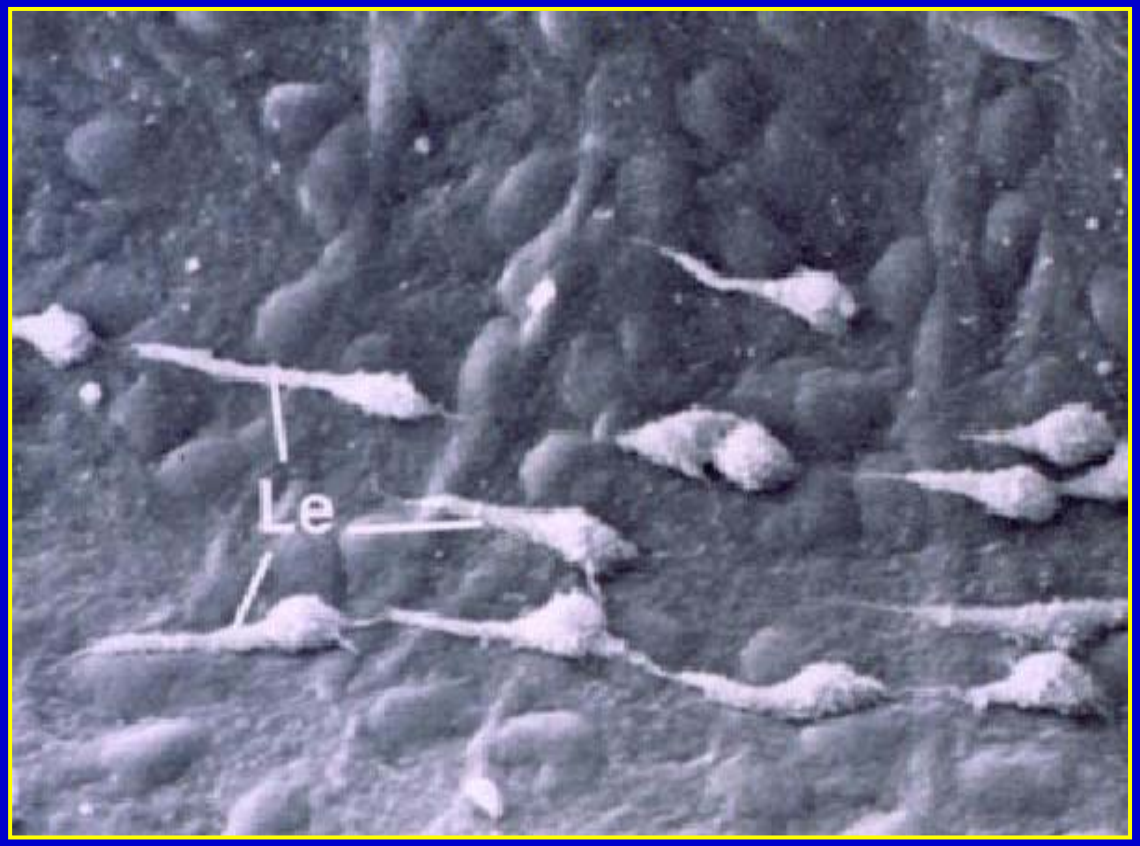
Tethering molecule

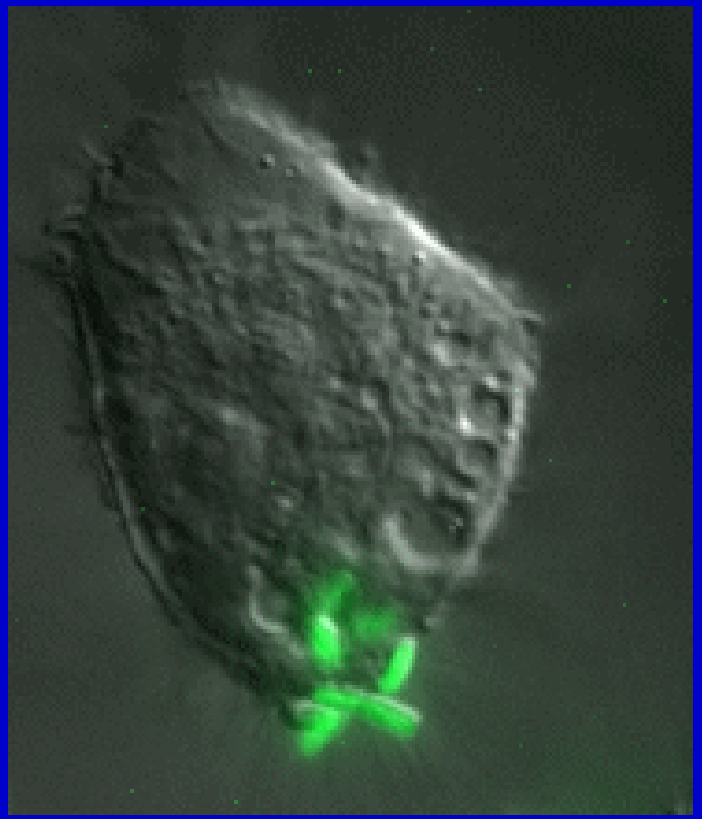
Ligand for tethering molecule

Signaling molecule

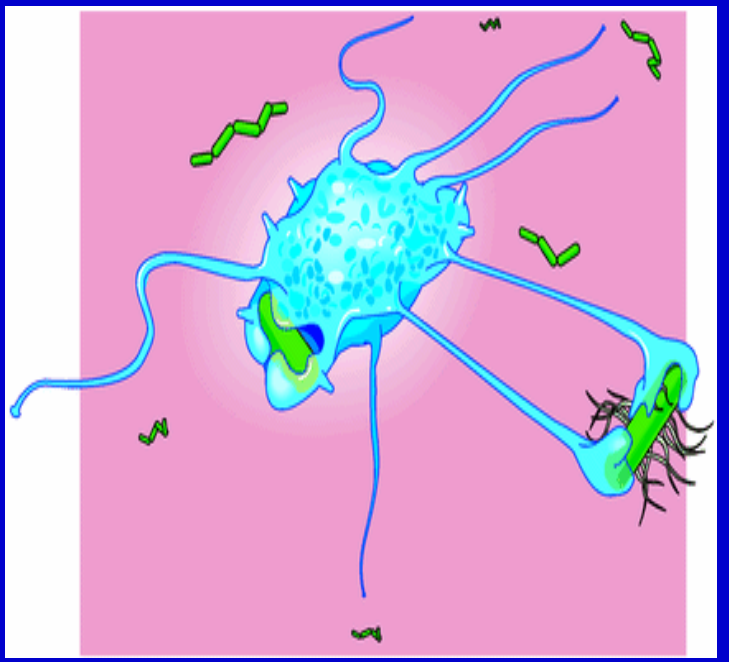
Receptor for signaling molecule

Preparação do recrutamento celular





Fagocitose



Moduladores

Mediadores (Dale, 1933)

1. Mimetização dos sinais da inflamação
2. Sintetizado durante a inflamação
3. Existência de sistemas de síntese e captação
4. Existência de enzimas catalíticas
5. Modulação farmacológica deve mudar a inflamação
6. Sua elevação ou redução deve mudar a inflamação
7. Existência de receptores.

Histamina


Nordsee-
Krabben
Lachs-
CAVIAR


PALMADA

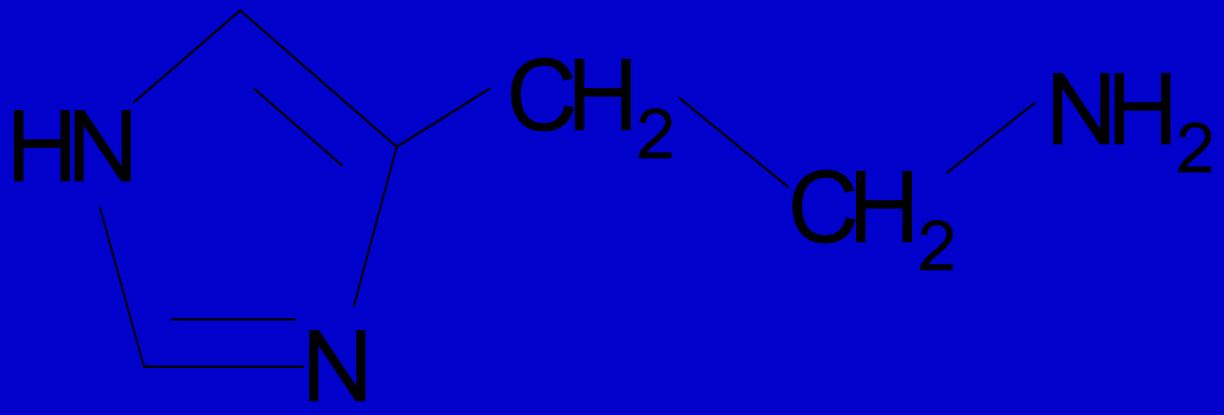
Histamina “in nature”

- **Presence - ubiquitous**
  - **plants**
  - **venoms and stings**
  - **synthesized by bacteria and certain fungi**
  - **found in human tissue**

Distribuição -Histamina

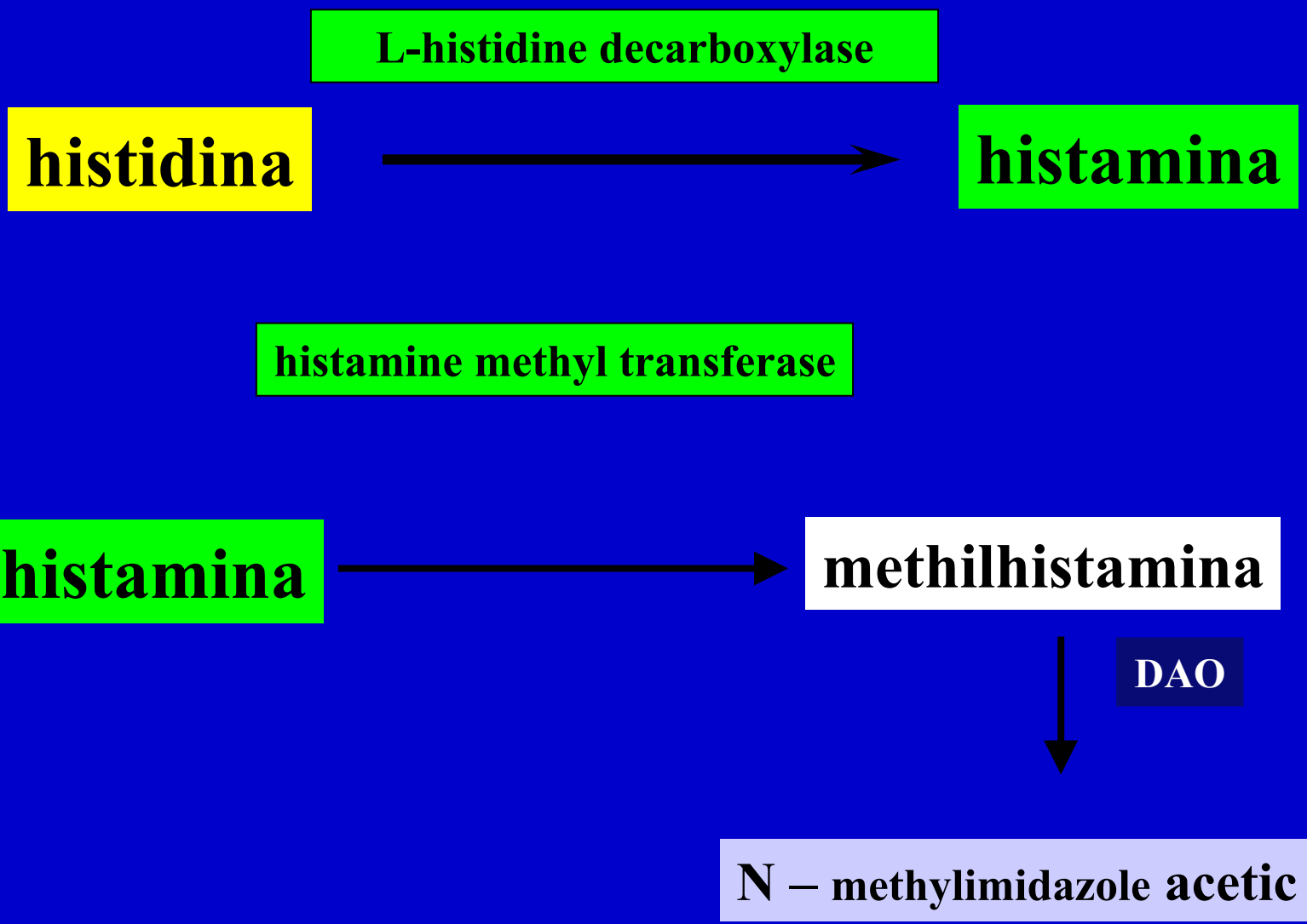
- **unevenly distributed throughout the body**
- **found in various cell types**
  - **mast cells**
  - **basophils - circulating counterpart of the mast cell**
  - **neurons – eg. CNS**
  - **enterochromaffin-like cells (ELC's) in the stomach**
- **tissues**
  - **skin**
  - **lung**
  - **gastrointestinal tract**

Histamina



Aminoethylimidazole

Síntese e Metabolismo



Receptores de Histamina

- types
  - H-1
  - H-2
  - H-3
- histamine stimulates all three
- certain drugs selectively stimulate and block these receptors

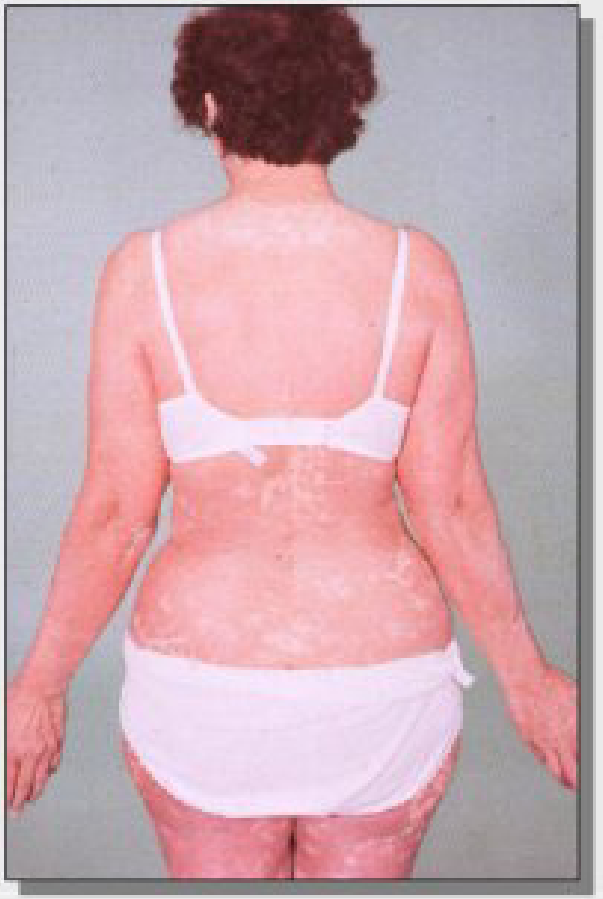
Histamina - Efeitos

- **H-1: GI and bronchial smooth muscle contraction**
- **H-2: cardiac stimulation, gastric secretion**
- **H-1 and H-2: dilation of arterioles and veins**
- **H-3:**
  - **Mainly in the CNS**
  - **Preterminal and autoreceptors**

Many organ are be affected by "allergy"

The skin

Urticaria
(hives)

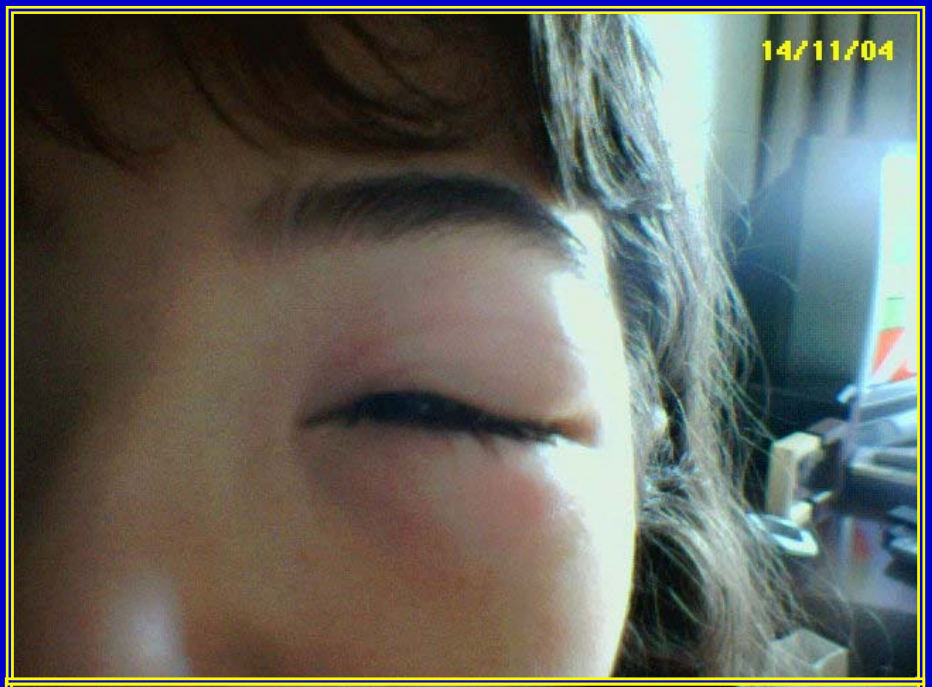


Ativação Seletiva – Bloqueio

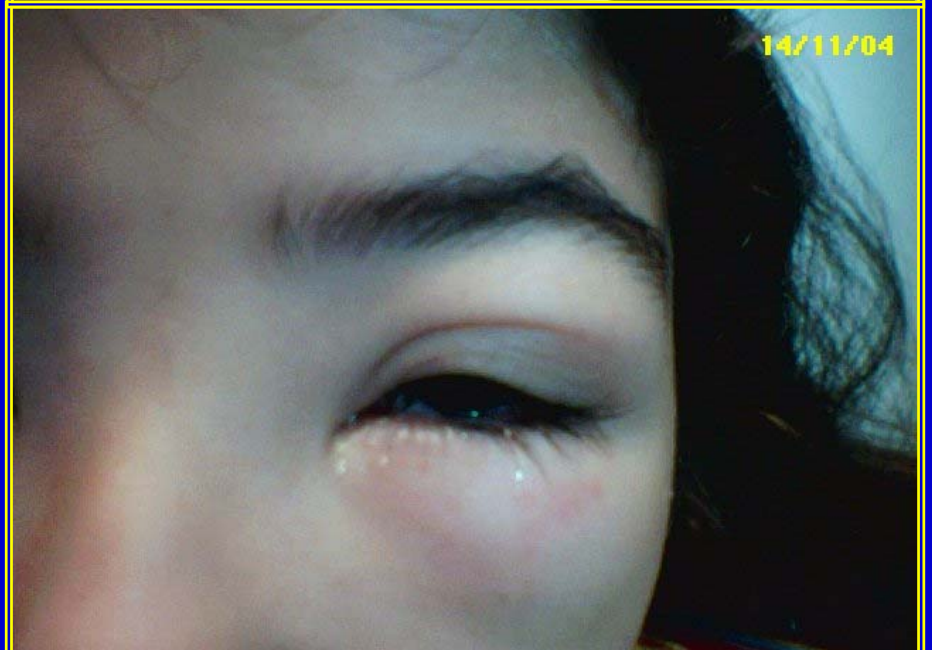
- **Ativação**
  - **H-1 agonists: 2-methylhistamine, betahistine**
  - **H-2 agonist: 4-methylhistamine**
- **Antagonismo**
  - **H-1 antagonists: pyrilamine**
  - **H-2 antagonists: cimetidine**

Hipersensibilidade Imediata (Tipo I)

Hidrocortisona
Anti-histaminico



12:30 hs

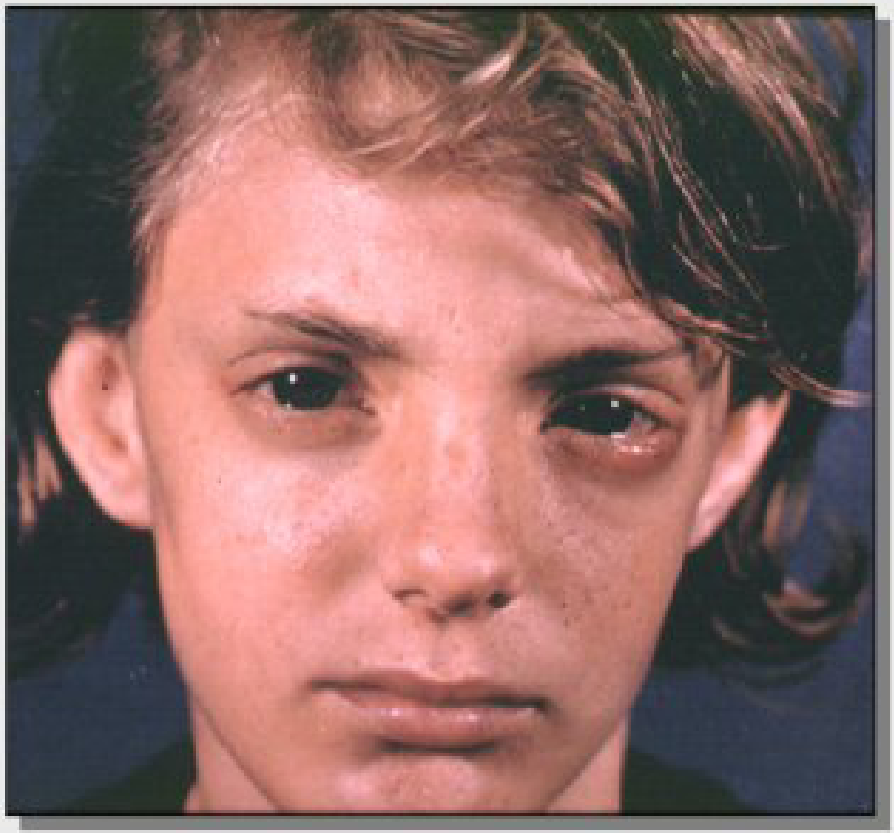


16:30 hs

Many organ are be affected by "allergy"

The eye

conjunctivitis



Efeitos- Cardiovasculares

- **Vasodilatation in the microcirculation – (H-1 & H-2 – receptors)**
- **Increased “post capillary venule" permeability: H-1 and perhaps H-2 receptors**

Efeitos- Cardiovasculares

- **Lowered systemic blood pressure**
- **Direct effects on the heart (complex)**
  - **H-2: positive inotropic and chronotropic**
  - **H-1: slowed AV conduction**
- **Indirect (reflex) effects on the heart**
  - **positive inotropic and chronotropic effect**
  - **due to decreased blood pressure**

Reação Tríplice

1. Redness at immediate injection site:

Direct vasodilation

2. Larger red area - called a flare:

Due to local axon reflex

3. Wheal: due to increased vessel permeability

Pain, itching (effects on nerves)

Efeitos

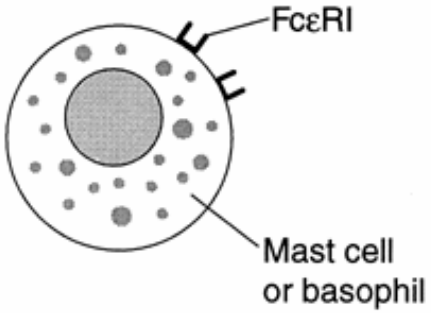
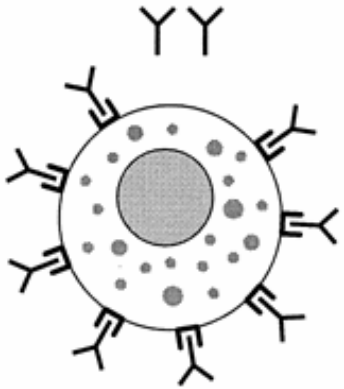
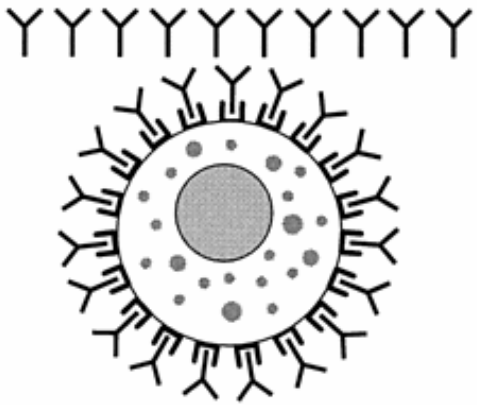
- **Extravascular smooth muscle**
  - **contraction of bronchial smooth muscle: H-1 mediated**
  - **contraction of GI smooth muscle: H-1 mediated**
- **increased gastric secretion: H-2 mediated**

Sumário

Bronchial contraction	**H-1**
GI contraction	**H-1**
Heart	**H-2, H-1 (AV node)**
Large artery contraction	**H-1**
Microvessel dilation	**H-1 & H-2**
Venule permeability	**H-1 & H-2 (?)**
Gastric acid secretion	**H-2**
CNS arousal	**H-1**

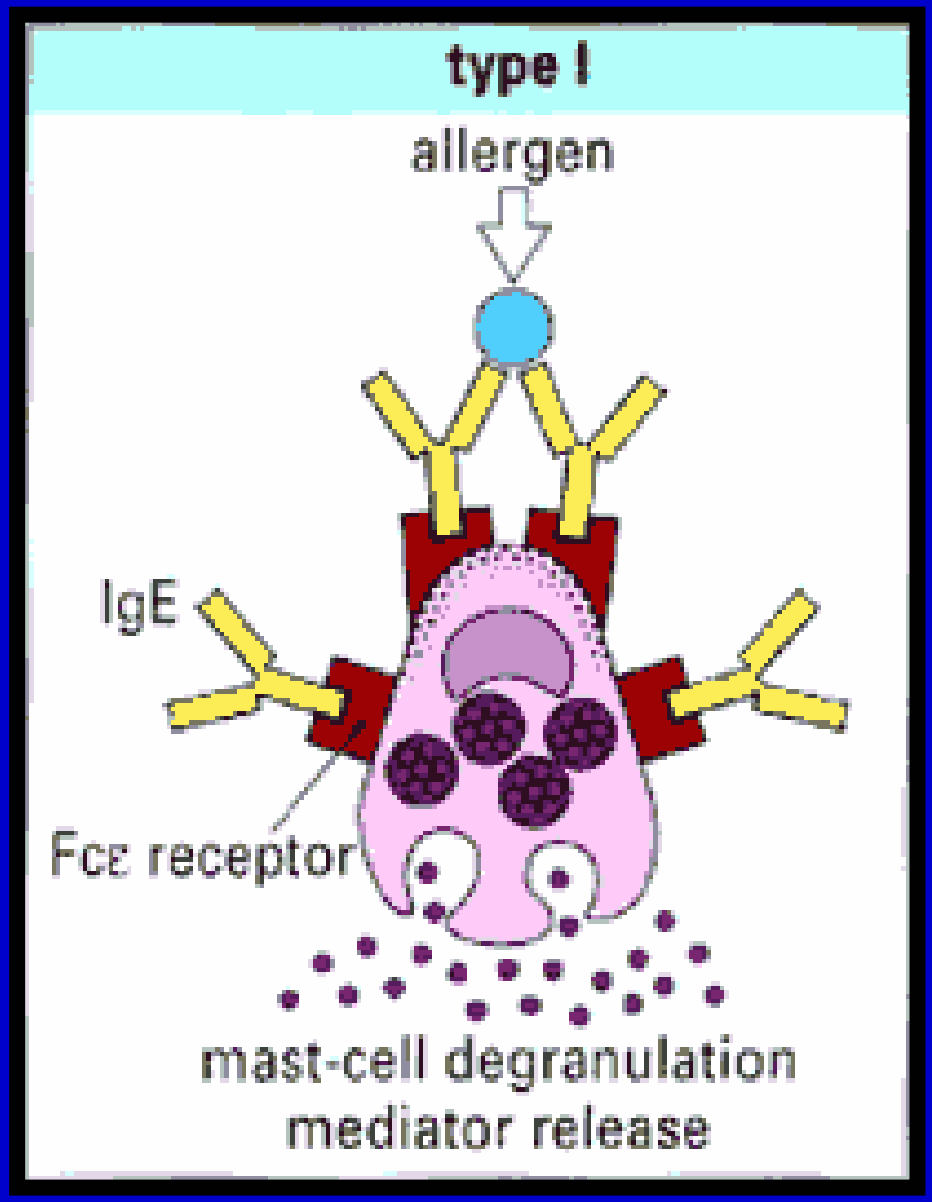
Liberação de Histamina

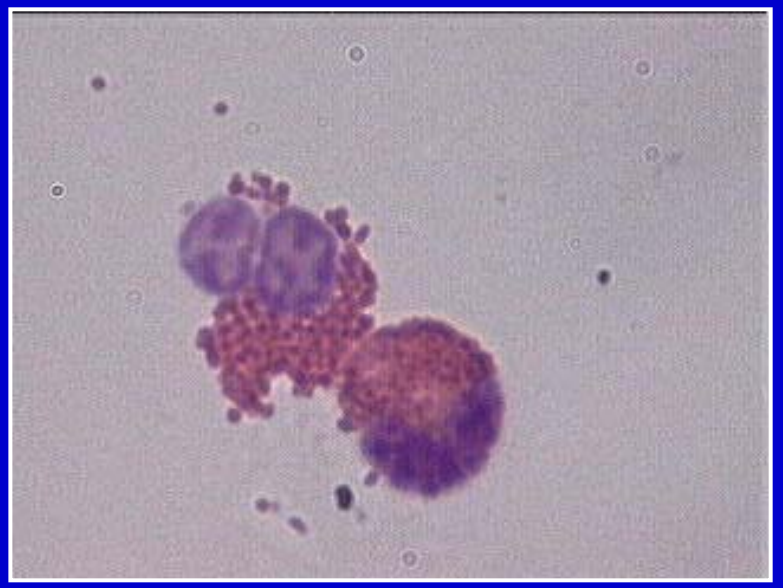
1. **Immunologic - Hypersensitivity reaction (IgE)**
2. **Pharmacological (direct release)**
   - **Morphine**
   - **Tubocurarine**
   - **Succinylcholine**
   - **Radiocontrast media**
   - **Carbohydrate plasma expanders**
3. **Physical stimuli – scratching the skin**

No IgE (in vitro or in IgE –/– mice)	Early phase of IgE-associated immune response	After persistent/repetitive allergen exposure
	Low levels of IgE 	High levels of IgE 
Low level surface expression of FcεRI	Enhanced surface expression of FcεRI	Greatly enhanced surface expression of FcεRI

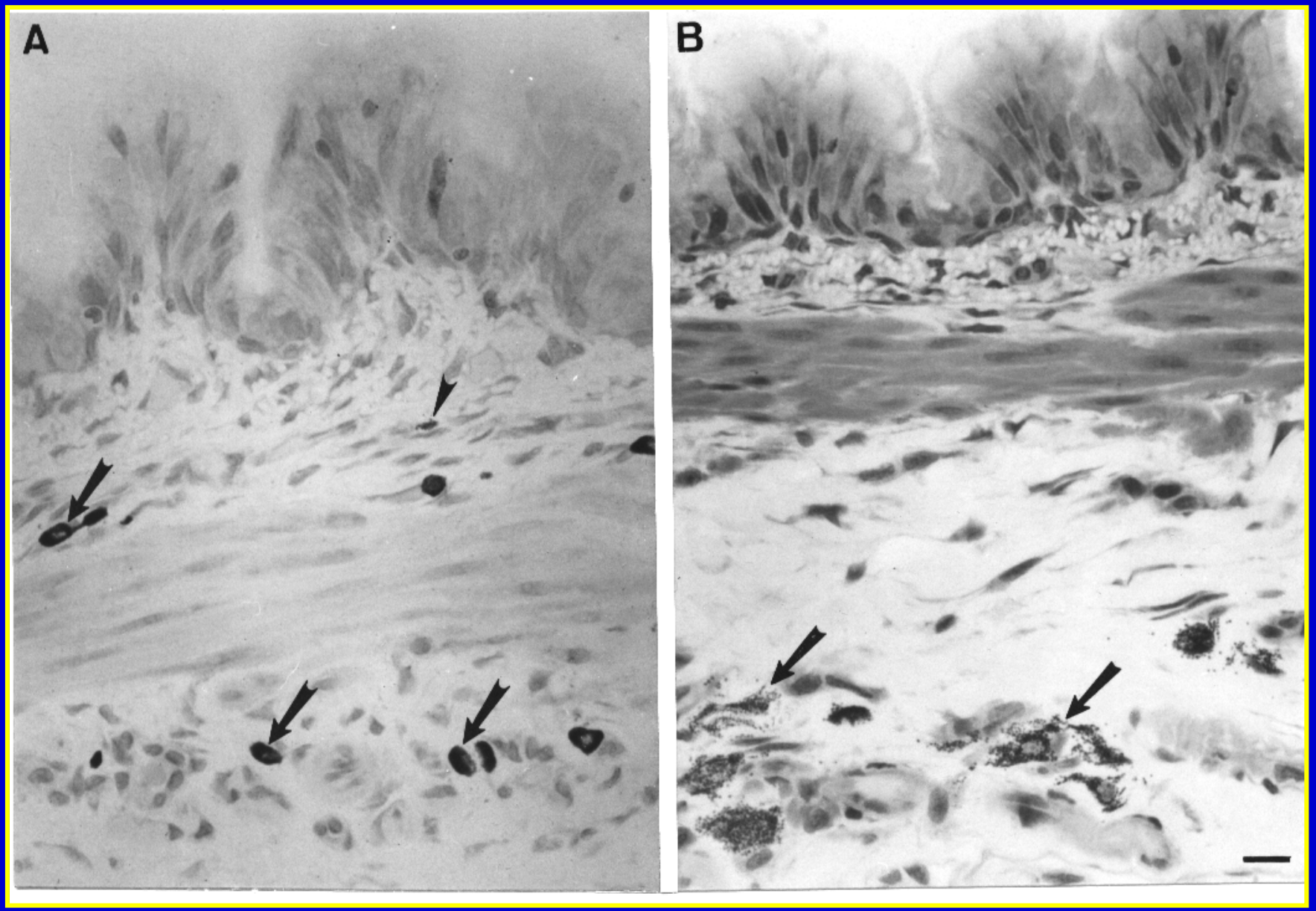
Mast cells which have undergone IgE-dependent up-regulation of FcεRI:

• Can be sensitized to respond to a larger set of different allergens	**Increased versatility** of immunological effector function
• Can release mediators and cytokines at lower concentrations of allergen	**Increased sensitivitity** of immunological responsiveness
• Can release larger amounts of mediators and cytokines in response to allergen	**Increased magnitude** of immunological effector function
• Can release certain products (including IL-4) in response to allergen which may not be detectably released at lower levels of FcεRI expression	**Change in pattern** of mediator/cytokine production; "positive feedback" effects on IgE production

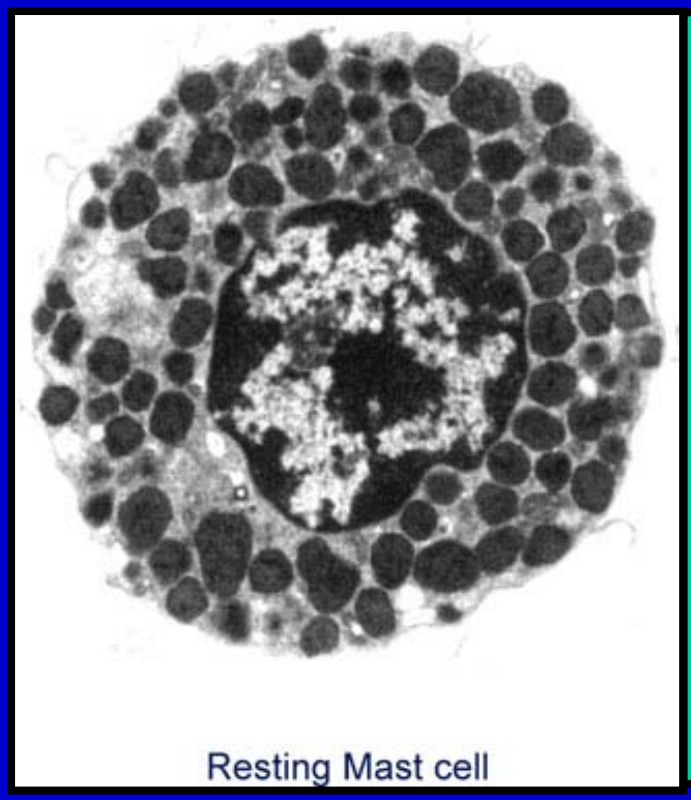

type I
allergen
IgE
Fcε receptor
mast-cell degranulation
mediator release



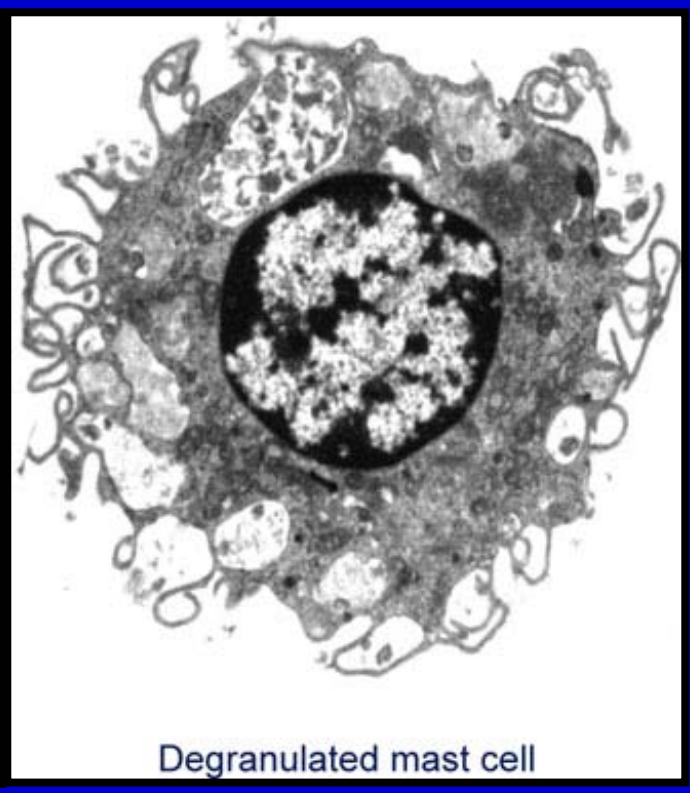
Mast cell integrity in rat trachea following OVA challenge



Damazo et al. 2001



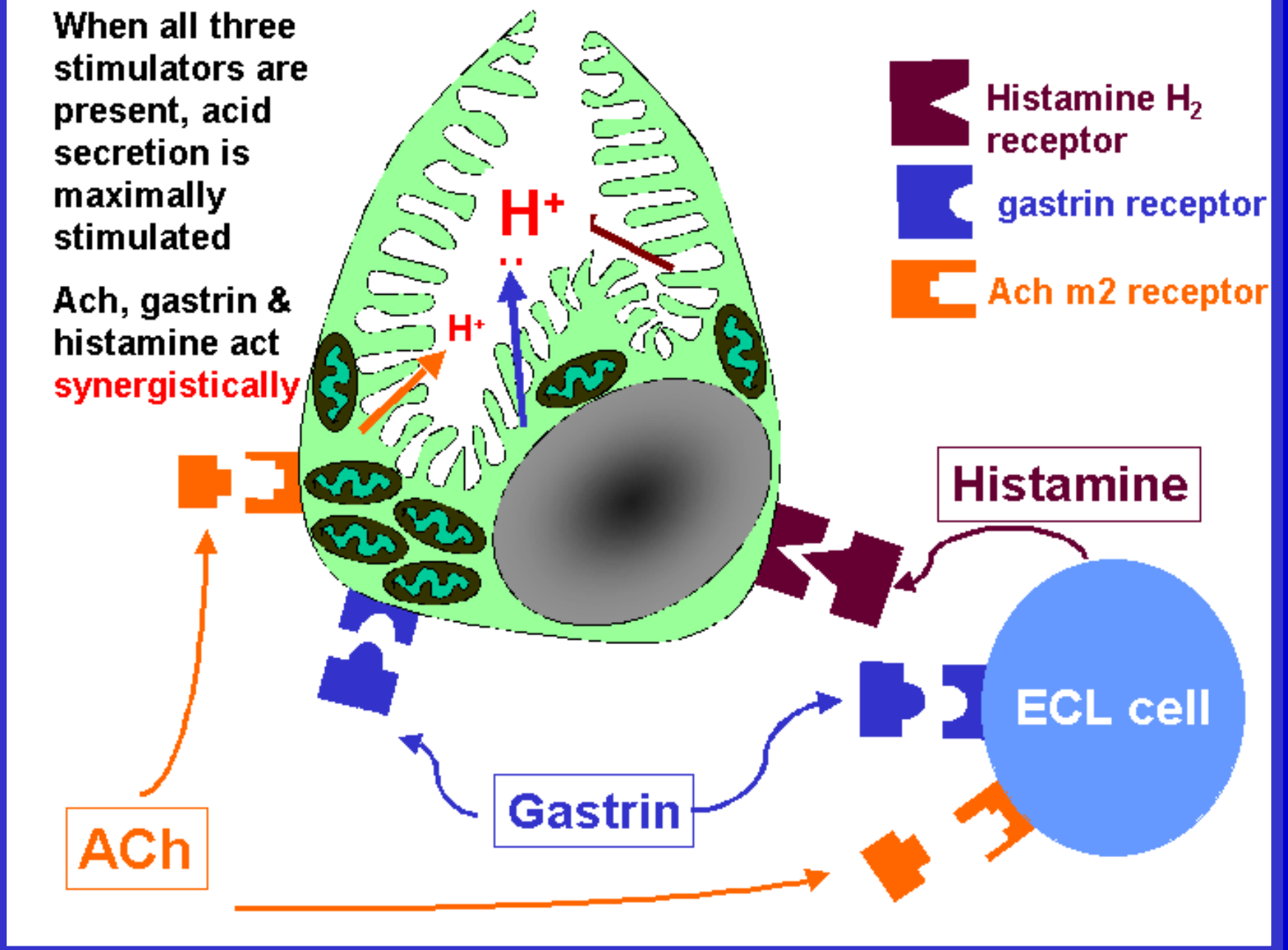
Resting Mast cell



Degranulated mast cell

Efeitos- Endógenos

1. **Mediator in local and systemic allergic reactions (*not the only one*)**
2. **Mediator in the tissue response to injury *(mechanical, thermal, infections, etc.)***
3. **Mediator of gastric acid secretion**
4. **Neurotransmitter in the CNS**



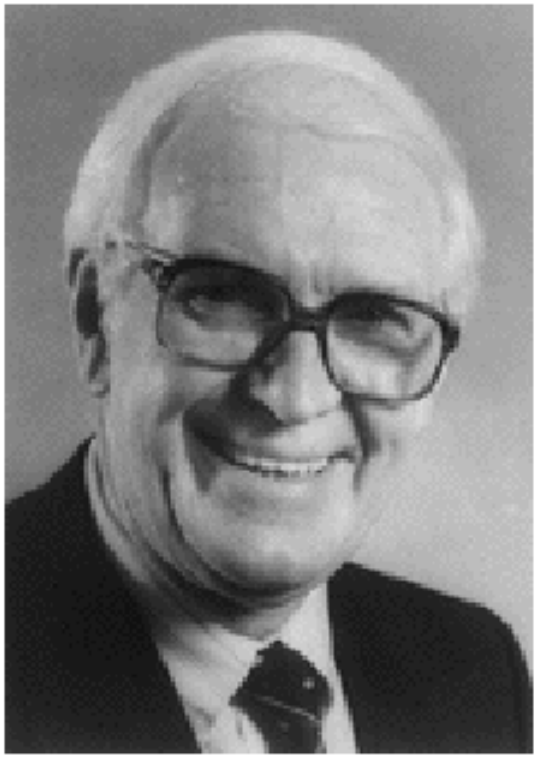
Ação não relacionada à inflamação

Antihistaminicos

- **H-1 blockers**
  - **1st Generation or classical (older)**
  - **2nd Generation or non-sedating (newer)**
- **H-2 blockers**
  - **Gastric acid blockers**

Gastric hormones

- **Histamine**



Sir James W. Black

Histamine is found in enterochromaffin-like cells (ECL cells) in the lamina propria of gastric glands

Histamine release is stimulated by:

- gastrin
- acetylcholine

Binds to histamine H_2 receptors and stimulates acid production from parietal cells

Acts synergistically with gastrin & Ach

Nobel 1988

Antagonistas Receptores H1

- **1st Generation antihistamines**
  - **Cause sedation in therapeutic doses**
  - **Affect autonomic receptors (cholinergic and adrenergic)**
- **2nd Generation antihistamines are sometimes called "non-sedating" antihistamines**

Antagonistas Receptores H1

1. **Block H-1 receptors competitively**
2. **Reduce local response to intradermal histamine**
3. **Antagonize the vasoconstrictor, and to a lesser extent, the vasodilator effects of histamine**

Antagonistas Receptores H1

4. Antagonize histamine-induced bronchospasm

Does not appreciably affect bronchospasm associated with anaphylaxis

5. inhibit gatrointestinal smooth muscle contractions

Antagonistas Receptores H1

6. **Some have good local anesthetic activity – *diphenhydramine* may be better than lidocaine**
7. **1st Generation antihistamines have some important central effects**
   - **sedation**
   - **antimotion sickness**
   - **excitation – rare in adults; more common in children**

Antagonistas Receptores H1

8. other

- **Anticholinergic properties - e.g. like atropine**
- **Alpha adrenergic blockade**
- **5-HT receptor blockade**

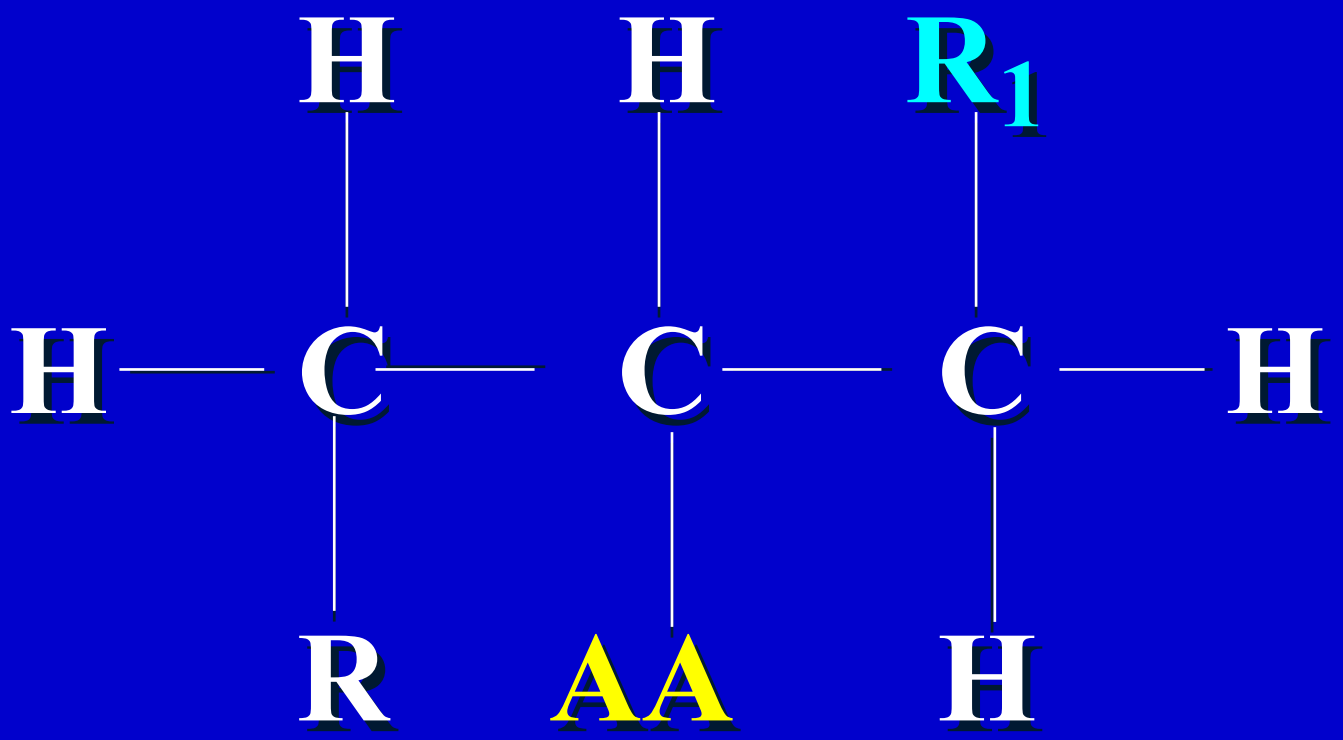
DERIVADOS DO ÁCIDO ARAQUIDÔNICO

Prostaglandinas

- 1930 – Descoberta na vesicula seminal e no plasma humano.
- 1960 – Demonstração de sua síntese a partir de ácido graxo essencial.
- 1970 – Drogas similares a aspirina previnem sua ação.
- 1990 - Múltiplas isoformas de cicloxigenases

Mecanismo de ação da aspirina

Fosfolipídeo de Membrana



R_1 = PAF?

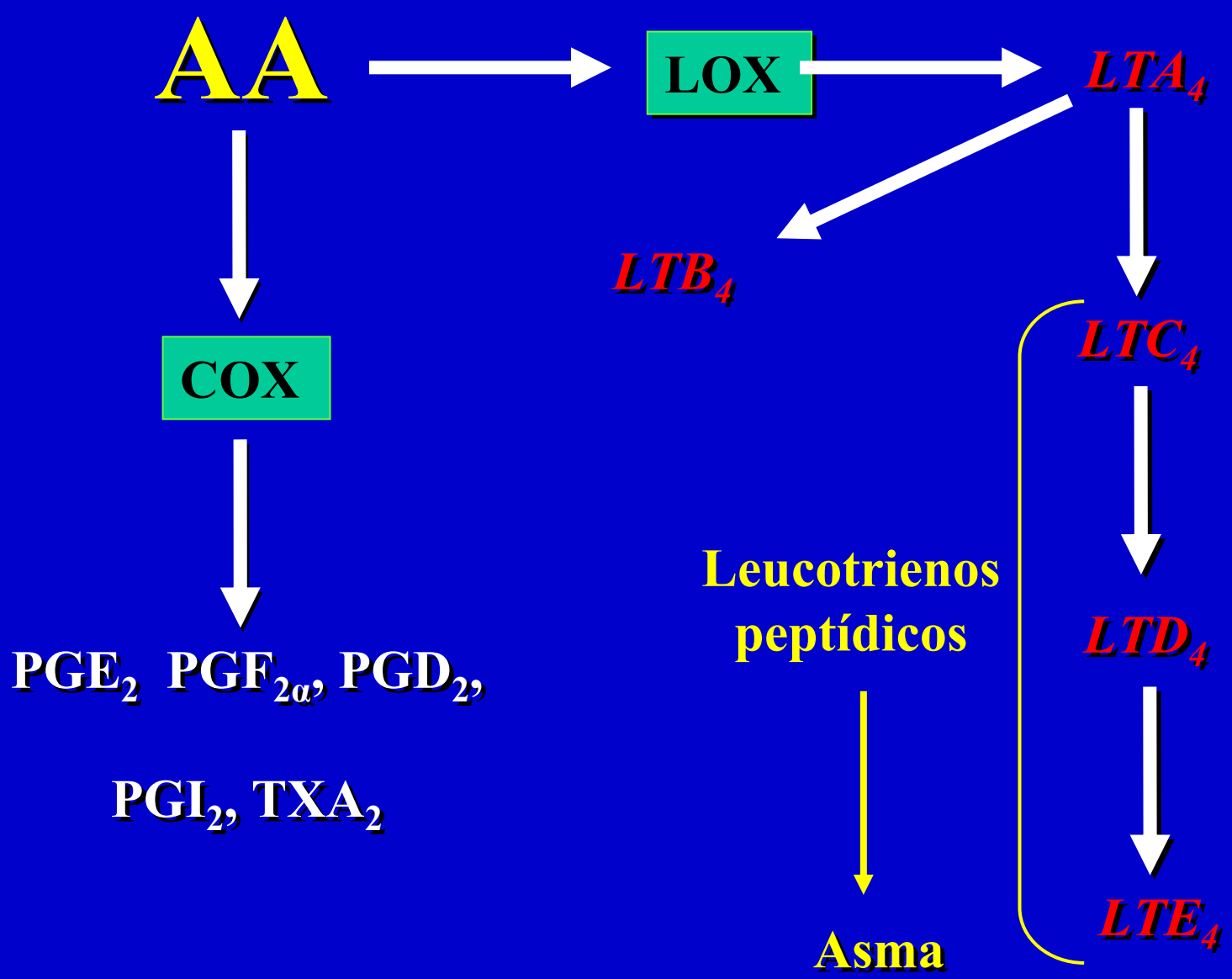
Ações dos Prostanoides

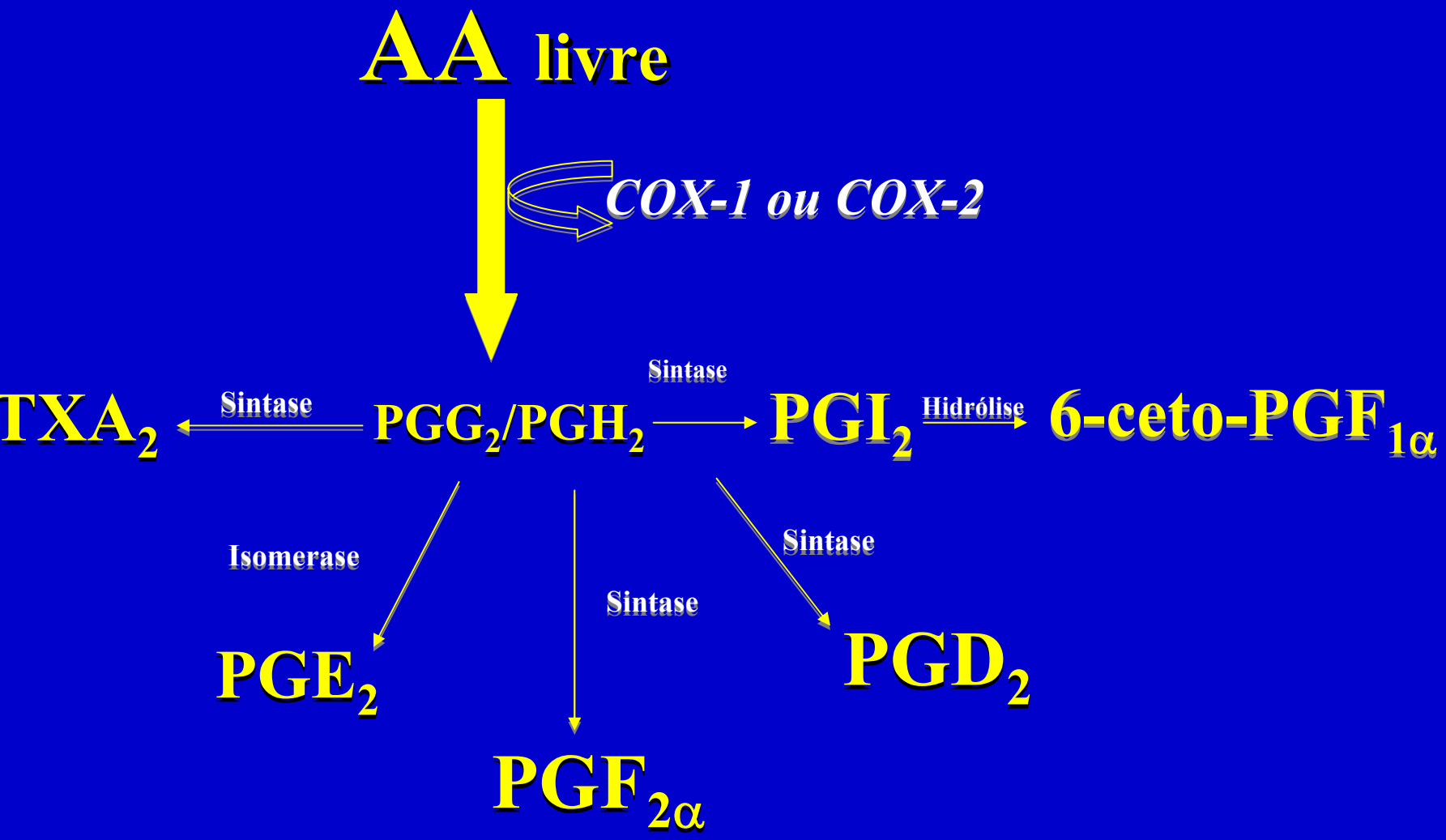
Relaxamento músculo liso, dor, febre (PGE_2)

Dilatação arteriolar, dor, febre (PGI_2)

Vasoconstrição, ativação de plaquetas (TXA_2)

Broncoconstrição (PGD_2)


AA
LOX
LTA_4
LTB_4
LTC_4
COX
LTD_4
LTE_4
Leucotrienos peptídicos
Asma
PGE_2 $PGF_{2\alpha}$, PGD_2,
PGI_2, TXA_2


AA livre
COX-1 ou COX-2
Sintase
TXA2
Sintase
PGG2/PGH2
PGI2
Hidrólise
6-ceto-PGF1α
Isomerase
Sintase
Sintase
PGE2
PGF2α
PGD2

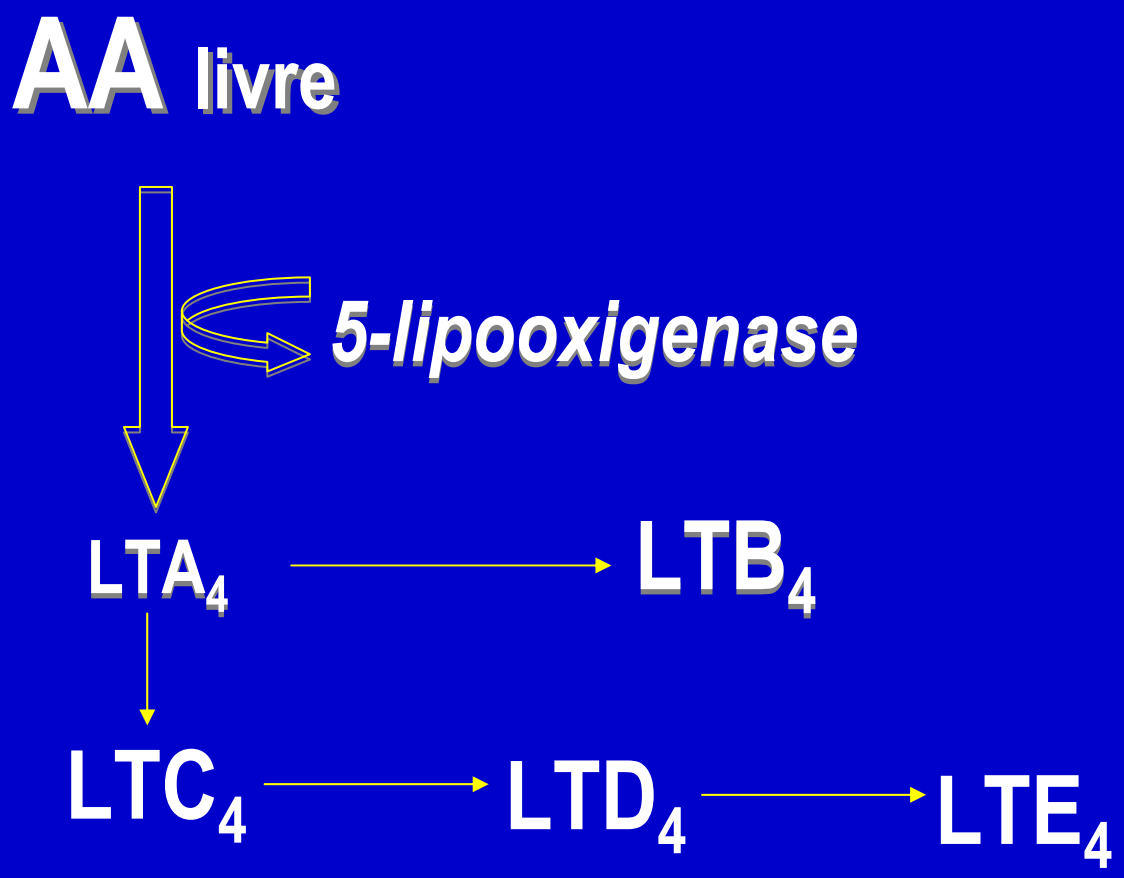
Transdução de sinal dos receptores para prostanoides

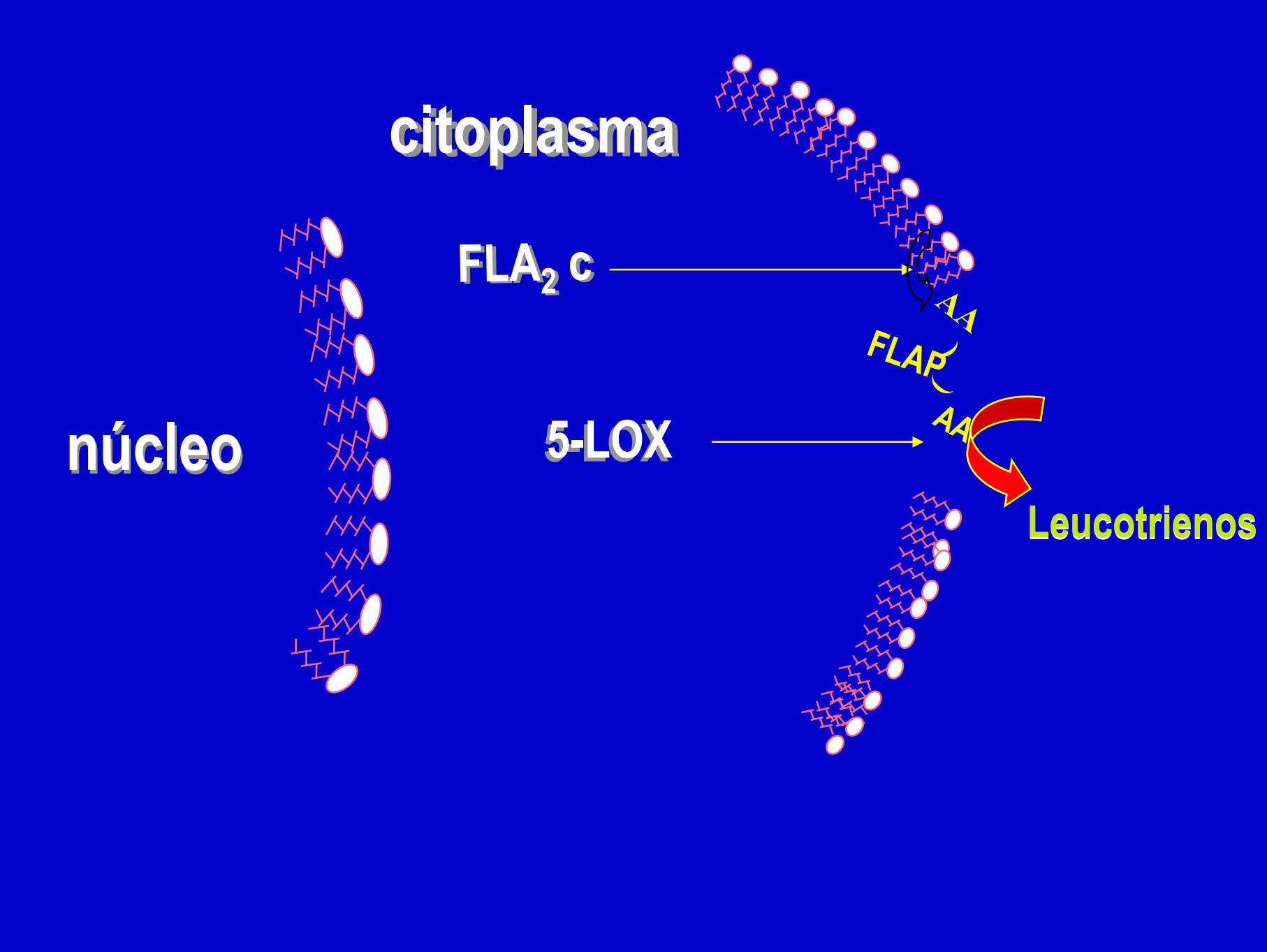
Tipo	Subtipo	Proteina G	Segundo mensageiro
FP		Gq	IP
IP		Gs, Gq	IP, cAMP ↑
TP	TPa	Gq,Gi	IP, cAMP ↓
	TPb	Gq,Gs	IP, cAMP ↑

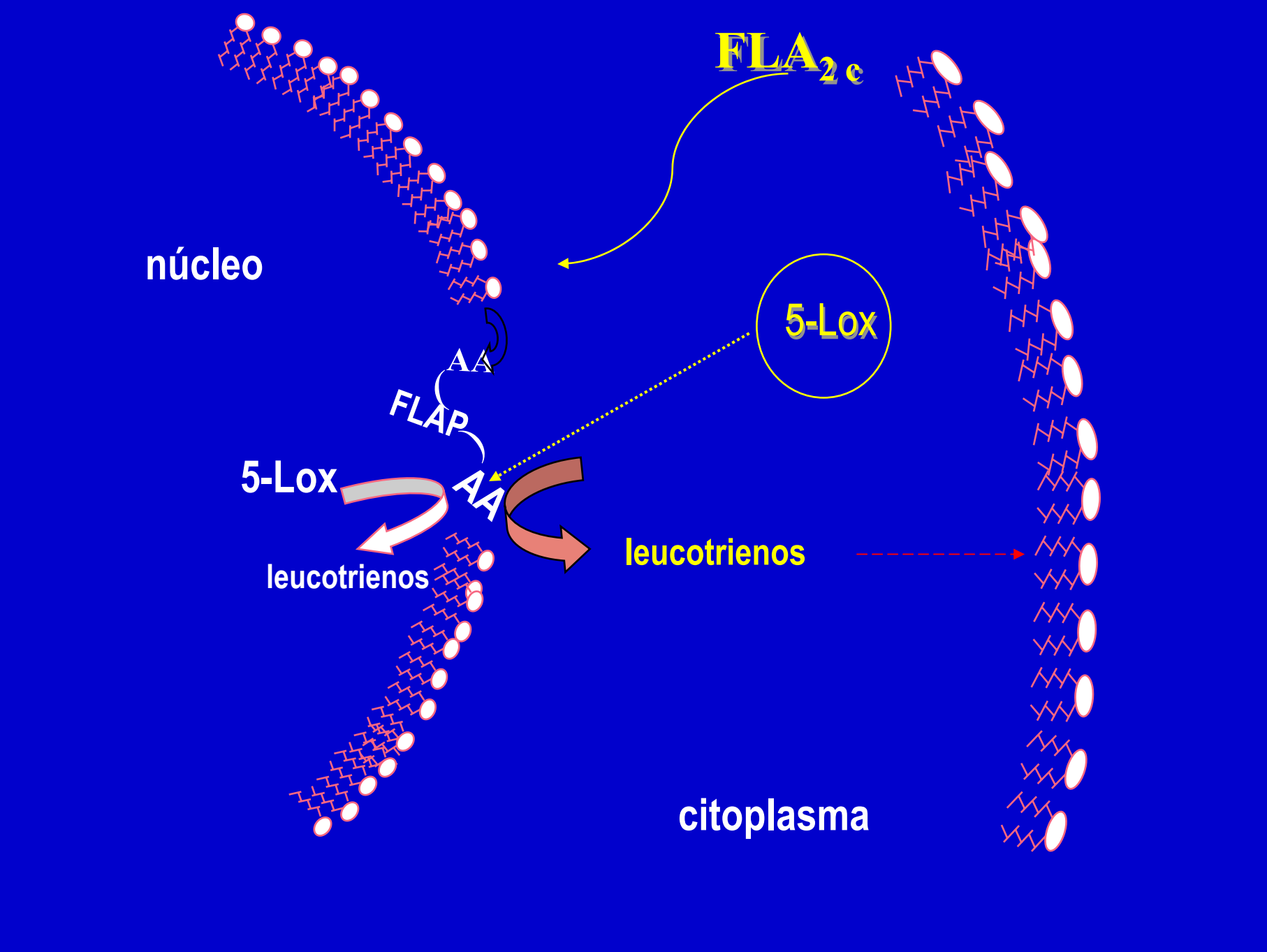
Receptores para Prostanóides

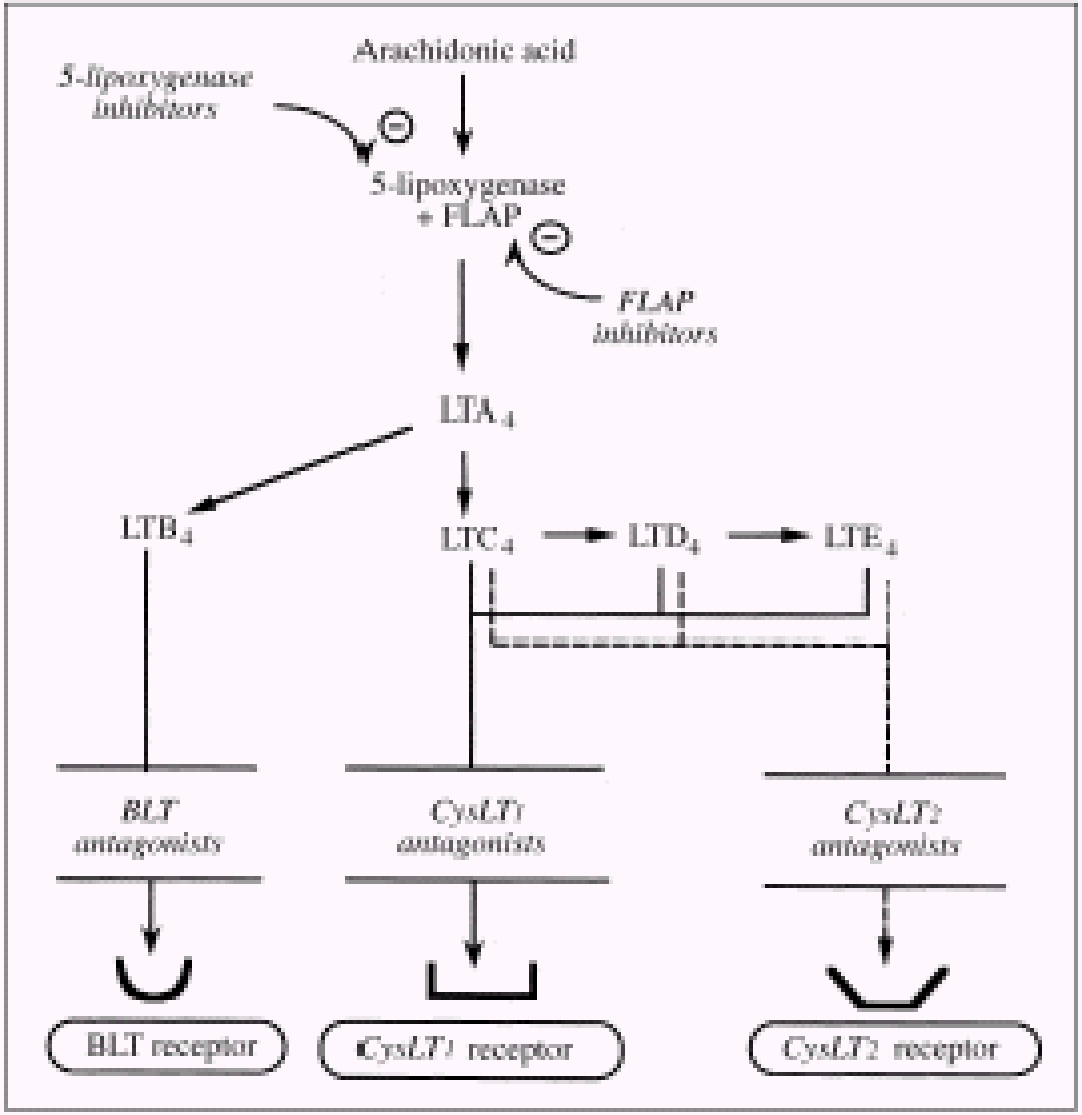
Tipo	Subtipo		Proteina G	Segundo mensageiro
DP			Gs	↑ AMPc
EP	EP1		?	↑ Ca
	EP2		Gs	↑ AMPc
	EP4		Gs	↓ AMPc
	EP3	A	Gi	↑ AMPc
		B	Gs	↑ AMPc
		C	Gs	↑ AMPc
		D	Gi,Gs,Gq	↑ ↓ AMPc,IP

Leucotrienos


AA livre
5-lipooxigenase
LTA4
LTB4
LTC4
LTD4
LTE4


citoplasma
núcleo
FLA2 c
AA
FLAP
AA
5-LOX
Leucotrienos


FLA2 c
núcleo
5-Lox
AA
FLAP
5-Lox
AA
leucotrienos
leucotrienos
citoplasma


Arachidonic acid
5-lipoxygenase inhibitors
5-lipoxygenase + FLAP
FLAP inhibitors
LTA4
LTB4
LTC4
LTD4
LTE4
BLT antagonists
CysLT1 antagonists
CysLT2 antagonists
BLT receptor
CysLT1 receptor
CysLT2 receptor

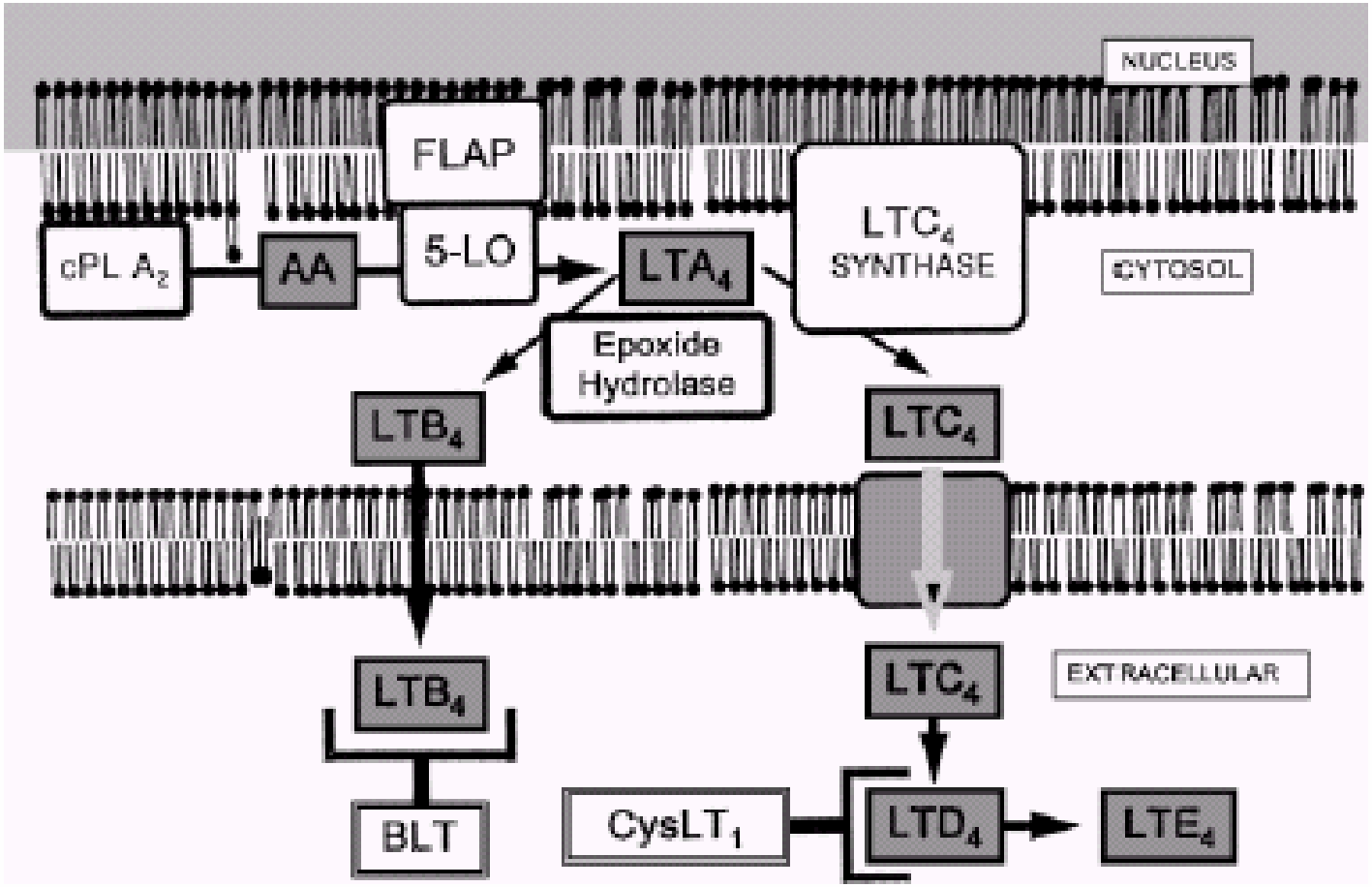

NUCLEUS
FLAP
cPL A_2
AA
5-LO
LTA_4
LTC_4 SYNTHASE
CYTOSOL
Epoxide Hydrolase
LTB_4
LTC_4
LTB_4
LTC_4
EXTRACELLULAR
BLT
$CysLT_1$
LTD_4
LTE_4

Table 1. Pharmacologic Actions of Leukotrienes

LTB_4	Cysteinyl LTs (LTC_4, LTD_4, LTE_4, LTF_4)
Aggregation of PMNs	Contraction of smooth muscle
Chemotaxis (PMNs)	Constriction of small airways
Chemokinesis of PMNs	Contraction of guinea pig parenchyma
Exudation of plasma	Secretion of mucus
Translocation of calcium	Leakage from postcapillary venules
Stimulation of PLA_2 (guinea pig lung)	Edema formation
Contraction of human bronchus*	Vasoconstriction
Contraction of guinea pig parenchymal strips*	Coronary arterial constriction
	Stimulation of PLA_2 (guinea pig lung)
	Antagonism by FPL 55712

TABLE 1 Cells secreting leukotrienes

Cell Type	LTB_4	cystLT	References
Polymorphonuclear leukocytes	+		5
Eosinophils	+	+	32, 35
Mast cells	+	+	4
Alveolar macrophages	+	+	63
Airway epithelial cells	+		64

Table 2. Overview of the CysLT Receptors and Their Antagonists

	$CysLT_1$	$CysLT_2$
Tissue	Human bronchus Guinea pig trachea Guinea pig gallbladder Rat lung strip	Human pulmonary vein Guinea pig trachea and ileum Sheep trachea and bronchus Ferret trachea
Antagonist	FPL 55712 LY 171 883 ICI 198 615/ICI 204 219 MK-571/MK-679/MK-476 SKF 104 353/SKF 106 203 ONO 1078/SB 205 312 RG 12 525 CGP 45715 BAY x7195 BAY u9773	BAY u9773*
Agonist	$LTC_4 = LTD_4 \geq LTE_4$	$LTC_4 \geq LTD_4 \gg LTE_4$

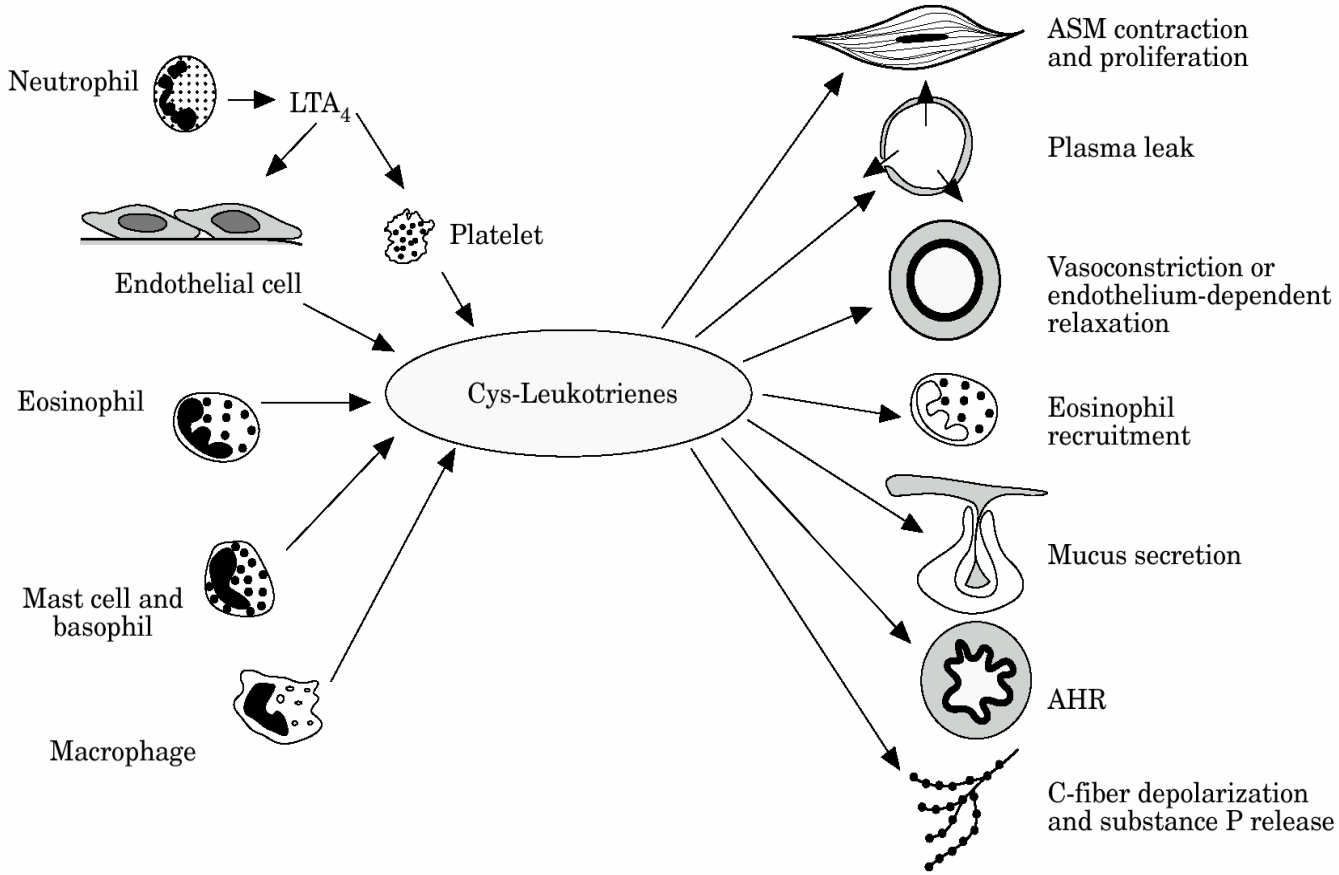


Fig. 1 Cellular sources of cysteinyl-leulotrienes and summary of their effects on airway and inflammatory cells.

Fator Ativador de Plaquetas (PAF)

Fator Ativador de Plaquetas

(1–O–Alquil–2–acil–glicerofosforil-colina)

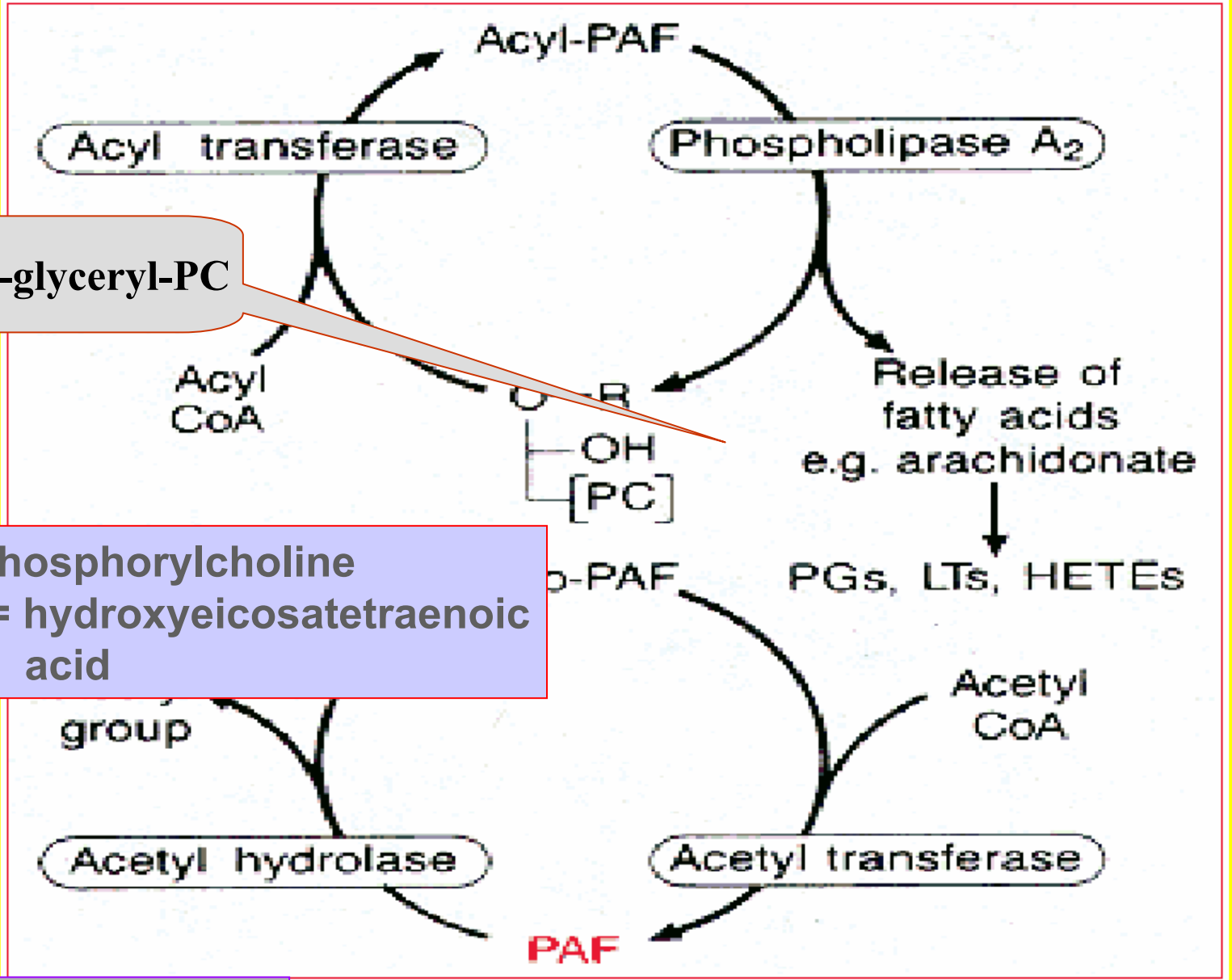
Origem

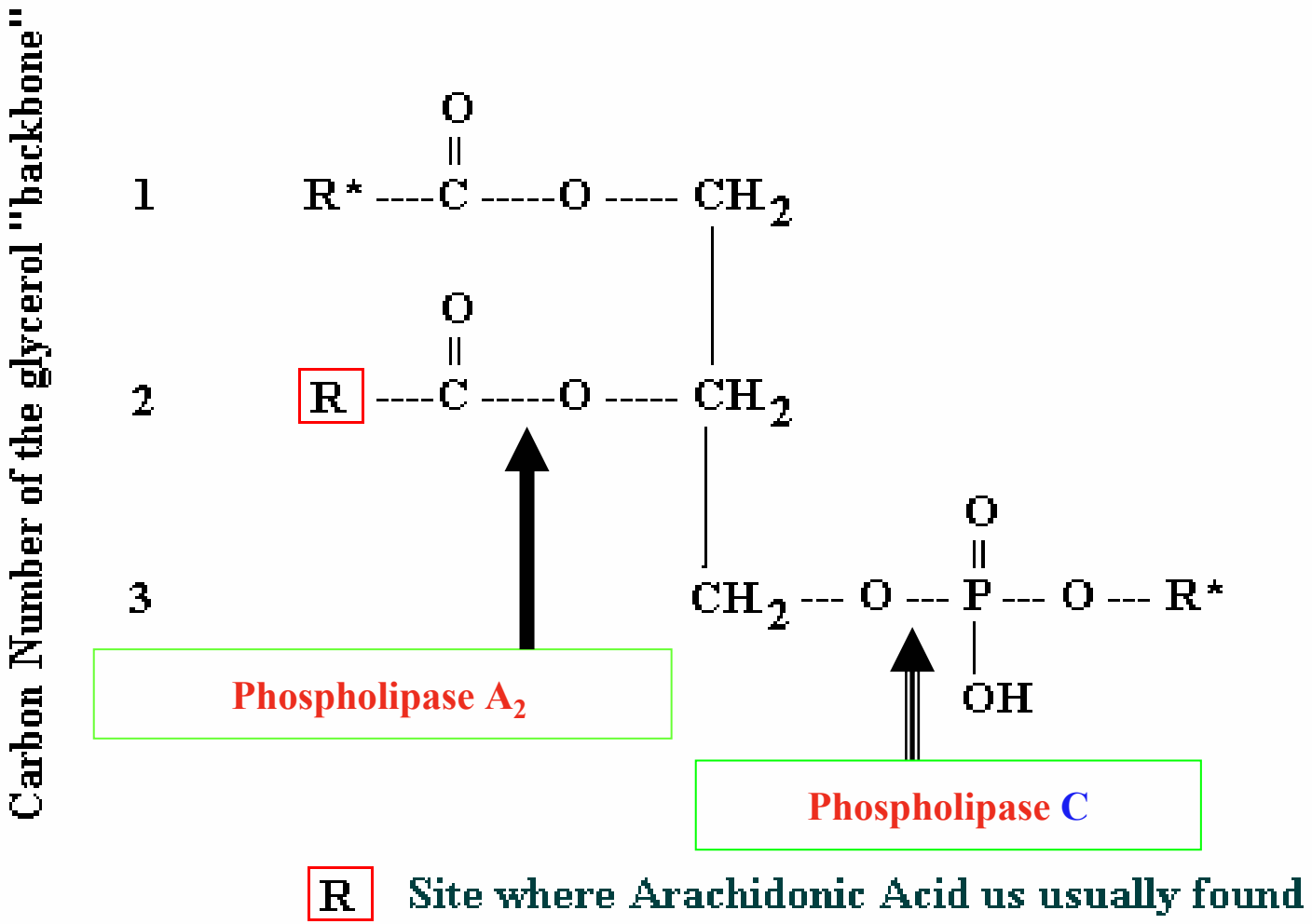
Basófilos sensibilizados e desafiados

Fonte

Diversos tipos celulares

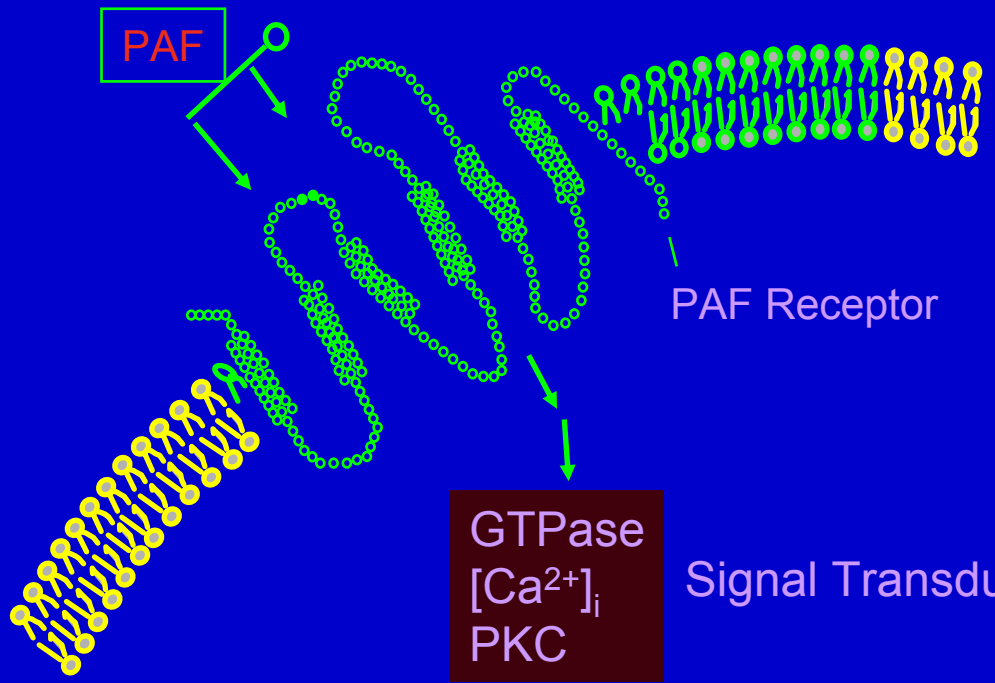
Synthesis and breakdown of PAF




Carbon Number of the glycerol "backbone"
1
2
3
R*
R
O
C
O
CH2
CH2
O
P
O
R*
OH
Phospholipase A2
Phospholipase C
R
Site where Arachidonic Acid us usually found

Rang et al

PAF Acts Via a Receptor



GTPase
$[Ca^{2+}]_i$
PKC

Signal Transduction Pathways

EFFECTOR MECHANISMS

- Priming
- β_2 Integrin CD11/CD18 Activation
- Shape Change
- Chemotaxis
- Degranulation
- Oxygen Radical Generation

PAF- Effects

Vasoconstriction/ Vasodilation

Hypotension & Cardiac Depression

Bronchoconstriction

Chemotaxis

Leukocyte- Endothelial Cell Adhesion

PAF- Effects

Leukocyte Emigration

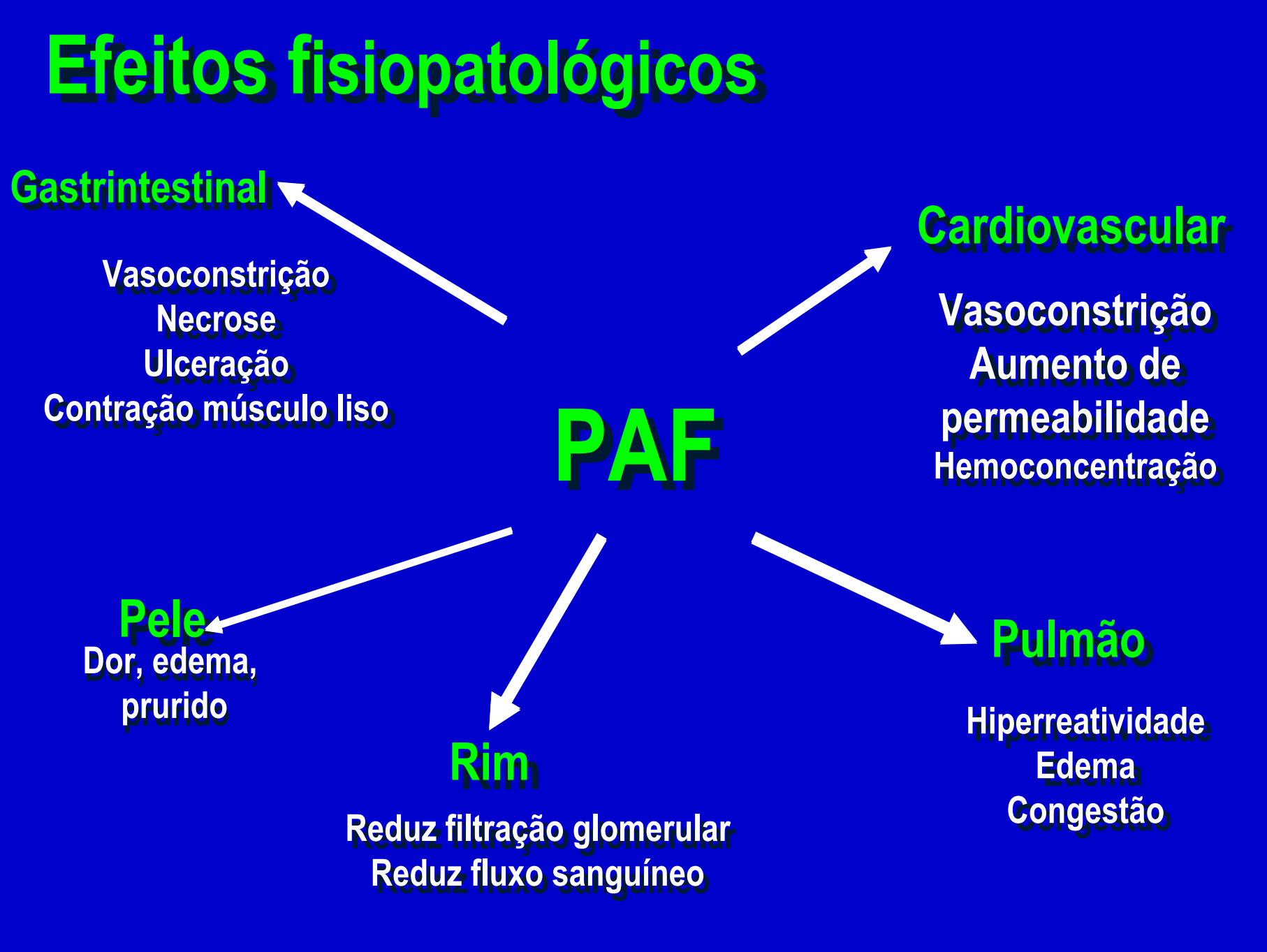
Vascular Leakage

Platelet Aggregation

Stimulates Leukotriene, PAF, Cytokine and

Oxygen Free Radical Release

Efeitos fisiopatológicos



Inflammation
Platelet Activating Factor (PAF)

Cell Activation (inflammatory, endothelial and tissue cells)

↓

Platelet Activating Factor (phospholipase A_2)

↓

Platelet Activation

- Granule release
- Aggregation & adhesion
- Production of Eicosanoids

Neutrophil/Macrophage Activation

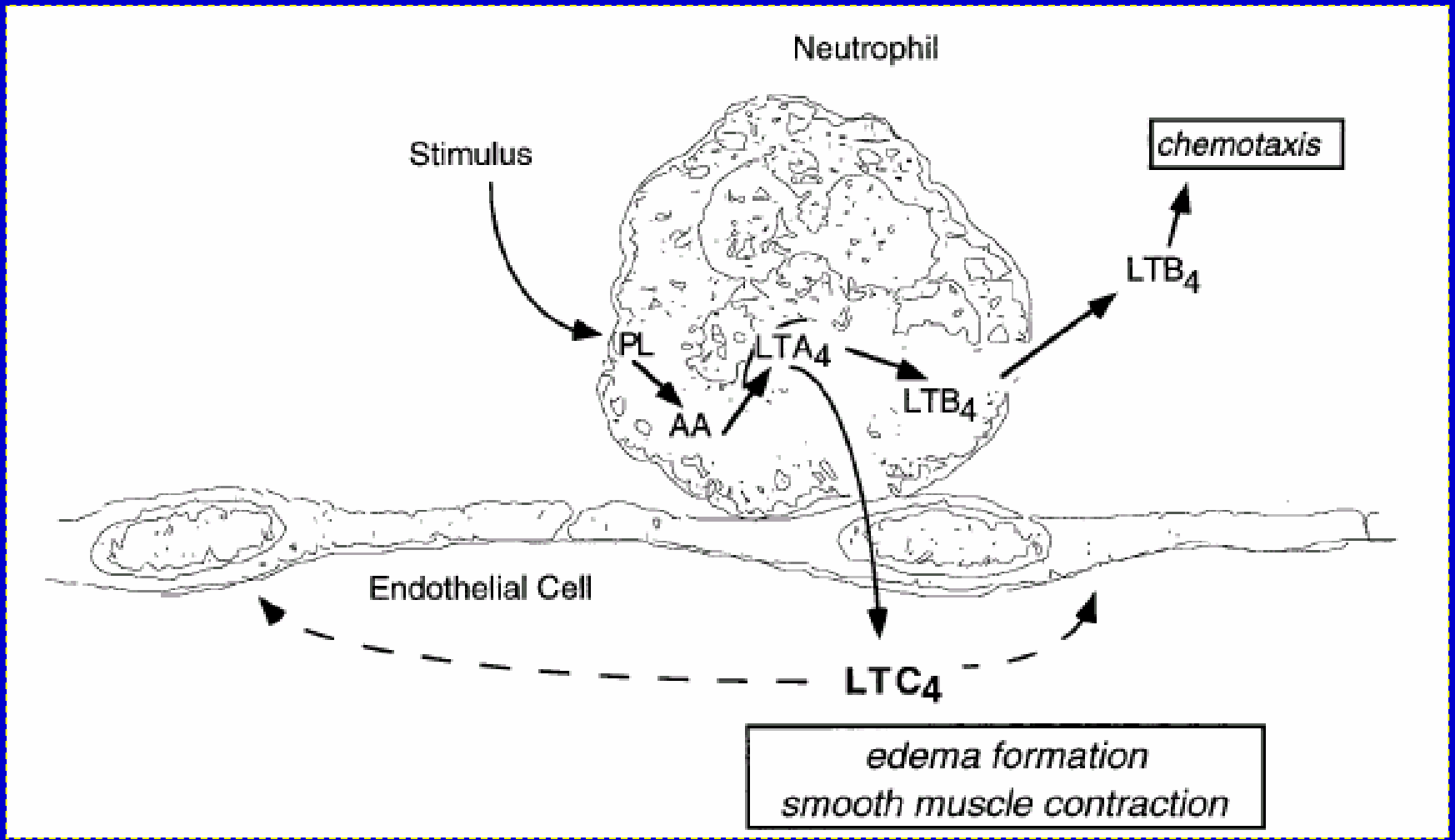
- Chemotaxis
- Production of oxygen inter-mediates

Vasculature

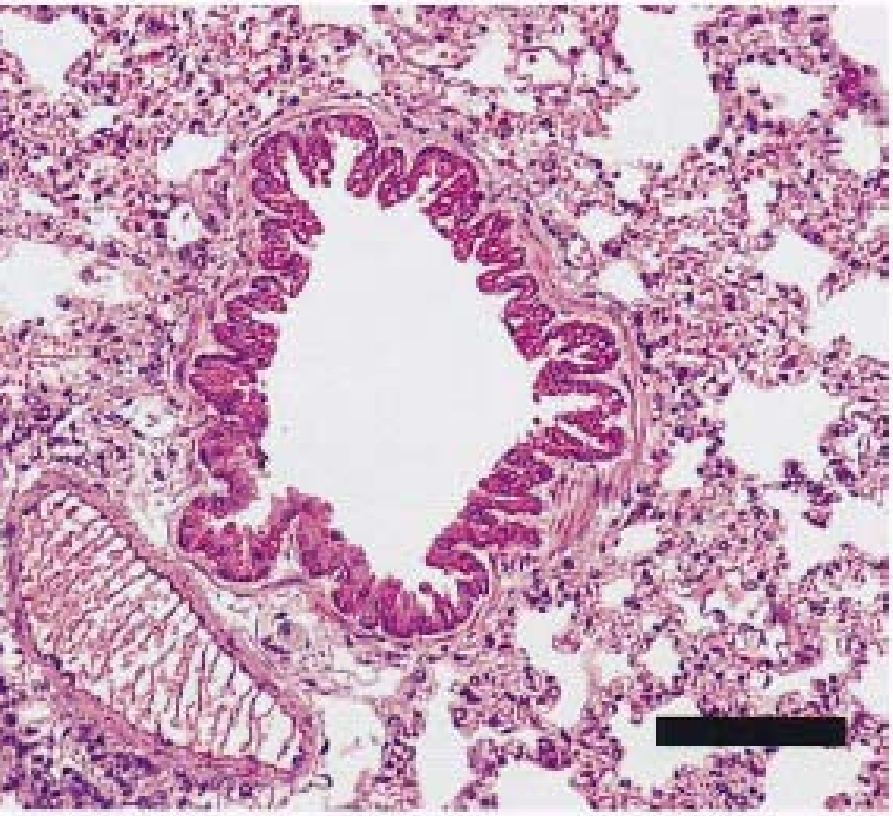
- Vasoconstriction
- Vasodilation*
- Increased vascular permeability*

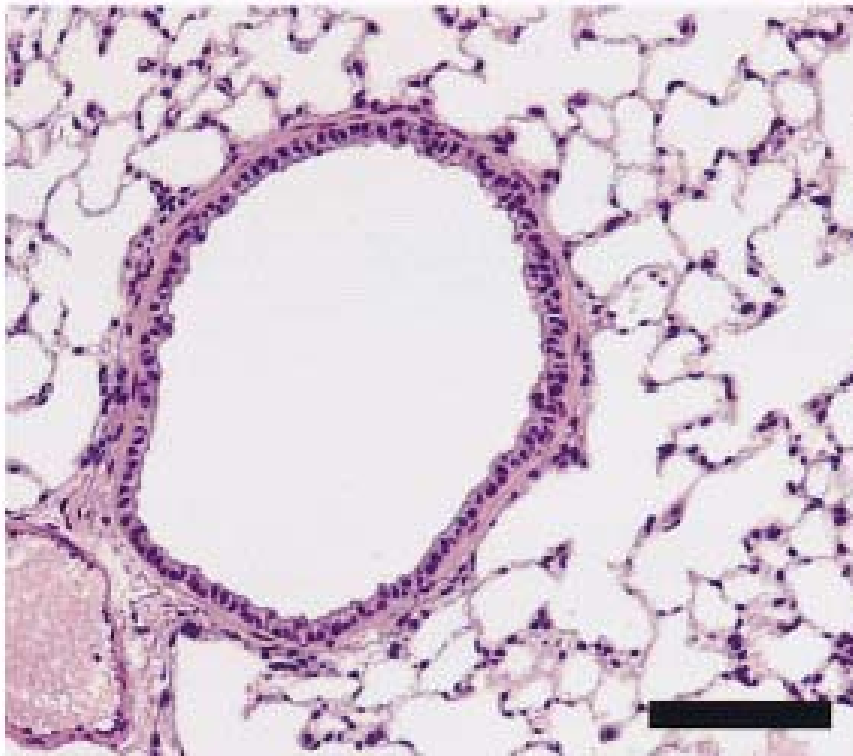
Smooth Muscle Contraction

* 100 to 10,000 times more potent than histamine


Neutrophil
Stimulus
chemotaxis
LTB4
PL
LTA4
AA
LTB4
Endothelial Cell
LTC4
edema formation
smooth muscle contraction

PAF e broncoconstriçã





Cytokines

Cytokines

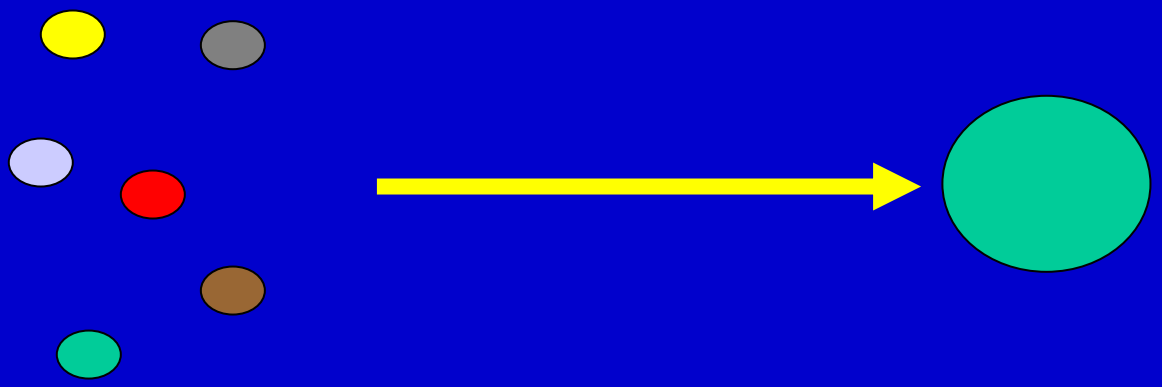
All produced by a variety of cell types:

Cytokines (e.g. TNF-α / IL-1/ IL-5)

- **cause expression of adhesive proteins on leukocytes / EC's + upregulate receptors**
- **induce chemoattractant production**
- **not chemotactic**

Característica funcional

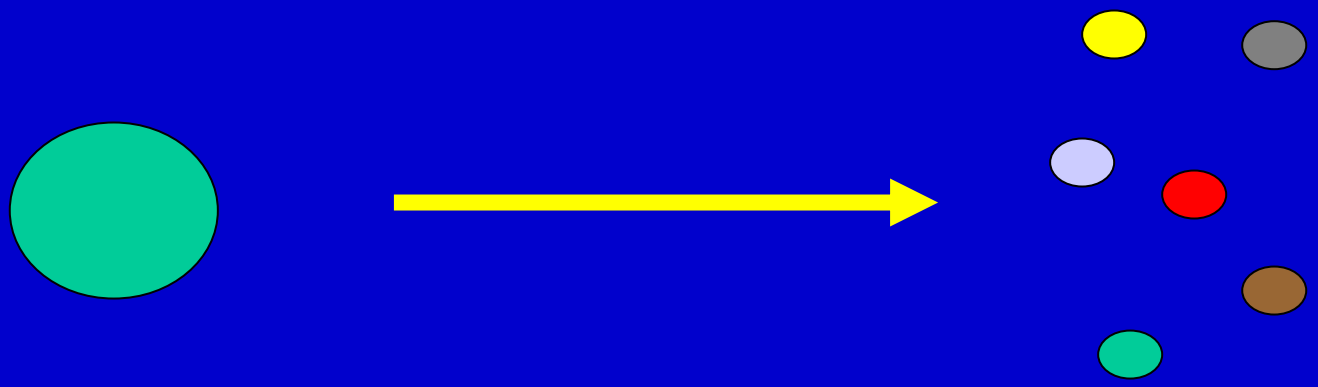
REDUNDÂNCIA



CITOCINAS *EFEITO*

Característica funcional

PLEIOTROPISMO



CITOCINA

EFEITOS

Efeitos das citocinas

Autócrino

Paracrino

Endócrino

Citocinas

Pró-inflamatórias

IL-1, TNF, IL-6, IL-11, GMCSF

Anti-inflamatórias

IL-10, IL-1r, IL-4, INFg, IL-12, IL -18

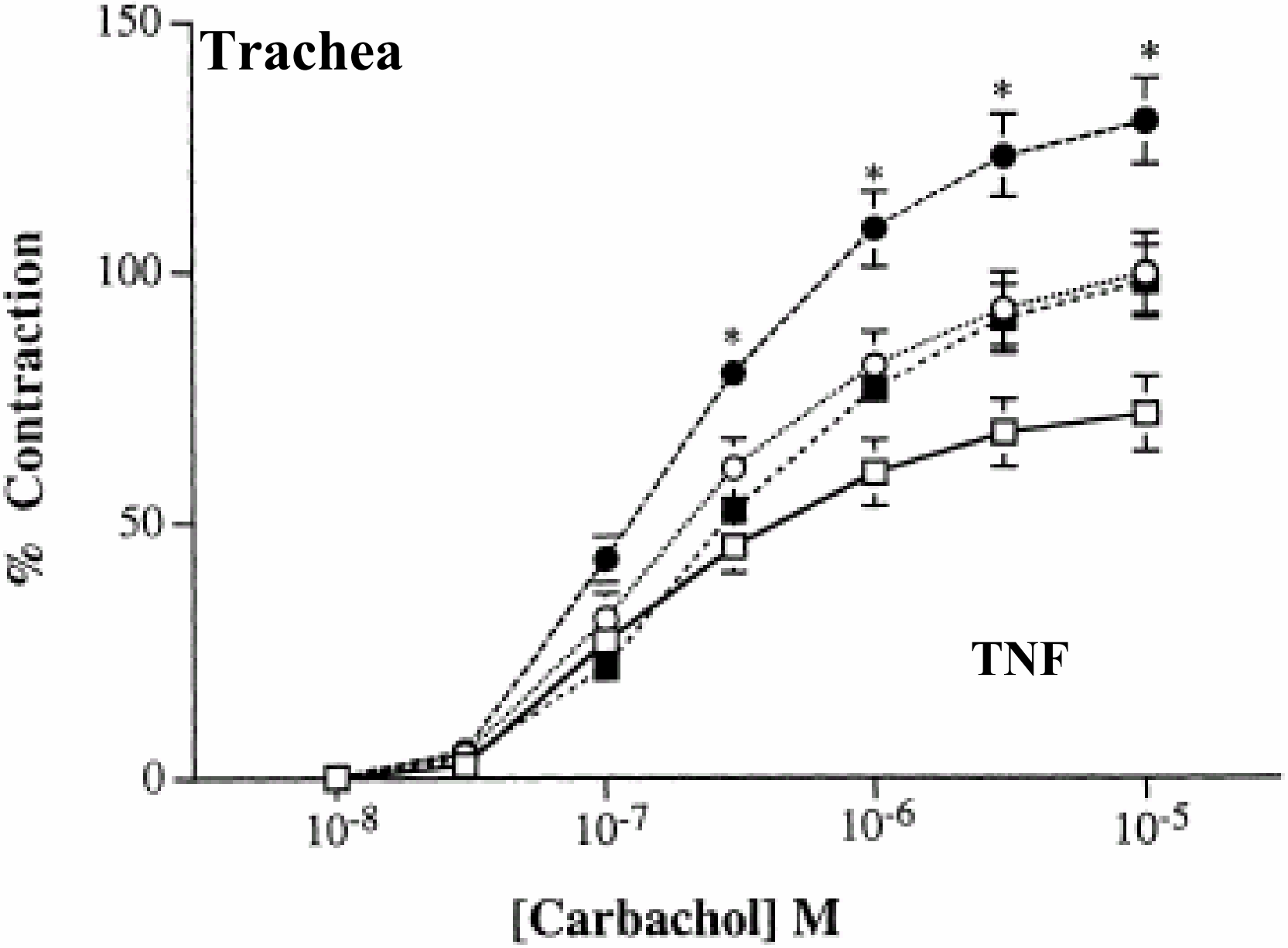
Fator de Necrose Tumoral

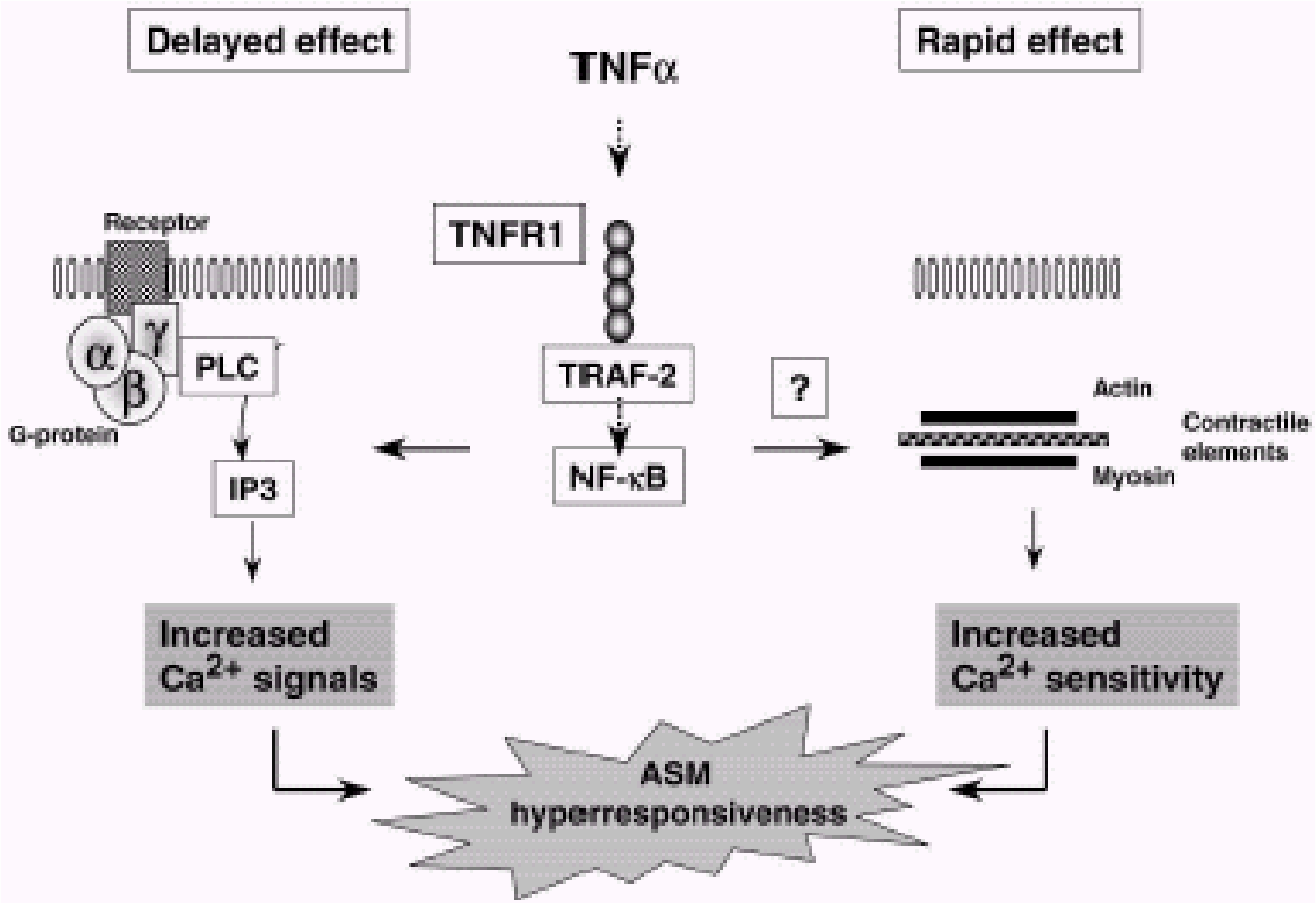
Fonte

Mácrófagos, monocitos; PMN, Eosinofilos, Astrócitos.
(Receptores TNF R-1 e TNF R-2)

Efeitos

Aumento de aderência de PMN ao endotélio
Permeabilidade vascular
Edema pulmonar
Hipóxia
Insuficiência de múltiplos órgãos e sistemas (I-MOS)
Indução de NO, PGs, LTs, PAF


Trachea
150
100
50
0
% Contraction
10-8
10-7
10-6
10-5
[Carbachol] M
TNF
*
*
*
*


Delayed effect
TNFα
Rapid effect
Receptor
TNFR1
α
γ
β
PLC
G-protein
TRAF-2
?
Actin
Contractile elements
Myosin
IP3
NF-κB
Increased Ca2+ signals
Increased Ca2+ sensitivity
ASM hyperresponsiveness

Óxido Nítrico

History of NO discovery (1)

1980, Furchgott and Zawadski reported:

- ACh-induced relaxation of rabbit aorta required the presence of endothelial cells.
- No relaxation in the absence of endothelium.
- Relaxation restored by the addition of exogenous endothelial cells.
- Proposed "Endothelial Derived Relaxing Factor" (EDRF).
- ACh-mediated relaxation was
  - Ca^{2+} dependent
  - mediated via rises in cGMP in the muscle cells
  - Mimicked by NO donors.
- Suggested NO ≡ EDRF and was a trans- or inter-cellular signaling molecule.

Óxido Nítrico

NOS: Sintase de óxido nítrico

Constitutiva - dependente Ca^{++}

Neurônios, célula endotelial

Induzida - independente Ca^{++}

Células inflamatórias, endotélio

Nitric Oxide has mixed effects

Second messenger role requires

- low concentrations of NO (<2μM)
- occurs usually via guanylate cyclase.

Cytotoxic effects due to a combination of

- Elevated concentration (>10μM)
- Interaction with oxygen radicals (e.g. inflammation/reperfusion injury)
- Formation of highly reactive peroxynitrite
- Kinetics of formation (short-term Ca^{2+} regulated vs long-term Ca^{2+} independent.

Properties of NOS isozymes

	Type I	Type II	Type III
Tissue in which first described	Cerebellum (Neuronal)	Immunologically activated Macrophages	Vascular Endothelial Cells
Tissue based terminology	nNOS	mNOS	eNOS
Expression	Constitutive	Inducible	Constitutive
Expression based terminology	cNOS	iNOS	cNOS
Intracellular free Calcium	Regulates	No Effect	Regulates

Tissue Expression of NOS

Type I (nNOS)	Type II (iNOS)	Type III (eNOS)
Neurons	Macrophages	Endothelium
Macula Densa	Cardiomyocytes	GI Interstitial Cells
Photoreceptor Inner segments	Vascular Smooth Muscle	Neurons
Gastric Epithelium	Hepatocytes	
Bronchial Epithelium	Intestinal Epithelium	
Skeletal Muscle	Megakaryocytes	
Endothelium	Keratinocytes	
Mast Cells	Chondrocytes	

NO derived from nNOS

Peripheral Effects (ANS)

NO generated from autonomic Non-Adrenergic, Non-Cholinergic (NANC) nerves acts as a neurotransmitter to relax smooth muscle in:

- **Gastrointestinal tract**
- **Urogenital tract**
- **Airways**

NO derived from eNOS

NO derived from eNOS in vascular endothelial cells contributes to the relaxation of smooth muscle. Effects include:

- **Regulation of blood pressure**
- **Raised tissue perfusion**
  - **Cardiac Perfusion**
  - **Erectile tissue**

Other effects include:

- **Inhibition of platelet adhesion and aggregation:**

NO derived from iNOS

Synthesized *de novo* within selected cell types after exposure to:

- **bacterial endotoxins or to**
- **a range of different cytokines.**

Significant in pathophysiological situations e.g.

- **Body's defensive response to disease and infection.**

NO- efeitos fisiológicos

Regula a pressão sanguínea
Mantém o tônus vasodilatador
Mantém a integridade do endotélio
Inibe proliferação do músculo liso vascular
Previne a interação leucócito~endotélio
Atua como neurotransmissor no SNC

NO- efeitos patofisiológicos

Migração celular

Edema

Atividade tumoricida e bacteriana ($M_{\varnothing}$)

Hipotensão choque séptico

Asma

Parasitas intracelulares

Clin Exp Immunol 2002; **129**:4–10

REVIEW

Nitric oxide: a regulator of mast cell activation and mast cell-mediated inflammation

J. W. COLEMAN *Department of Pharmacology and Therapeutics, University of Liverpool, UK*

(Accepted for publication 1 May 2002)

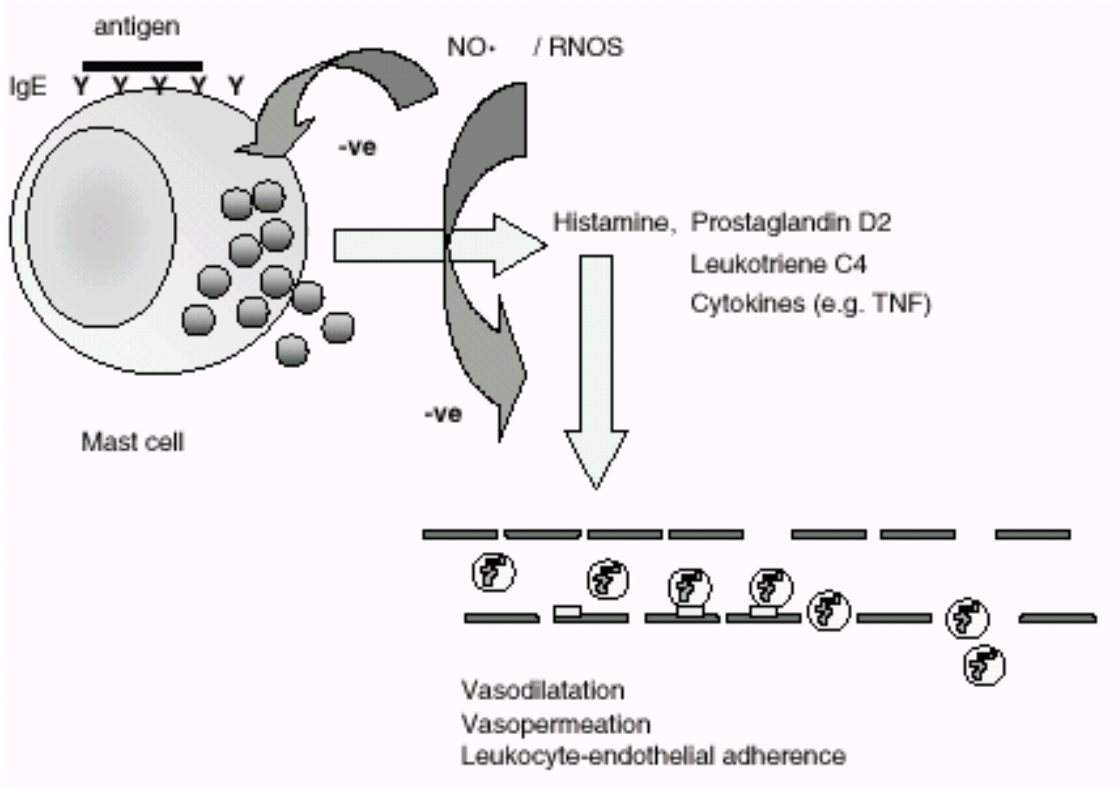
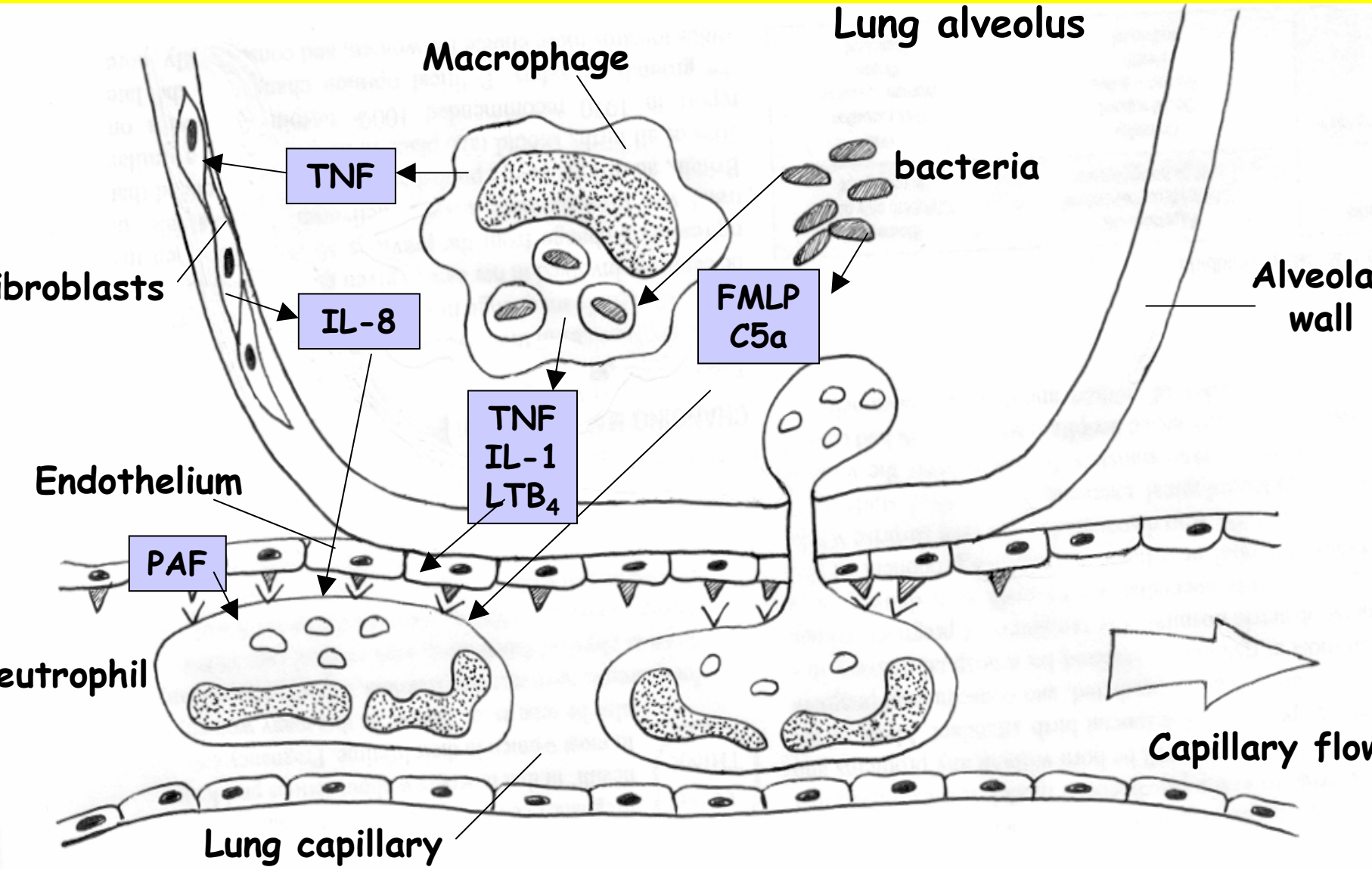


Fig. 4. Inhibitory effects of NO on mast cell activation, mediator release and mast cell dependent vascular inflammatory events.

Initiation of the Acute Inflammatory response



Vamos acordar ??