Aula Magna Univesità della Calabria
19 Aprile 2008

Il counseling sui Farmaci Anti-Infiammatori Non Sterodei

G. Bagetta
L. Morrone

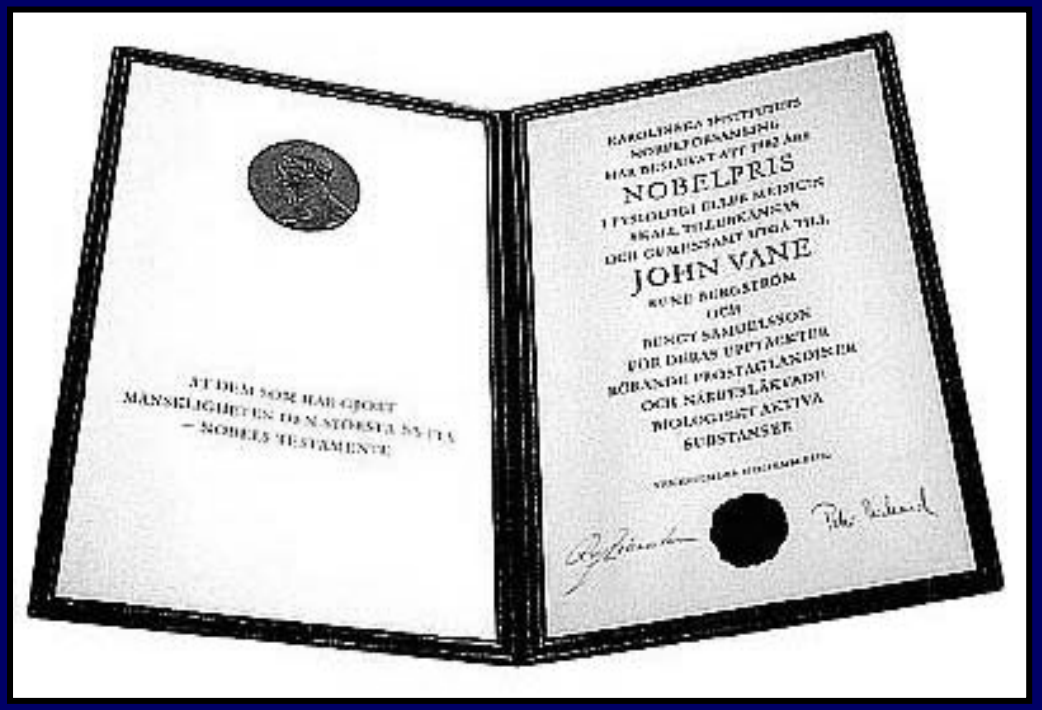
Dr. F. Hoffman, the Father of Acetylsalicylic Acid

1897

In 1971, Dr. John Vane, published in Nature is studies on the action mechanism of Aspirin.

1982 Dr. Vane would win the Nobel Prize in Medicine for his work in this respect.

1927-2004



FARMACI ANTINFIAMMATORI NON STEROIDEI (FANS)

Condividono la capacità di inibire la biosintesi dei prostanoidi attraverso l'inibizione dell'attività ciclossigenasica delle PGH sintasi 1 e/o 2

Azione antipiretica
Azione analgesica
Azione antinfiammatoria
Azione antiaggregante piastrinica

FARMACI ANTINFIAMMATORI NON STEROIDEI (FANS)

Azione antipiretica
Azione analgesica
Azione antinfiammatoria
Azione antiaggregante piastrinica

- **Come antipiretici**
- **Come analgesici**
- **Artrite reumatoide, artrite giovanile, spondilite anchilosante, osteoartrite**
- **Trattamento o profilassi malattie caratterizzate da iperaggregabilità delle piastrine**

Isoforme della PGH sintasi (COX)

COX-1

- Presente in forma costitutiva in quasi tutti i tessuti (es. piastrine, cellule endoteliali, tratto G.I., rene)

- Ruolo:

  Regolazione di funzioni omeostatiche (es. integrità mucosa gastrica, funzionalità piastrinica, flusso ematico renale)

COX-2

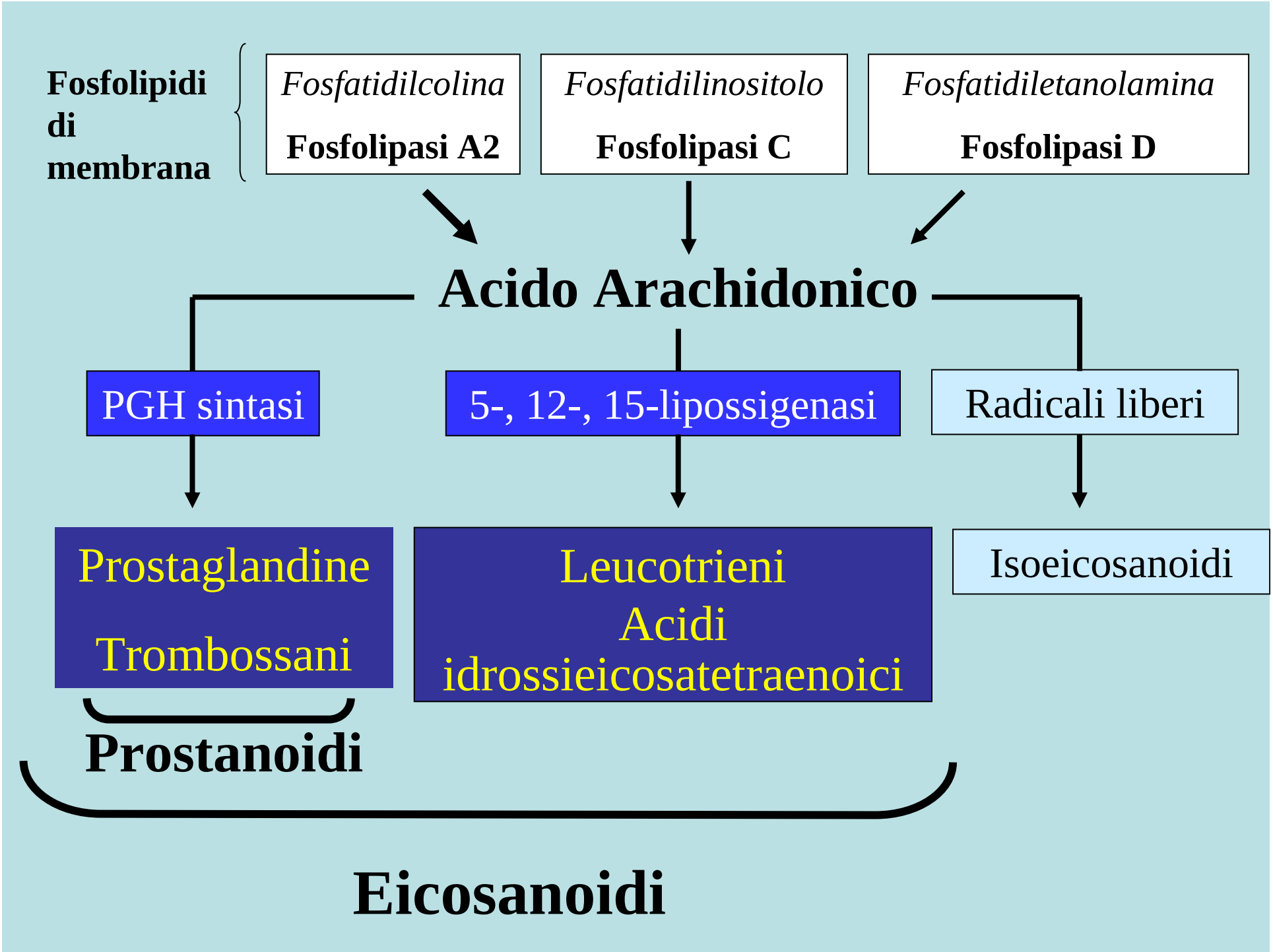
- Presente in forma costitutiva solo in alcuni tessuti (es. cervello, rene, testicoli, prostata, ovaio)

- L'espressione è rapidamente indotta in risposta a stimoli pro-infiammatori (citochine, endotossine, fattori di crescita, …) in monociti, macrofagi, sinoviociti, condrociti, cellule endoteliali …

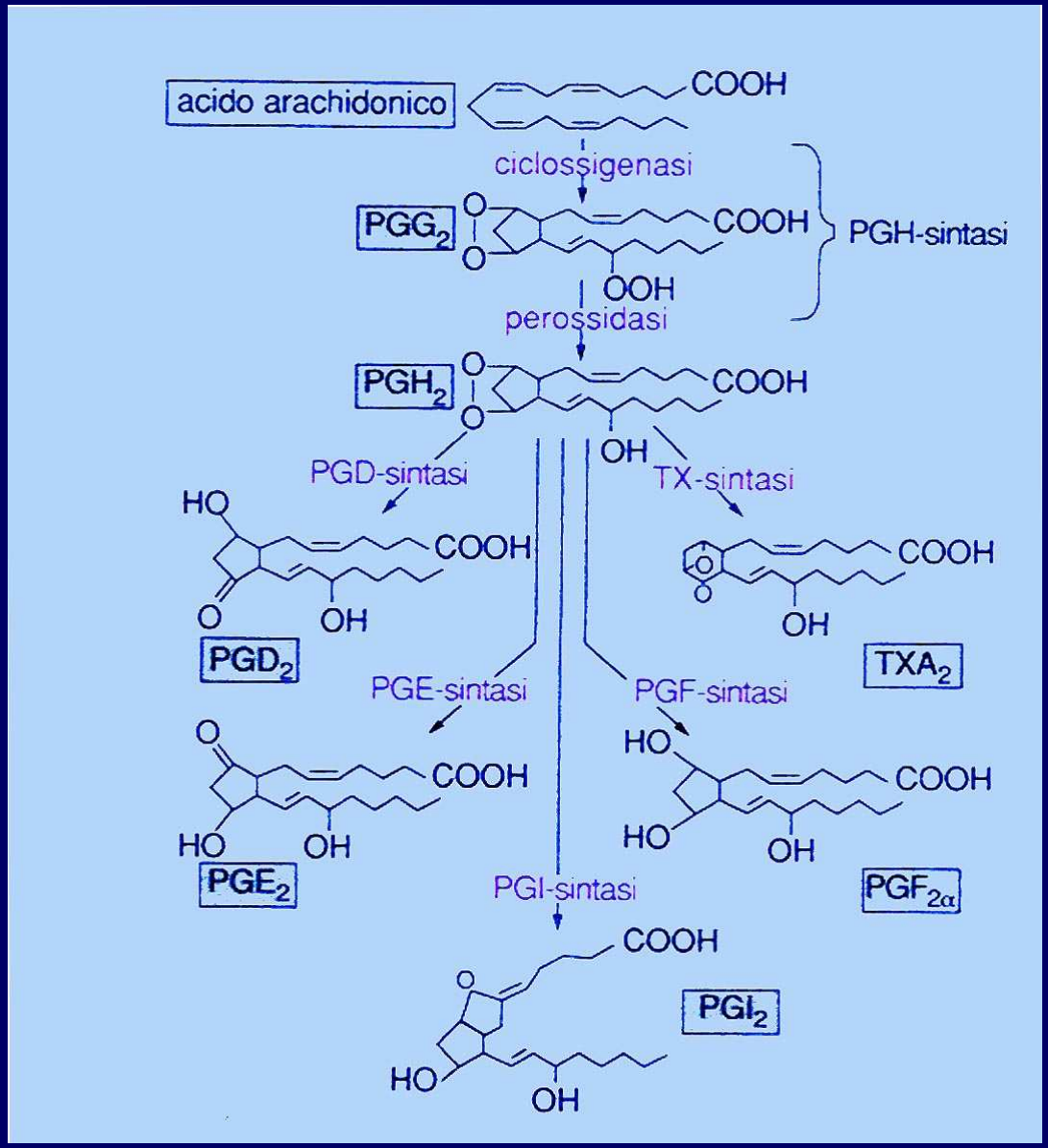
Table 1—*Structure, Distribution, and Regulation of COX-1 and COX-2*

Variables	COX-1	COX-2
DNA	Chromosone 9 (22 kB)	Chromosone 1 (8.3 kB)
Messenger RNA	2.8 kB	4.8 kB
Protein	72 kd (599 amino acids)	72 kda (604 amino acids)
Homology	Amino acids 90% homologous between species for both isoforms; similar Vmax and Km values for arachidonic acid	
Differences	Glucocorticoids inhibit expression of COX-2, not COX-1; the active site of COX-2 is larger than that of COX-1	
Regulation	Predominantly constitutive. Increased twofold to fourfold by inflammatory stimuli	Predominantly inducible (10-fold–20-fold) Constitutive in certain organs
Tissue expression	Most tissues, but particularly platelets, stomach, and kidney	Induced by inflammatory and mitogenic stimuli in monocytes/macrophages, synoviocytes, chondrocytes, fibroblasts; induced by laminar shear stress and platelet microparticles in vascular endothelial cells; induced by hormones in the ovaries and fetal membranes; constitutive in the CNS, kidney, testes, and tracheal epithelial cells.

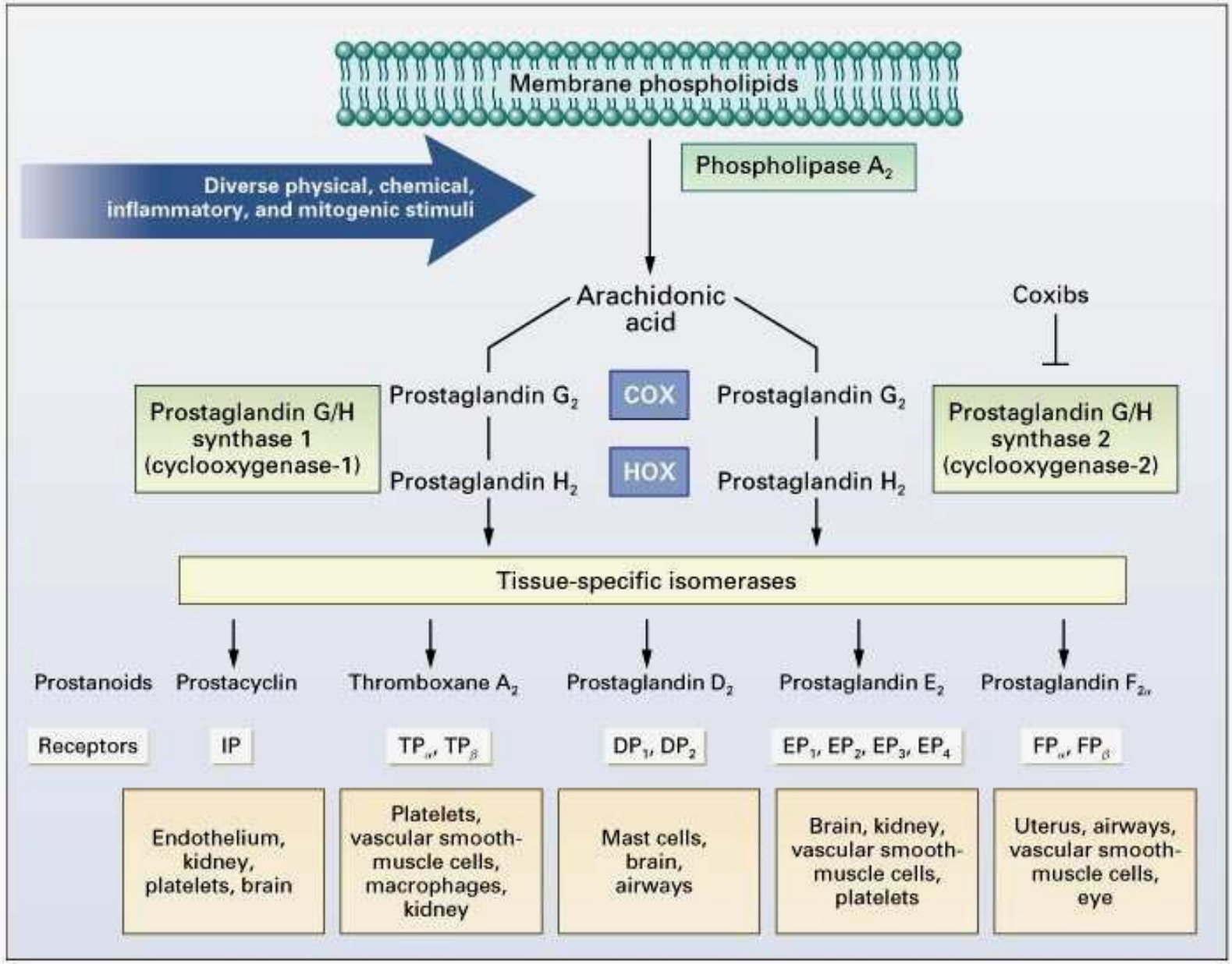
Patrono et al., 2004, *Chest* 126:234-264


Fosfolipidi di membrana
Fosfatidilcolina
Fosfolipasi A2
Fosfatidilinositolo
Fosfolipasi C
Fosfatidiletanolamina
Fosfolipasi D
Acido Arachidonico
PGH sintasi
5-, 12-, 15-lipossigenasi
Radicali liberi
Prostaglandine
Trombossani
Leucotrieni
Acidi idrossieicosatetraenoici
Isoeicosanoidi
Prostanoidi
Eicosanoidi

La via della PGH-sintasi



Production and Actions of Prostaglandins and Thromboxane



FitzGerald, G. A. et al. N Engl J Med 2001;345:433-442

Classificazione dei recettori dei prostanoidi

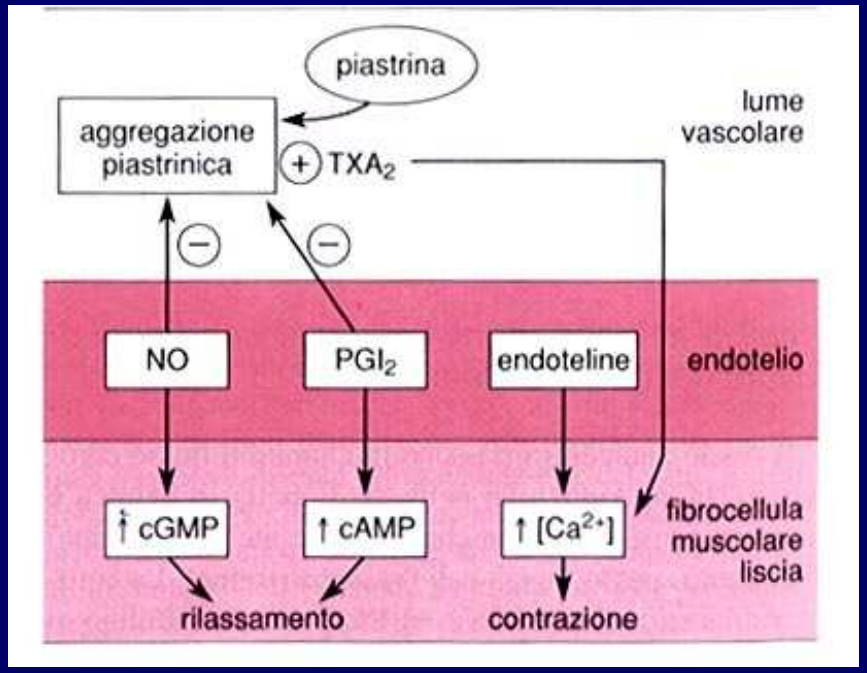
Tipo	Sottotipo	Proteina di accoppiamento	Meccanismo di trasduzione del segnale
DP		G_s	↑ cAMP
EP	**EP_1**	$G_{q/11}$	↑ IP_3/DAG/Ca^{2+}
	EP_2	G_s	↑ cAMP
	EP_3	$G_{q/11}/G_{i/o}/G_s$	↓ cAMP ↑ IP_3/DAG/Ca^{2+}
	EP_4	G_s	↑ cAMP
FP		$G_{q/11}$	↑ IP_3/DAG/Ca^{2+}
IP		G_s	↑ cAMP
TP		$G_{q/11}$	↑ IP_3/DAG/Ca^{2+}

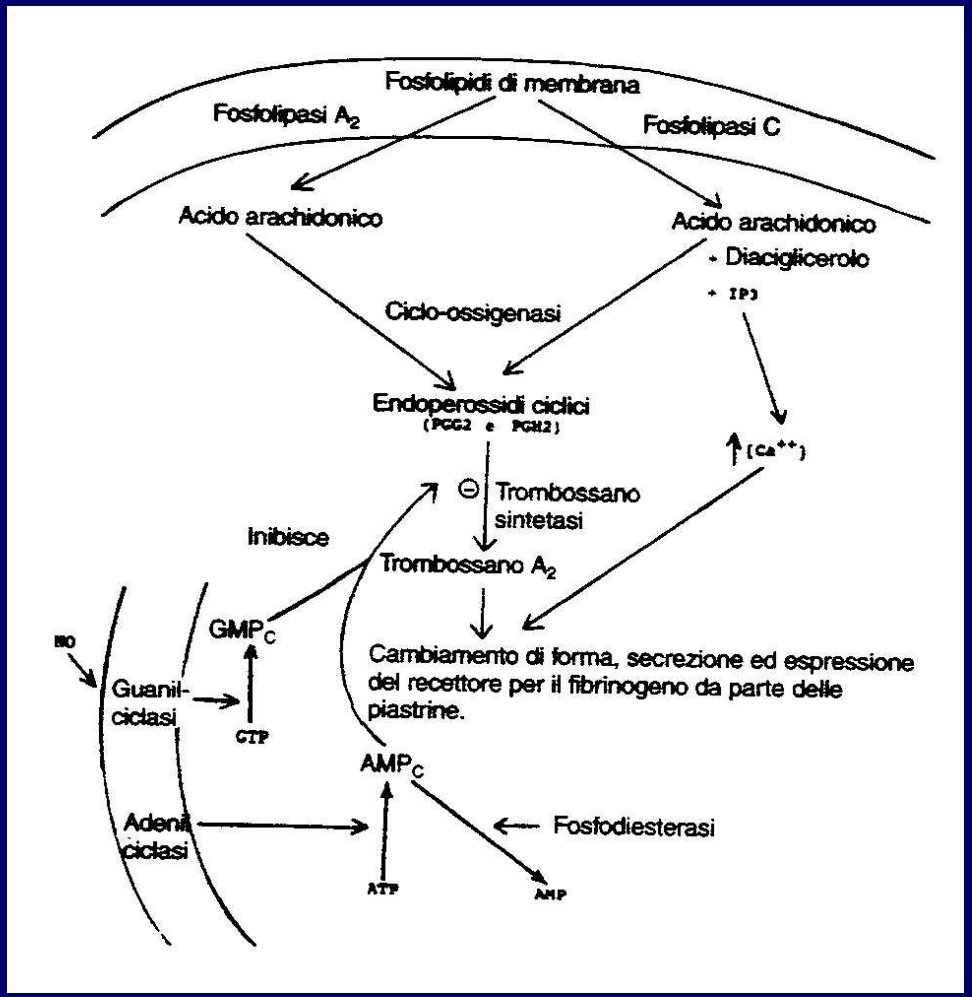
Prostanoide Sede di produzione Attività biologiche

TXA_2	Piastrine, polmone, corticale renale	↑ aggregazione e degranulazione piastrinica ↑ tono della muscolatura liscia vasale e bronchiale ↓ flusso ematico renale e filtrazione glomerulare
PGE_2	Monociti, midollare renale, ipotalamo timo	↓ tono della muscolatura liscia; secrezione gastrica ↓ liberazione di neurotrasmettitori del SN autonomo ↓ lipolisi negli adipociti ↓ soglia del dolore ↑ diuresi, natriuresi ↑ temperatura corporea ↑ regolazione della differenziazione linfocitaria
$PGF_{2\alpha}$	Utero, midollare renale, piastrine	↑ tono della muscolatura liscia ↑ luteolisi
PGI_2	Vasi, corticale renale	↓ tono della muscolatura liscia aggregazione piastrinica ↑ flusso ematico renale e filtrazione glomerulare

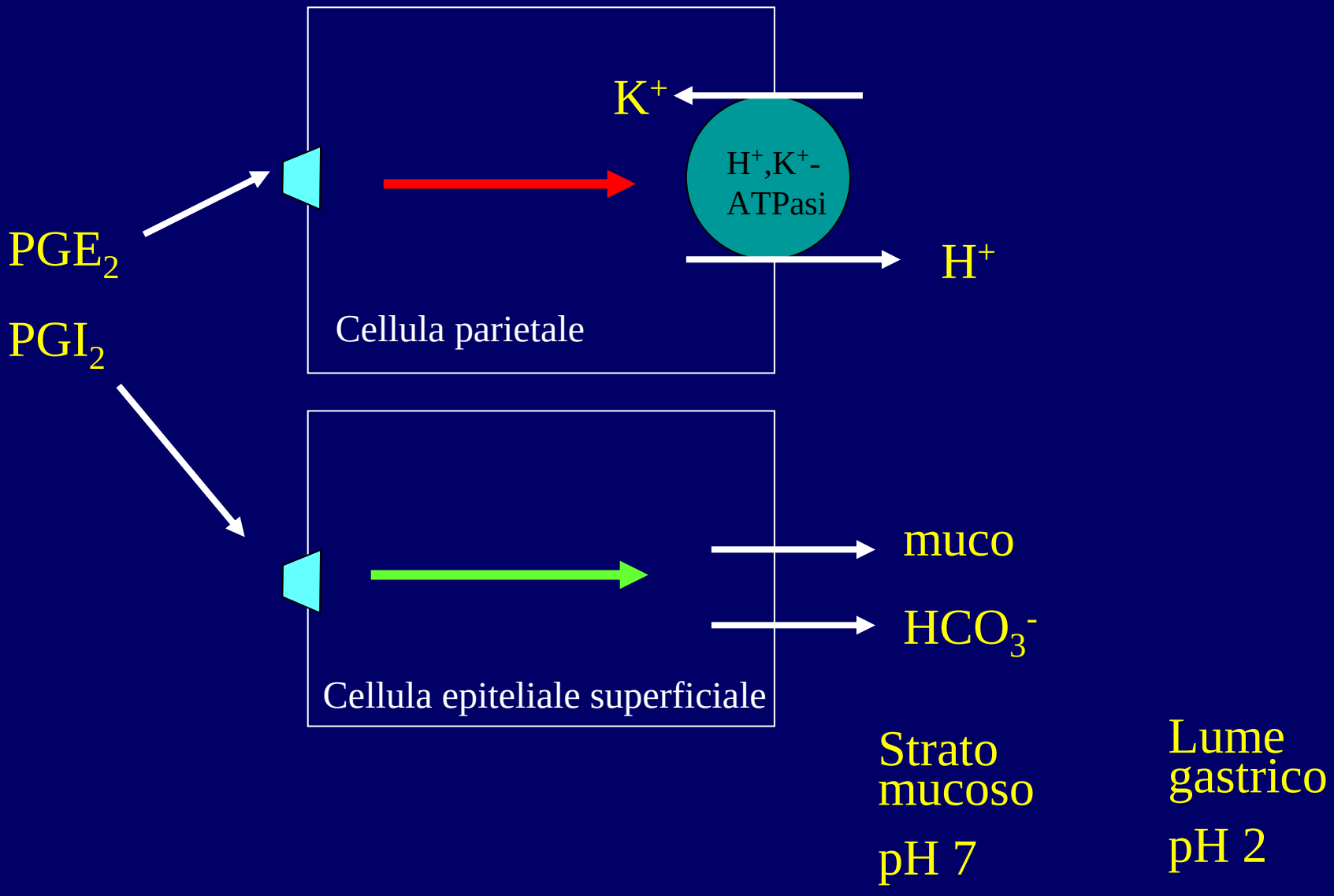
Leucotriene isoprostano	Sede di produzione	Attività biologiche
LTC_4	Monociti, eosinofili	↑ tono della muscolatura liscia vasale e bronchiale ↑ permeabilità vasale
LTB_4	Monociti, neutrofili	↑ aggregazione, degranulazione e chemiotassi dei polimorfonucleati neutrofili
8-iso-$PGF_{2\alpha}$	Membrane cellulari LDL	↑ aggregazione piastrinica ↑ tono della muscolatura liscia vasale ↑ proliferazione delle cellule muscolari lisce

Prostaciclina e Trombossano: effetti su muscolatura liscia vasale e piastrine





Prostaglandine: agenti citoprotettivi della mucosa gastrica

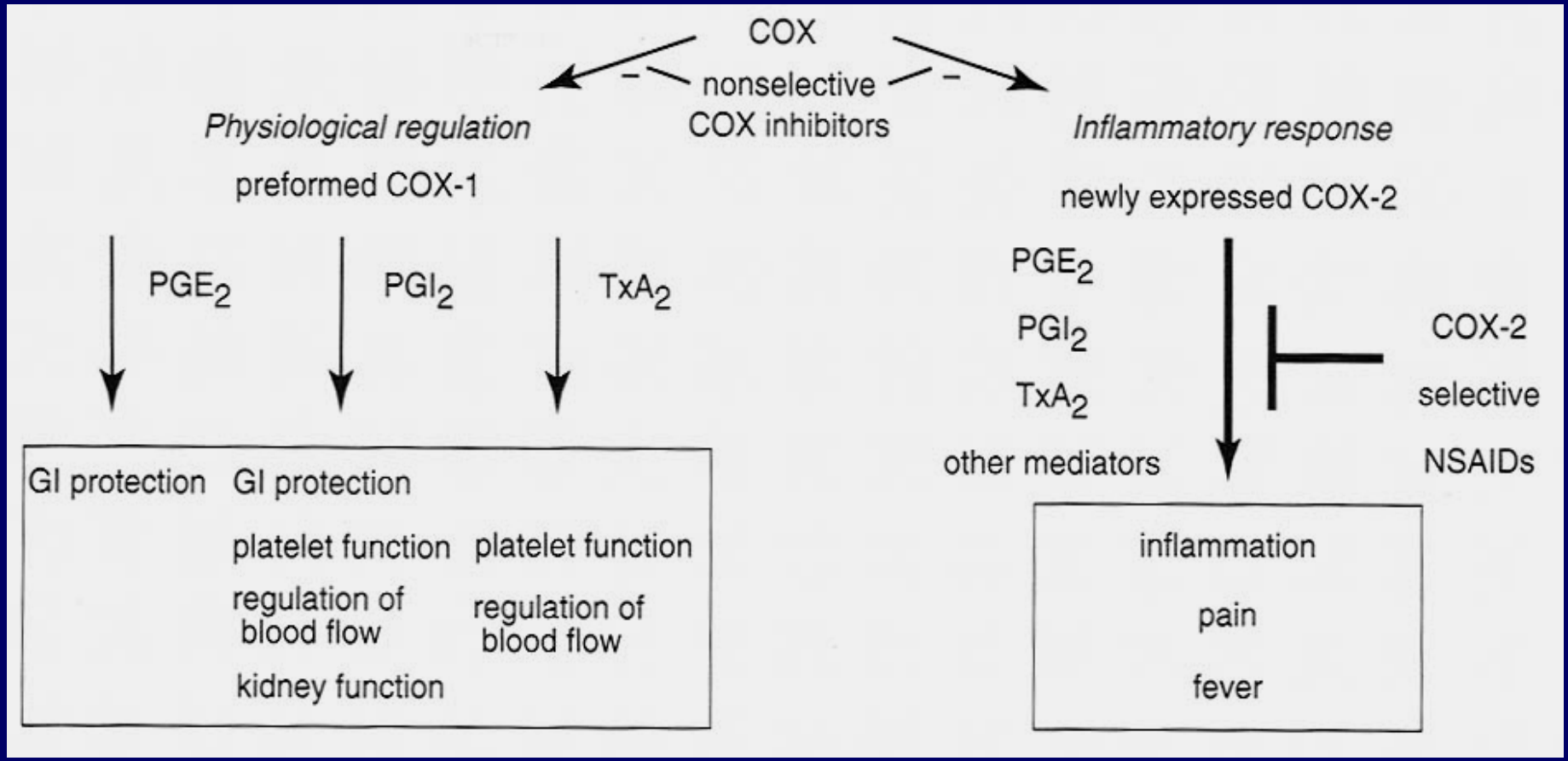


Prostaglandine e rene

IMPLICATE IN:

- Controllo della secrezione di renina
- Regolazione del tono vascolare
- Controllo della funzione tubulare

- Aumentano il flusso ematico renale
- Si oppongono agli effetti dell'angiotensina II attraverso la dilatazione dei vasi renali
- Inibiscono il riassorbimento di Na
- Stimolano la secrezione di renina in risposta a ipovolemia
- Si oppongono al riassorbimento di H_2O indotto dall'ormone antidiuretico (ADH)



Frolich JC 1997 TiPS 18: 30-34

Danno tissutale Agenti infettivi Reazioni immunitarie

Processo infiammatorio:

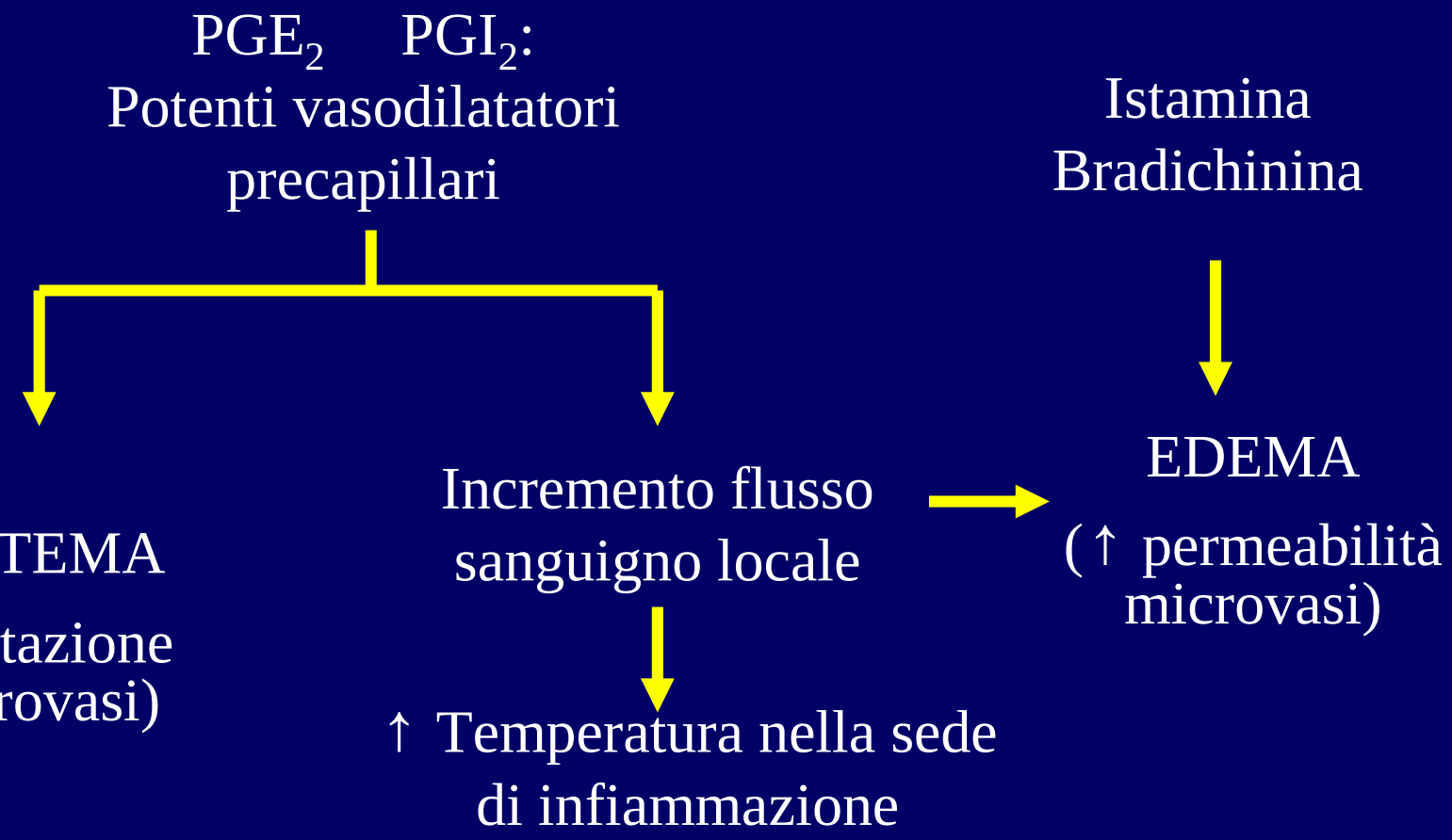
- Eritema
- Aumento temperatura locale
- Edema
- Iperalgesia
- Dolore
- Alterata funzione tessuto ed organo interessati

Le prostaglandine contribuiscono ai segni clinici dell'infiammazione

FANS → Inibizione COX

↓

Riduzione eritema ed edema



FANS

↓

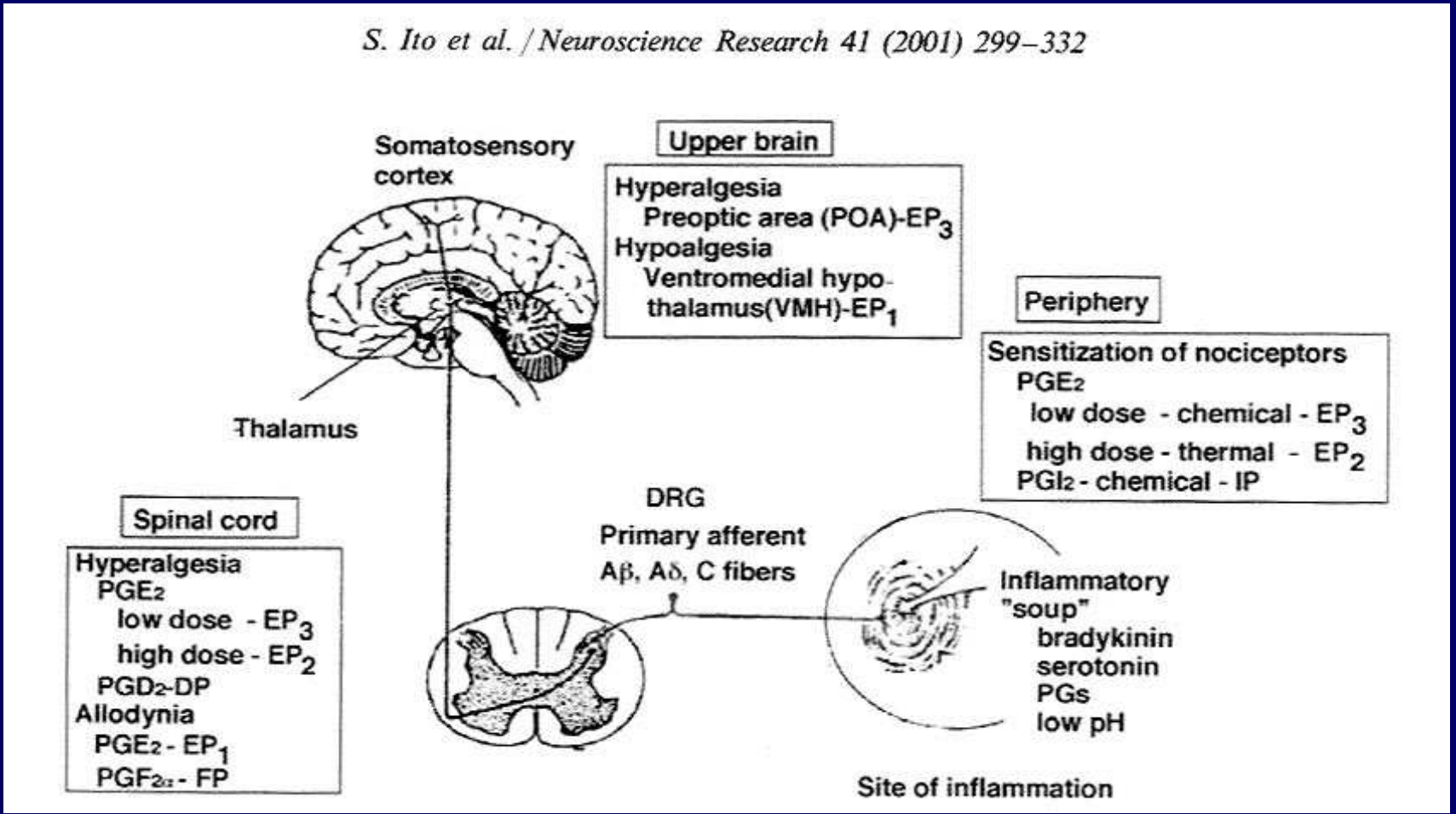
Inibizione sintesi prostaglandine

↓

EFFETTI ANALGESICI

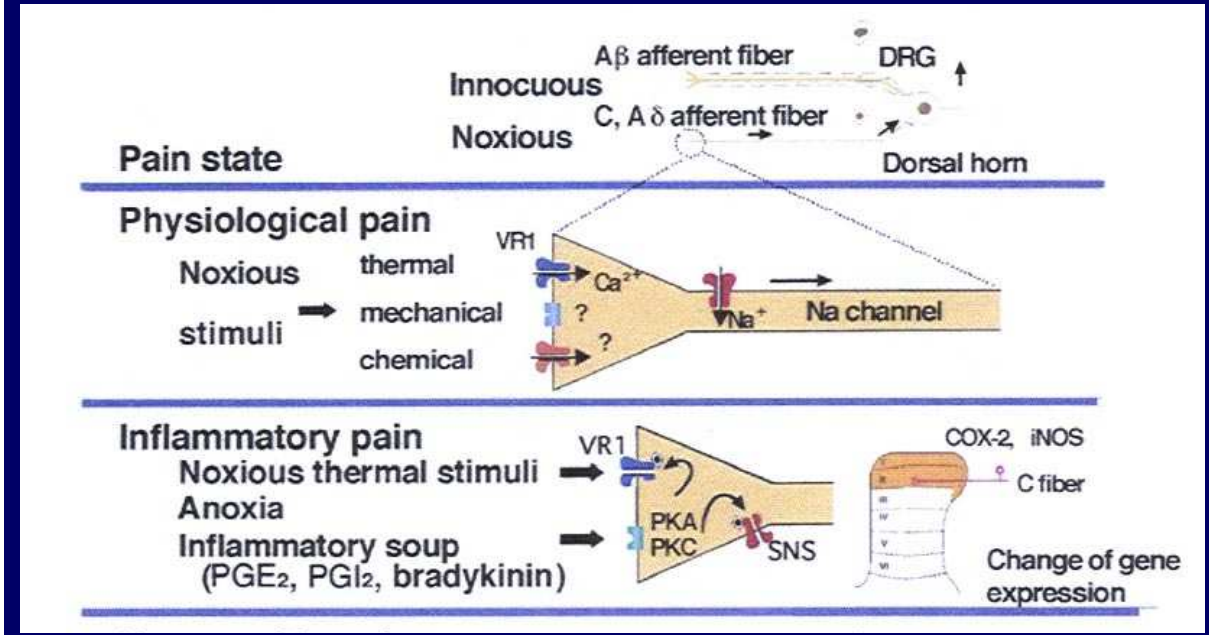
Dolore che accompagna l'infiammazione e il danno tissutale

Prostaglandine e nocicezione



Prostaglandine: meccanismi di sensitizzazione periferica

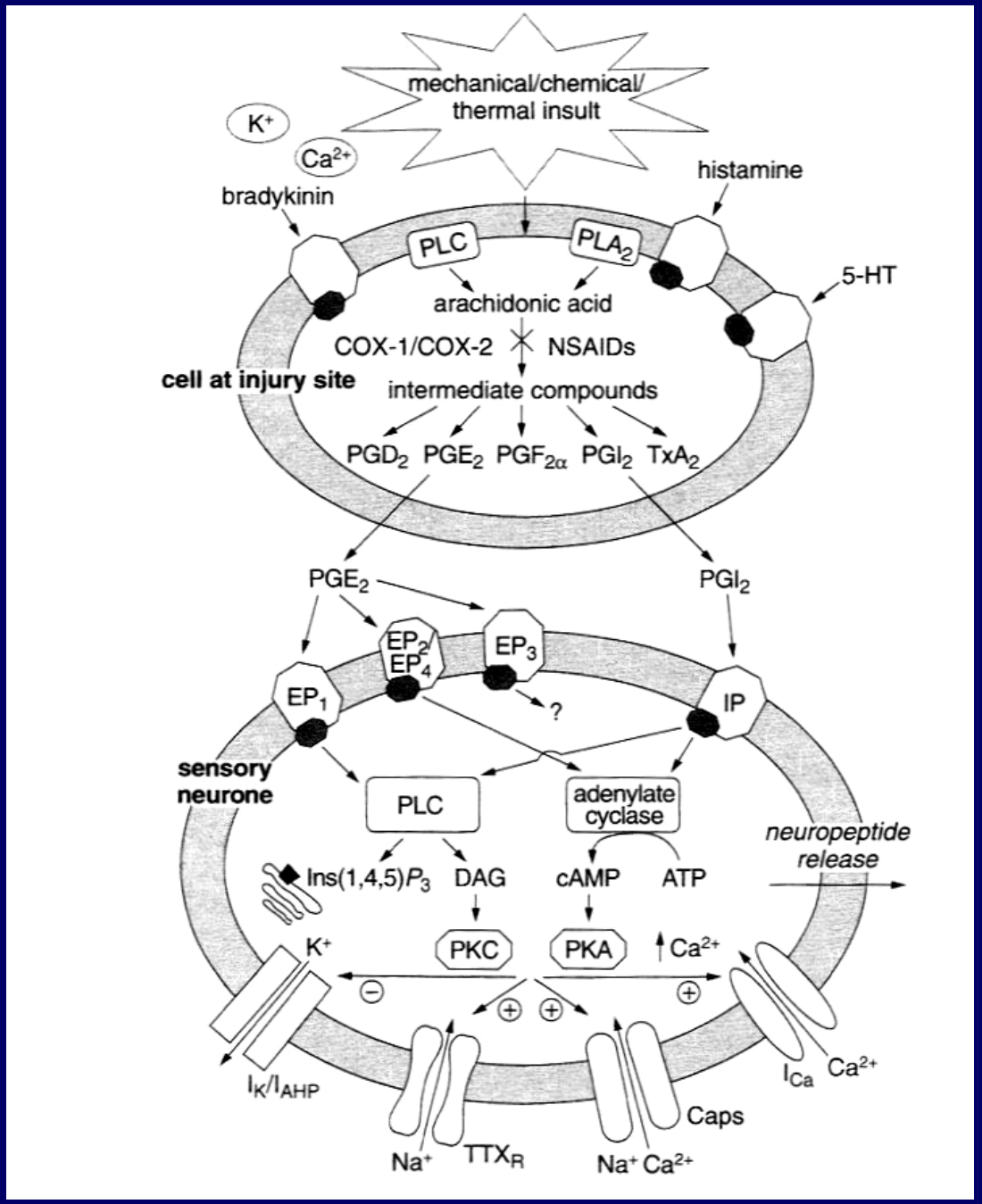
Sensibilizzano i nocicettori agli effetti algogeni di mediatori (es. bradichinina, istamina) liberati durante il processo infiammatorio



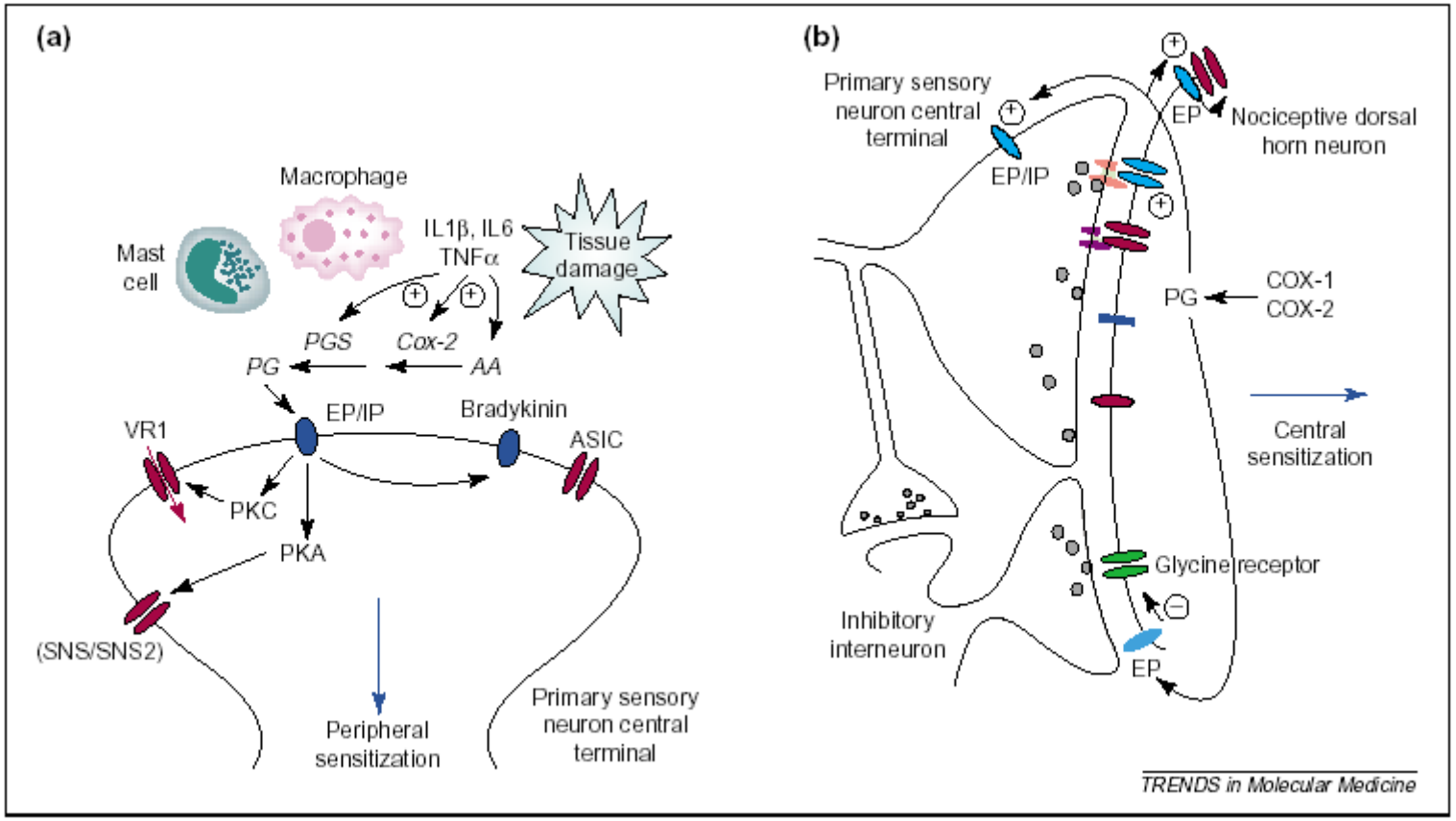
Modulano l'attività di canali ionici in periferia

- Aumento dell'attività dei canali (VR 1) sensibili alla capsaicina (effetto PKA-dipendente)

- Fosforilazione PKA-dipendente di canali al Na^+ voltaggio-dipendenti:
  - *–Abbassamento della soglia di attivazione delle fibre primarie afferenti*



Bley et al., TiPS 1998 vol. 19:141-147



TRENDS in Molecular Medicine Vol.8 No.8 August 2002

Neuroni midollo spinale: espressione costitutiva di COX-1 e COX-2

Infiammazione locale periferica

↓

IL-1β

↓

induzione COX-2 neuroni midollo spinale ed altre aree SNC (es. ipotalamo)

↓

aumento PGE_2 liquor

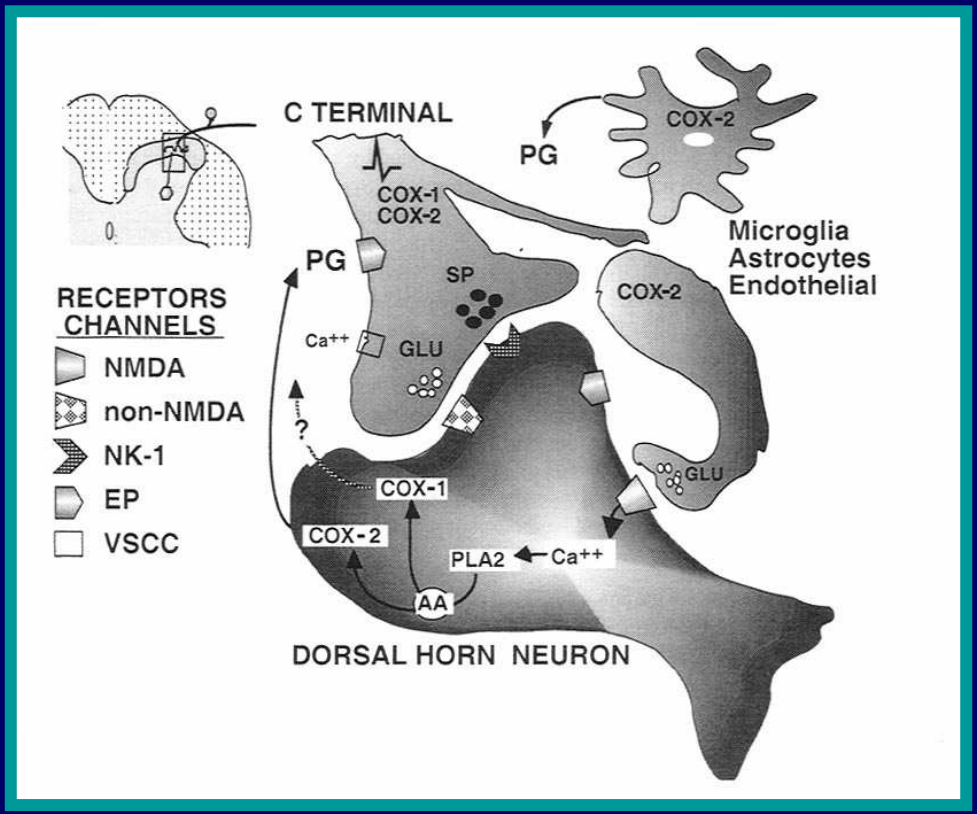
↓

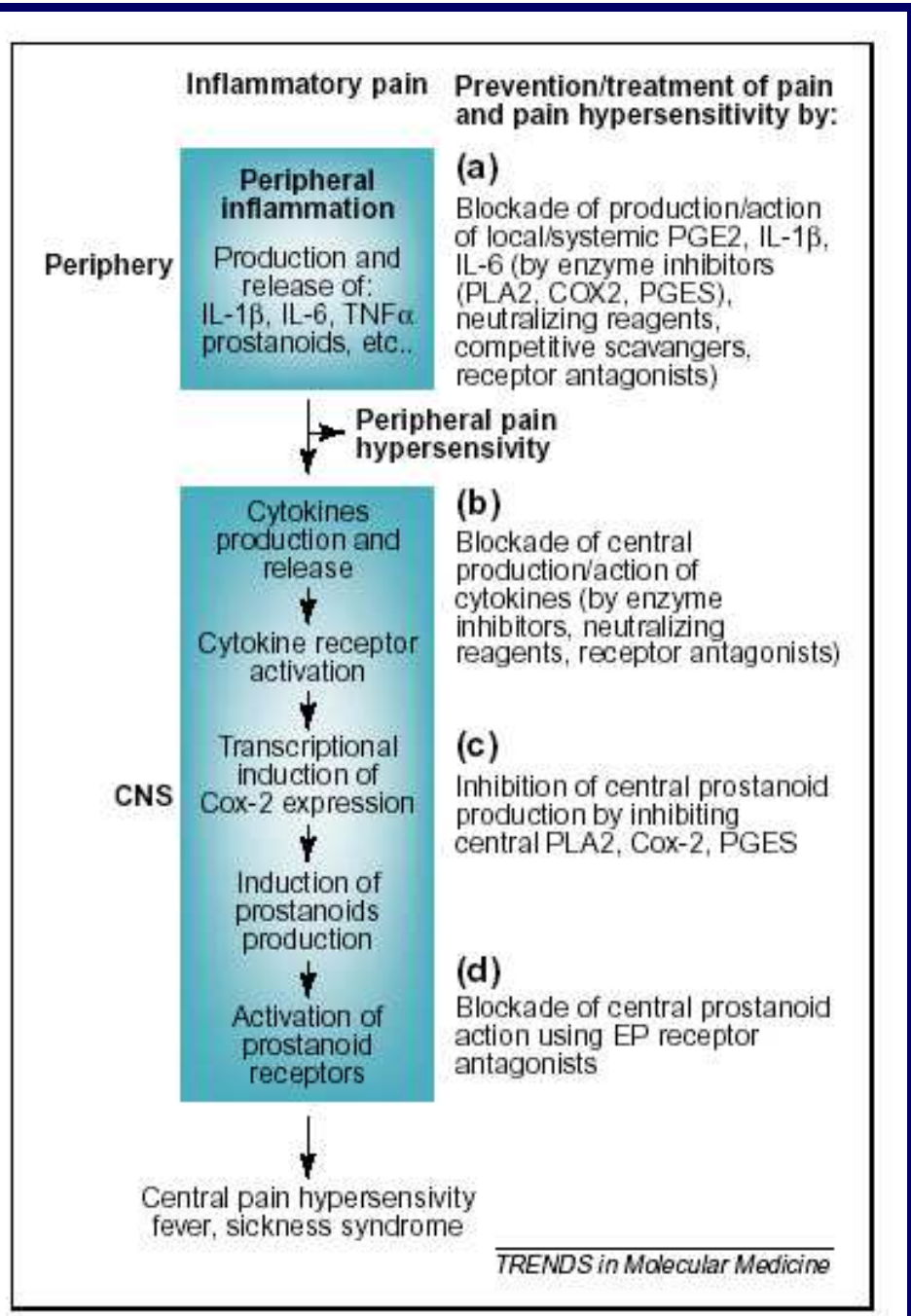
iperalgesia (ma anche altri sintomi: febbre, letargia, anoressia)

L'inibizione selettiva della COX-2 ma non della COX-1 riduce i livelli di PGE_2 nel liquor in modelli sperimentali di infiammazione periferica

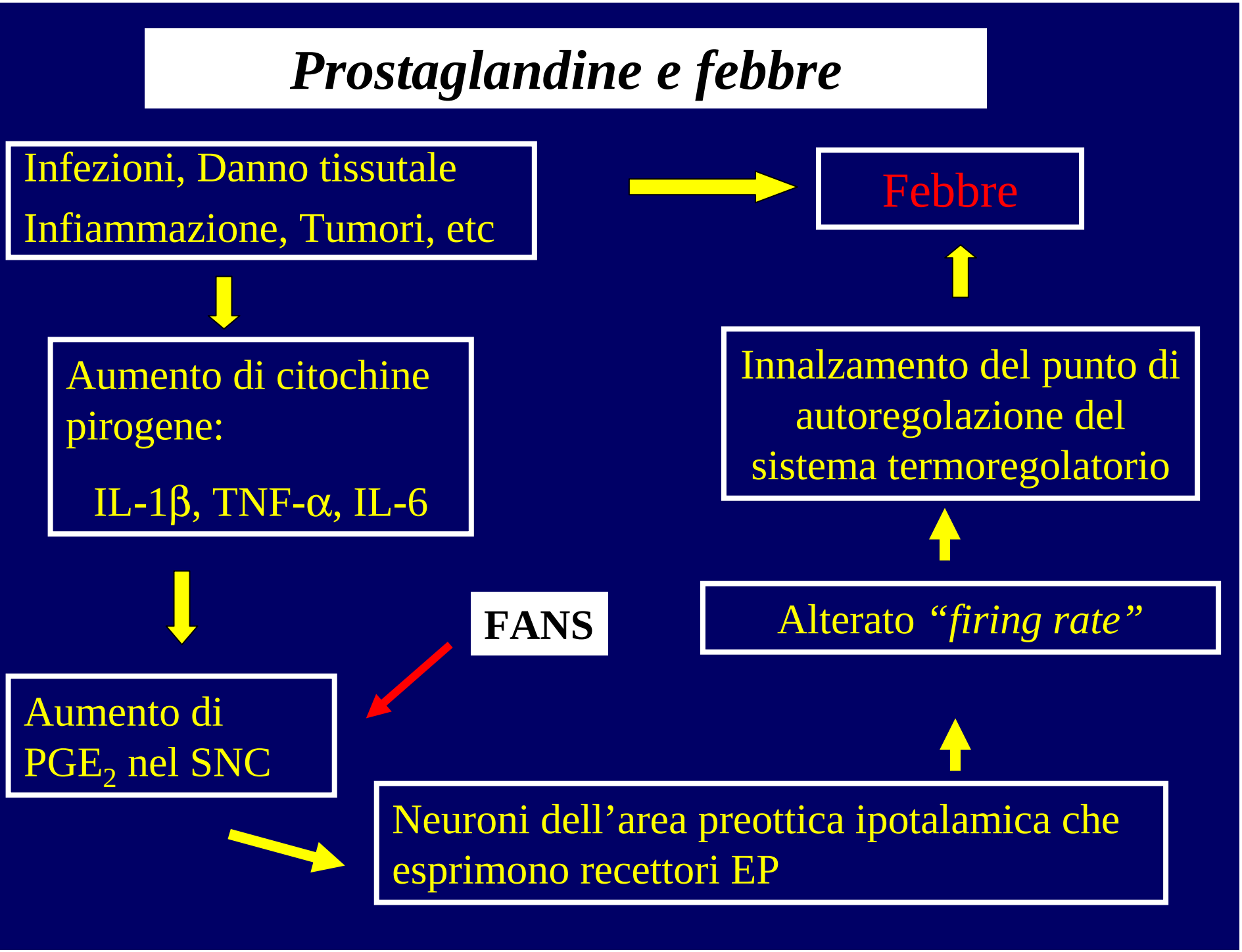
FANS: Effetti analgesici

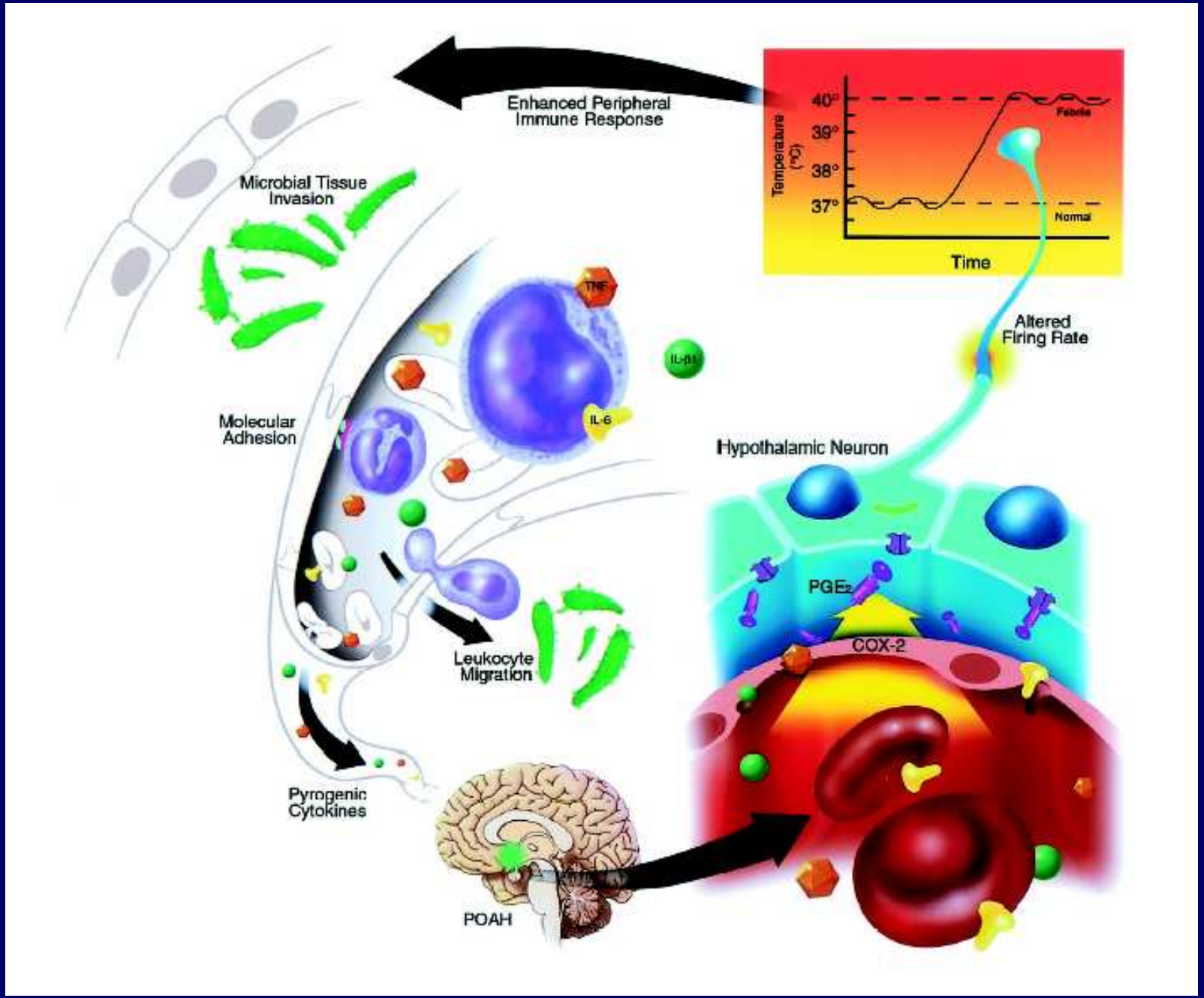
Possono essere coinvolte anche azioni centrali




Inflammatory pain
Prevention/treatment of pain and pain hypersensitivity by:
Periphery
Peripheral inflammation
Production and release of: IL-1β, IL-6, TNFα prostanoids, etc..
(a)
Blockade of production/action of local/systemic PGE2, IL-1β, IL-6 (by enzyme inhibitors (PLA2, COX2, PGES), neutralizing reagents, competitive scavangers, receptor antagonists)
Peripheral pain hypersensivity
CNS
Cytokines production and release
Cytokine receptor activation
Transcriptional induction of Cox-2 expression
Induction of prostanoids production
Activation of prostanoid receptors
(b)
Blockade of central production/action of cytokines (by enzyme inhibitors, neutralizing reagents, receptor antagonists)
(c)
Inhibition of central prostanoid production by inhibiting central PLA2, Cox-2, PGES
(d)
Blockade of central prostanoid action using EP receptor antagonists
Central pain hypersensivity fever, sickness syndrome
TRENDS in Molecular Medicine

Prostaglandine e febbre




Enhanced Peripheral Immune Response
Temperature (°C)
40°
39°
38°
37°
Febrile
Normal
Time
Microbial Tissue Invasion
TNF
IL-β1
IL-6
Molecular Adhesion
Altered Firing Rate
Hypothalamic Neuron
PGE2
COX-2
Leukocyte Migration
Pyrogenic Cytokines
POAH

FANS: effetti antipiretici

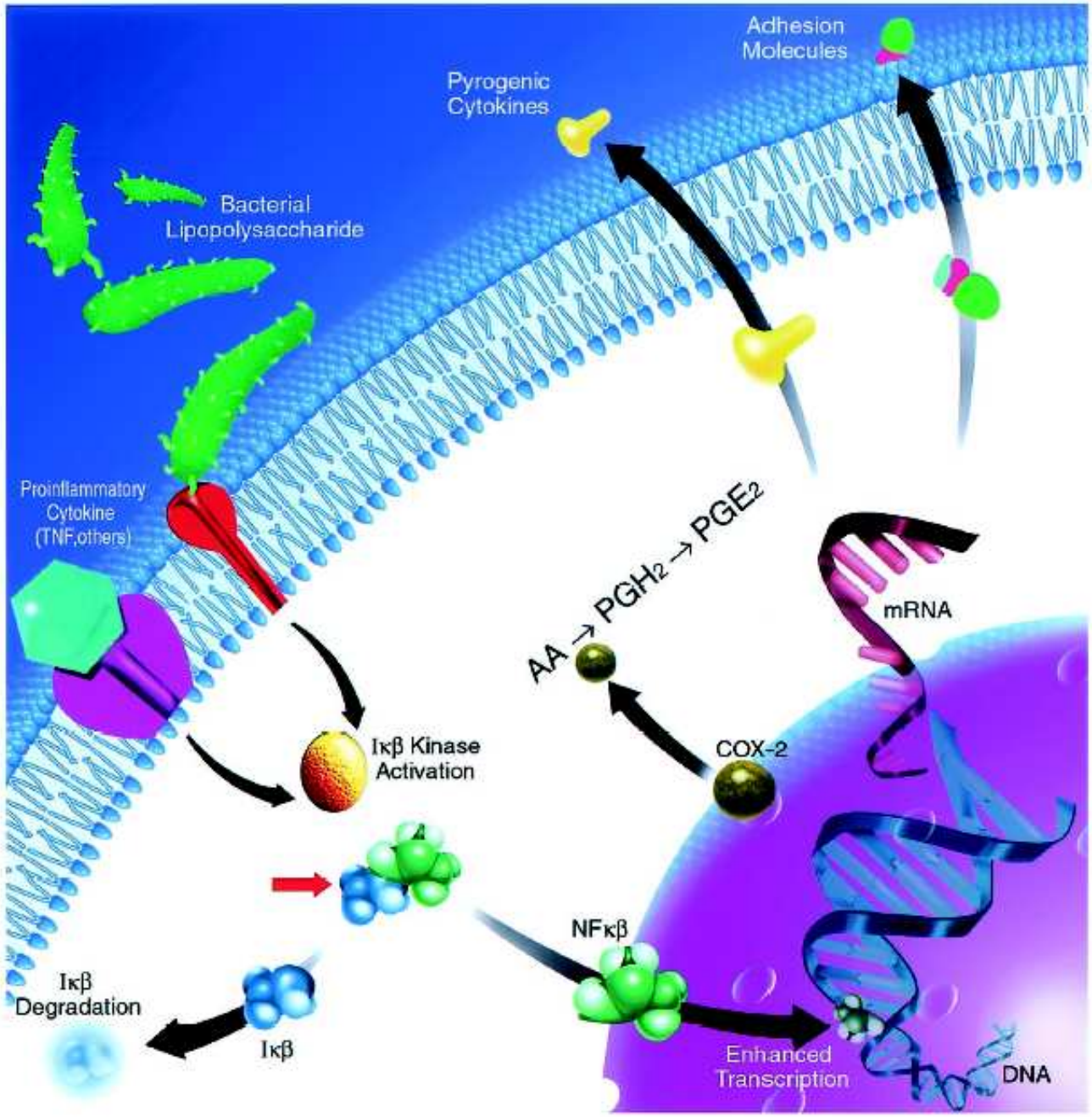
COX-1 o COX-2?

- Inibitori selettivi COX-2 esplicano azione antipiretica
- La risposta febbrile all'LPS è prevenuta nei topini COX-$2^{-/-}$ ma non nei topini COX-$1^{-/-}$

Meccanismi COX-indipendenti?

Suggeriti da osservazioni sperimentali:

- Inibizione infiltrazione leucocitaria nella sede di infiammazione
  - Riduzione espressione molecole di adesione (ICAM-1, VCAM-1, L-selectina)
- Riduzione produzione citochine
- Stimolazione mediatori antinfiammatori (adenosina, 15-epi-lipoxin A_4e B_4)


Adhesion
Molecules
Pyrogenic
Cytokines
Bacterial
Lipopolysaccharide
Proinflammatory
Cytokine
(TNF,others)
AA → PGH2 → PGE2
mRNA
Iκβ Kinase
Activation
COX-2
NFκβ
Iκβ
Degradation
Iκβ
Enhanced
Transcription
DNA

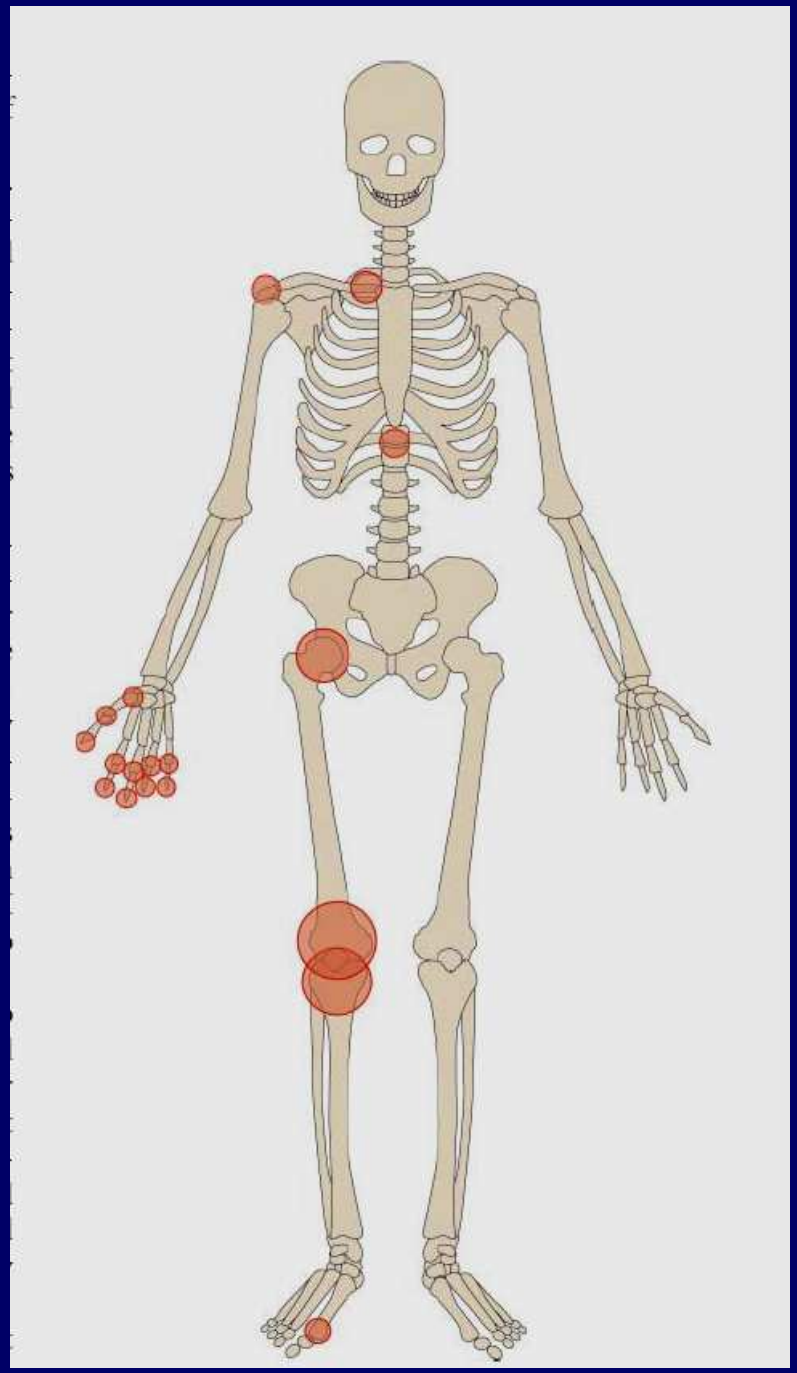


Table 1 | **Osteoarthritis epidemiology***

Country	2002	2007	2012
United States	13.2	14.4	15.5
Europe	14.5	15.2	15.8
Japan	6.6	6.9	7.2
OA total prevalent cases	34.3	36.5	38.6
RA total prevalent cases	2.8	3.1	3.4

*Number (in millions) of diagnosed total prevalent cases of OA (see REF. 7 for more details). Adapted from REF. 7. OA, osteoarthritis; RA, rheumatoid arthritis.

Figure 1 | **Common target sites for osteoarthritis.** The most common target joint affected by osteoarthritis (OA) is the knee joint, whereas hip, shoulder, spine and toe are less frequently affected. OA has a slow, insidious onset and mostly affects only one or a few joints (in contrast to rheumatoid arthritis, which is a systemic multi-joint disease). OA is a leading cause of disability and has a substantial economic impact[2].

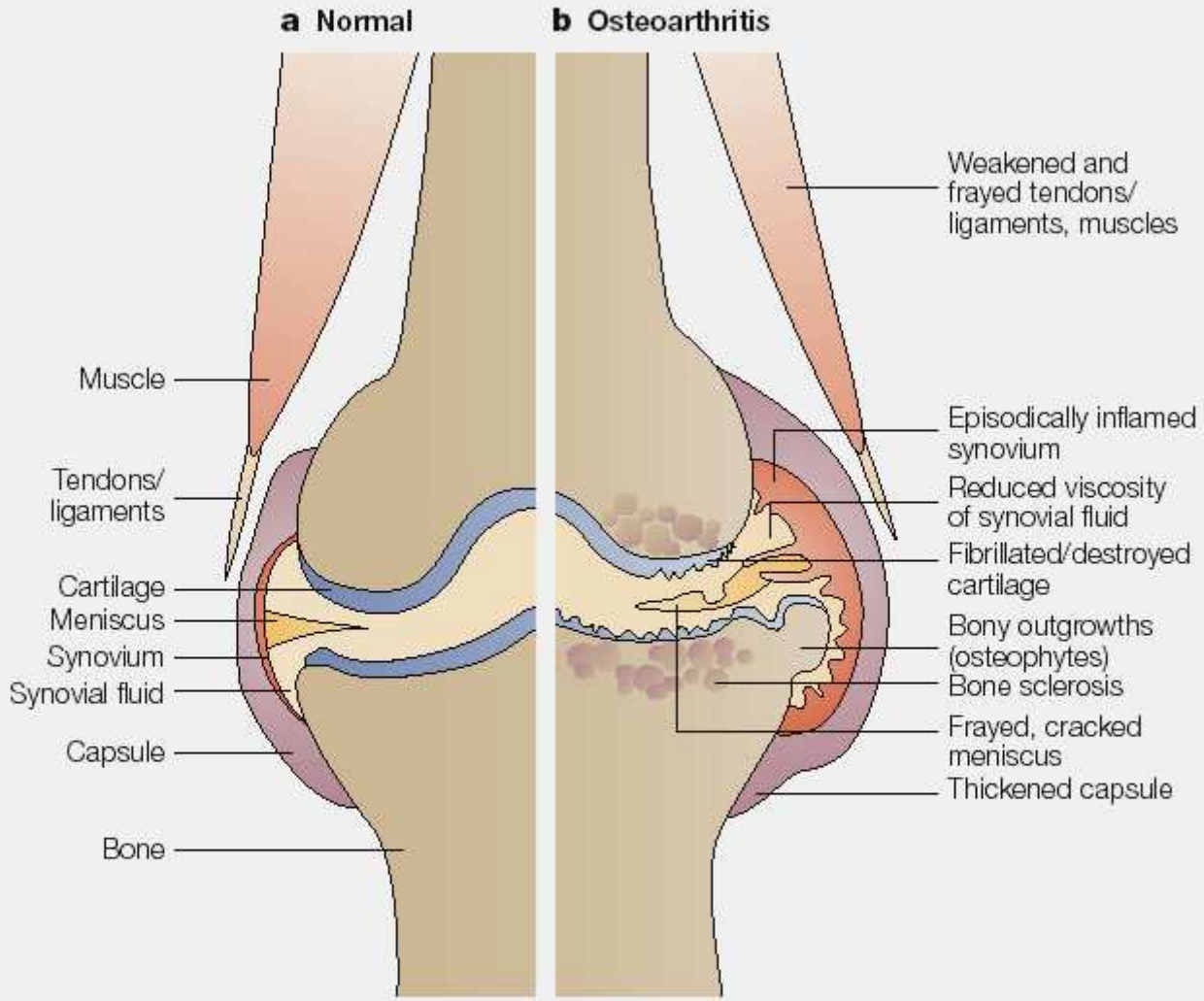


Figure 2 | **Articular structures that are affected in osteoarthritis. a** | Healthy tissue is shown: normal cartilage without any fissures, no signs of synovial inflammation. **b** | Early focal degenerate lesion and 'fibrillated' cartilage, as well as remodelling of bone, is observed in osteoarthritis. This can lead to bony outgrowth and subchondral sclerosis.

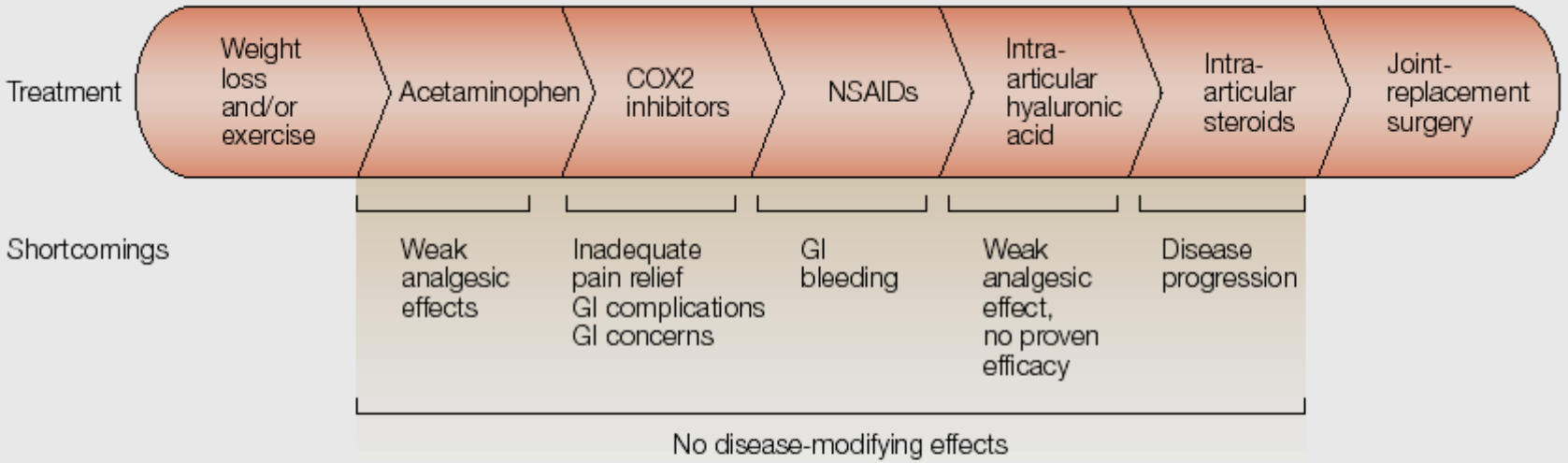


Figure 3 | **Current osteoarthritis treatment options.** The current treatment options as issued in the guidelines from the American College of Rheumatology are fairly limited. In addition to non-pharmaceutical measures such as weight loss and physical exercise they include only symptomatic treatment of limited efficacy with analgesics, non-steroidal anti-inflammatory agents or intra-articular administration of steroids or hyaluronic acid. Because no drugs exist that prevent or halt osteoarthritic joint destruction, the ultimate measure is joint replacement. COX2, cyclooxygenase 2; GI, gastrointestinal; NSAID, non-steroidal anti-inflammatory drug.

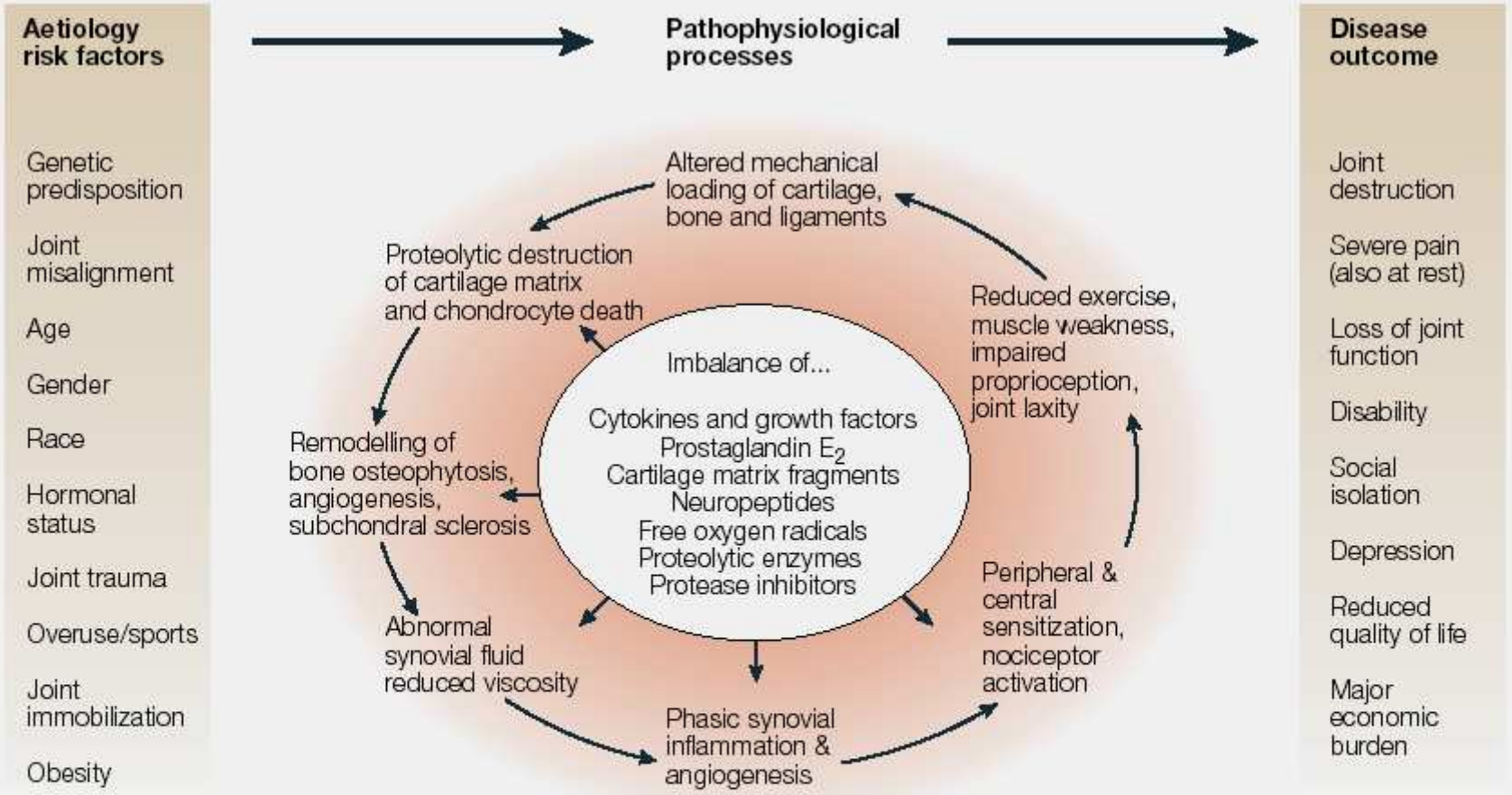
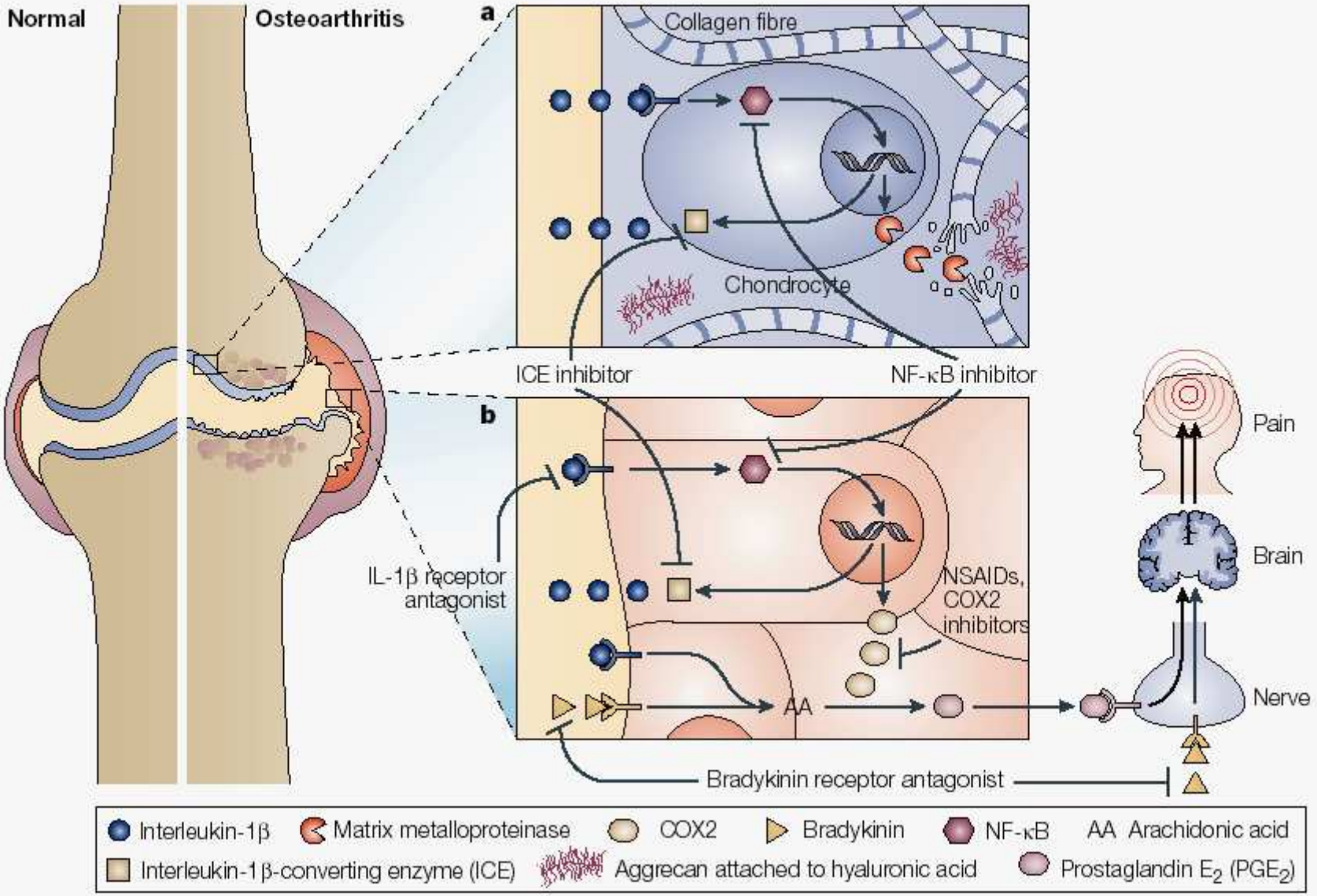


Figure 4 | **Vicious cycle of osteoarthritis.** This is a simplified scheme showing the intricate relationship between aetiological factors (left), pathophysiological processes (central) and disease outcome (right). The pathophysiological processes influence and often amplify each other in a vicious cycle. For instance, joint mis-alignment (left) can contribute to cartilage destruction and subchondral bone sclerosis. As a consequence, pain originates due to mechanical and chemical nociceptor activation, and reduces quality of life, often resulting in disability and social isolation (right). The dysregulation of certain biochemical factors shown in the inner cycle drives the disease process that finally leads to joint destruction. The individual trigger of disease onset is often unknown.


Normal
Osteoarthritis
a
Collagen fibre
Chondrocyte
ICE inhibitor
NF-κB inhibitor
b
IL-1β receptor antagonist
NSAIDs, COX2 inhibitors
AA
Pain
Brain
Nerve
Bradykinin receptor antagonist
Interleukin-1β
Matrix metalloproteinase
COX2
Bradykinin
NF-κB
AA Arachidonic acid
Interleukin-1β-converting enzyme (ICE)
Aggrecan attached to hyaluronic acid
Prostaglandin E2 (PGE2)

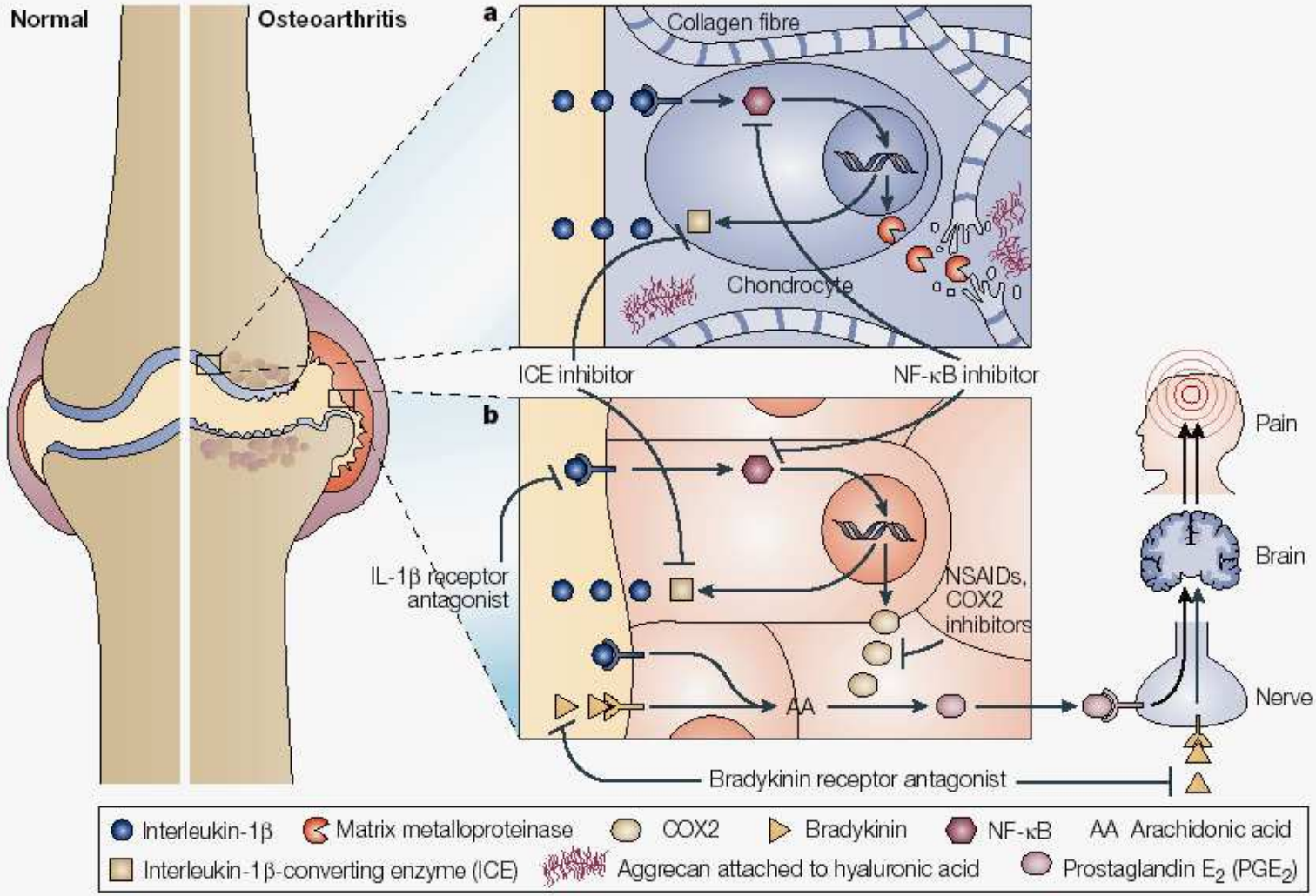
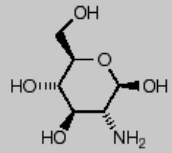
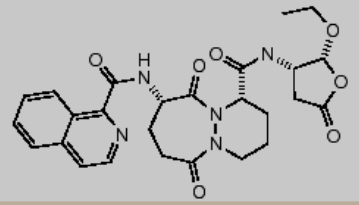
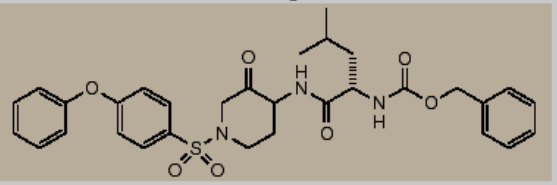
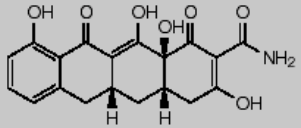
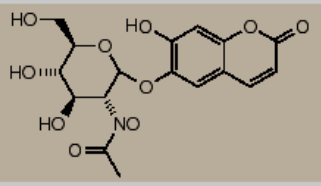
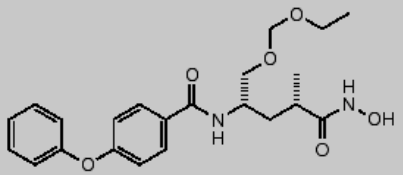


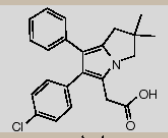
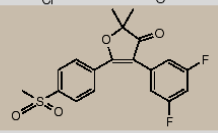
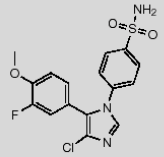
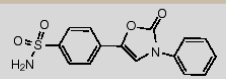
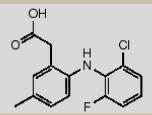
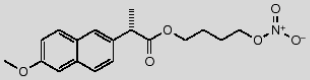
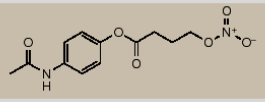
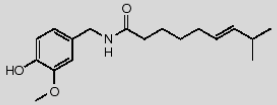
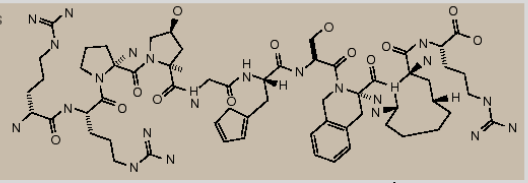
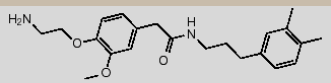
Figure 5 | **Targets for the development of disease- (a) or symptom-modifying (b) drugs for osteoarthritis. a** | Degenerative processes in cartilage, and potential targets for disease modification. A chondrocyte embedded in the network of collagen fibres and aggrecan is shown. IL-1β induces the expression of matrix proteases, which degrade the matrix components (shown on the right of panel **a**). The matrix metalloproteinases are targets with potential for disease modification. Interleukin-converting enzyme (ICE) converts IL-1β to its active form and, therefore, represents another target for disease modification. **b** | Nociception and possible ways of interfering with it. Inhibiting the production of the inflammatory cytokine IL-1β or blocking its receptors or interrupting its subsequent intracellular signalling through nuclear factor-κB (NF-κB) and the blockade of bradykinin receptors are more recent approaches to developing symptom-modifying drugs with greater efficacy than non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit the formation of the pain mediator prostaglandin E_2. COX2, cyclooxygenase 2.

Table 2 | **Disease-modifying drugs currently in clinical trials for osteoarthritis**

Drug	Class	Phase	Company	Chemical structure
Glucosamine	Non-pharmaceutical	III	NIH	
VX-765	ICE inhibitor	I	Vertex	Unavailable
Pralnacasan	ICE inhibitor	II	Vertex/Sanofi-Aventis	
SB-462795	Cathepsin K inhibitor	I	GlaxoSmithKline	
Doxycycline	Antibiotic	III	FDA/NIH	
CPA-926	Inhibits MMP expression	II	Kureha	
ONO-4817	MMP inhibitor	I	Pfizer	
S-3536	MMP inhibitor	I	Shionogi	Unavailable
PG-530742	MMP inhibitor	II	Procter & Gamble	Unavailable
CP-544439	MMP inhibitor	I	Pfizer	Unavailable

FDA, Food and Drug Administration; ICE, interleukin-1β-converting enzyme; MMP, matrix metalloproteinase; NIH, National Institutes of Health.

Table 3 | **Symptom-modifying drugs currently in clinical trials for osteoarthritis**

Drug	Class	Phase	Company	Chemical structure
Licofelone	COX/LOX inhibitor	III	Merckle	
PAC-10549	COX2 inhibitor	I	Pacific	
Cimicoxib	COX2 inhibitor	I	Uriach	
GW-406381	COX2 inhibitor	II	GlaxoSmithKline	Unavailable
LAS-34475	COX2 inhibitor	II	Almirall	
CS-502	COX2 inhibitor	II	Sankyo	Unavailable
Prexige	COX2 inhibitor	III	Novartis	
Medinox	NSAID	I	Medinox	Unavailable
NO-naproxen	NO analgesic	II	NicOX	
NCX-701	NO analgesic	II	NicOX	
ALGRX-4975	NO analgesic	I	AlgoRx	
ADL-100116	Peripheral κ-opioid agonist	I	Adolor	Unavailable
AD827	Cytokine synthesis inhibitor	I	Arakis	Unavailable
HOE140	Bradykinin B_2 receptor antagonist	II	Sanofi-Aventis	
DA-5018	Capsaicin analogue	I	Dong-A	

COX2, cyclooxygenase 2; LOX, lipooxygenase; NO, nitric oxide; NSAID, non-steroidal anti-inflammatory drug.

FARMACI ANALGESICI, ANTIPIRETICI ED ANTINFIAMMATORI NON STEROIDEI

INIBITORI NON SELETTIVI DELLA COX

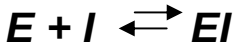
- aspirina, salicilato di sodio, salsalato, diflunisal, sulfasalazina, olsalazina
- paracetamolo
- indometacina, sulindac
- tolmetin, diclofenac, ketorolac
- ibuprofene, naproxene, flurbiprofene, ketoprofene, fenoprofene, oxaprozina
- ac. mefenamico, ac. meclofenamico
- piroxicam, meloxicam
- nabumetone

INIBITORI SELETTIVI COX-2

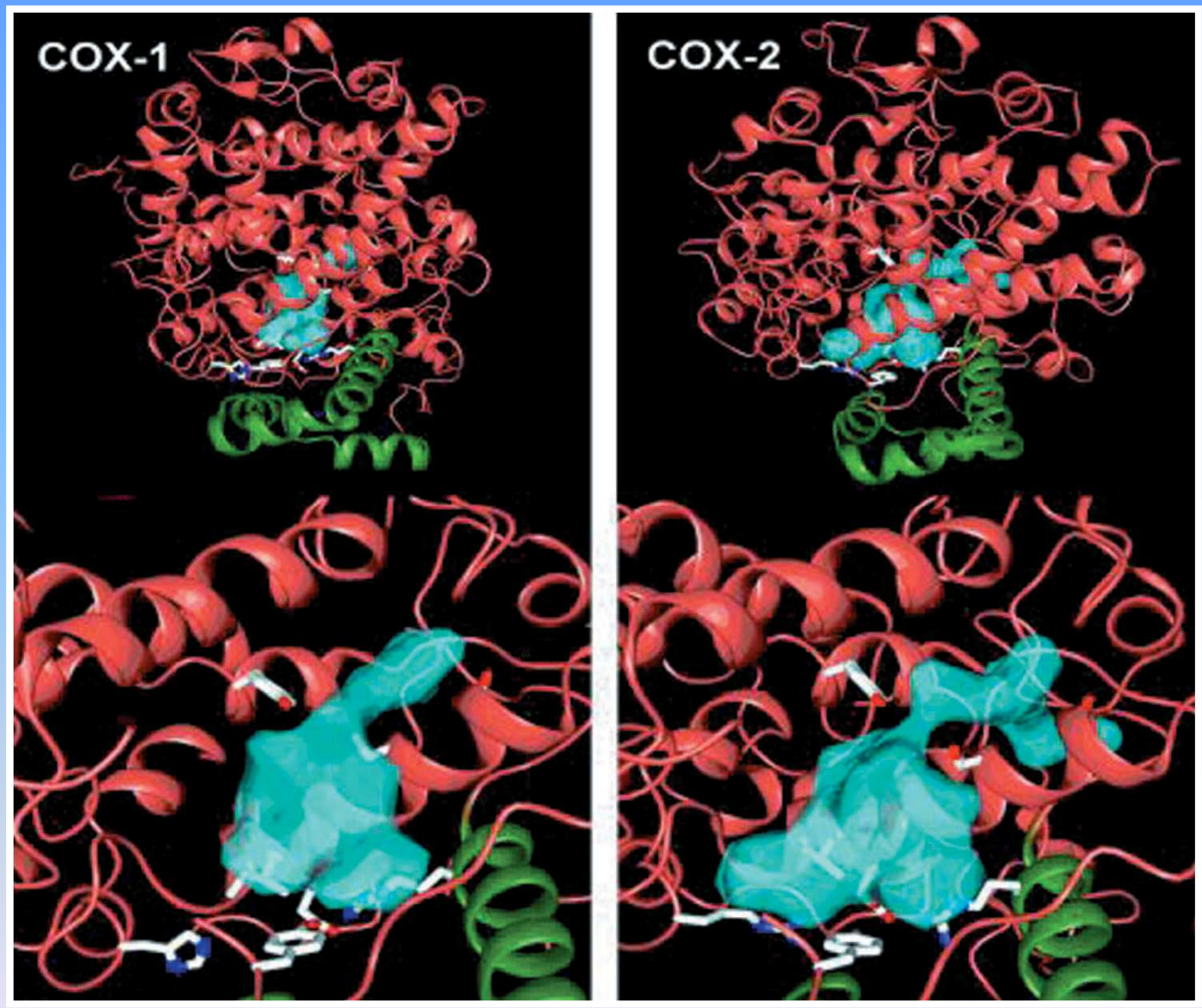
- rofecoxib • celecoxib • etodolac • nimesulide

Adattata da Goodman & Gilman –The Pharmacological Basis of Therapeutics- X Ed., McGraw Hill, 2001

Classificazione dei FANS in base al meccanismo di inibizione delle PGH-sintasi

Classe I	Classe II	Classe III
Meccanismo competitivo		
Semplice 	***Tempo-dipendente lentamente reversibile*** 	***Tempo-dipendente Irreversibile*** 
Ibuprofene	Indometacina	Ac. acetilsalicilico
Piroxicam	Flurbiprofene	
Sulindac solfuro	Ac. meclofenamico	
Naproxene	Diclofenac	
Ac. flufenamico	Inibitori selettivi PGHS-2[#]	
Ac. mefenamico		

[#] Inibitori reversibili tempo-dipendenti della PGHS-2; inibitori competitivi semplici della PGHS-1



Struttura tridimensionale della COX-1 e della COX-2.

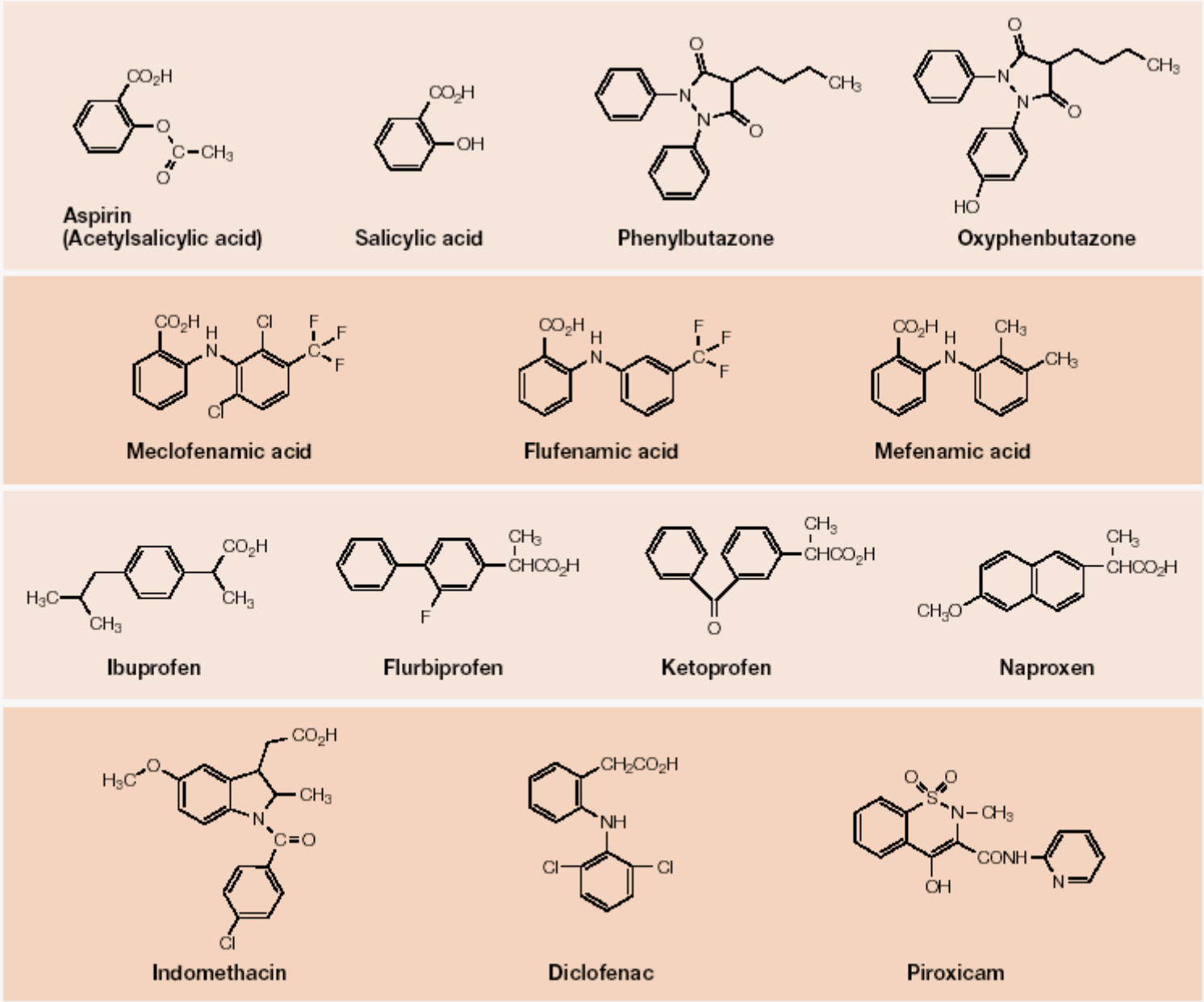


Figure 2 | **Chemical structures of NSAIDs and related compounds.** Structures of some 'classical' NSAIDs, including representative salicylates, pyrazolones, fenamates, proprionates, oxicams and indomethacin. Note the general presence of a carboxylic-acid moiety.

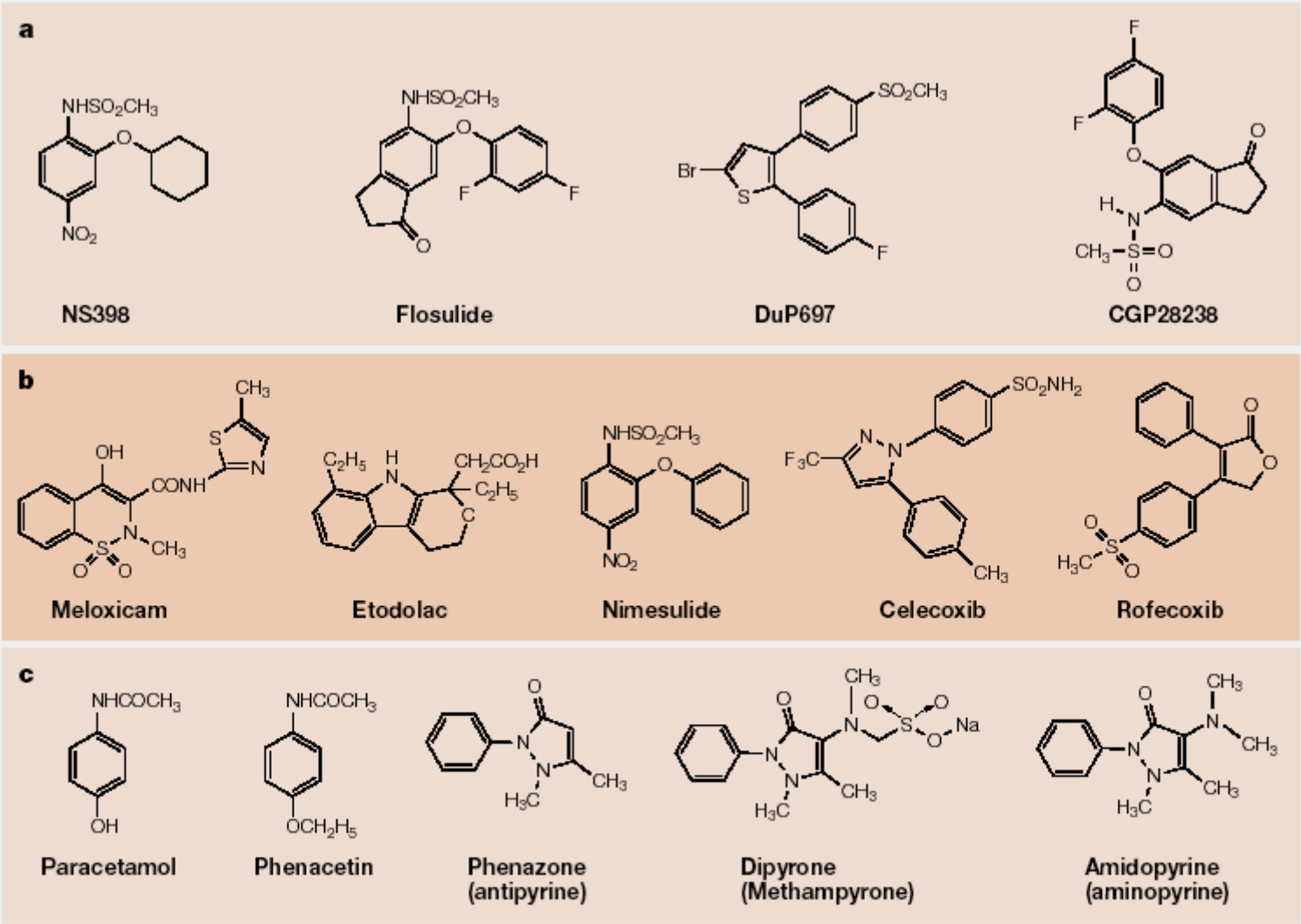


Figure 3 | **Chemical structures of NSAIDs and related compounds. a** | Structures of DuP697, NS398 and other similar compounds. **b** | Selective COX2 inhibitors that were discovered as a result of a search for selective isoform inhibitors (celecoxib and rofecoxib) or that were 'revealed' as being COX2 selective (meloxicam, etodolac and nimesulide). **c** | Structures of some compounds that are more effective inhibitors of COX3 according to Simmons[124].

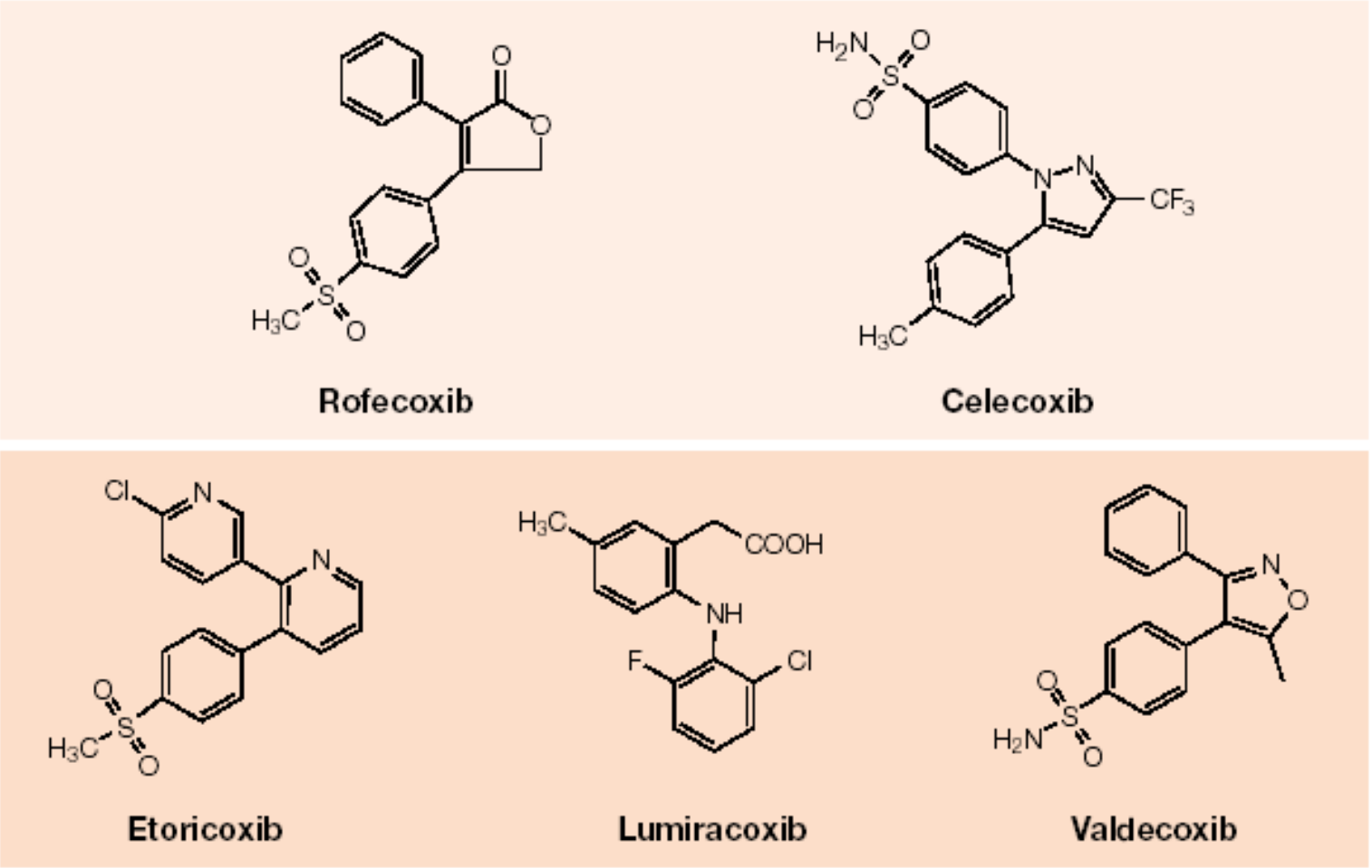


Figure 3 | **Selective inhibitors of COX-2.** The structures of first-generation (that is, rofecoxib and celecoxib) and second-generation (that is, etoricoxib, lumiracoxib and valdecoxib), purpose-designed selective inhibitors of cyclooxygenase-2 (COX-2).

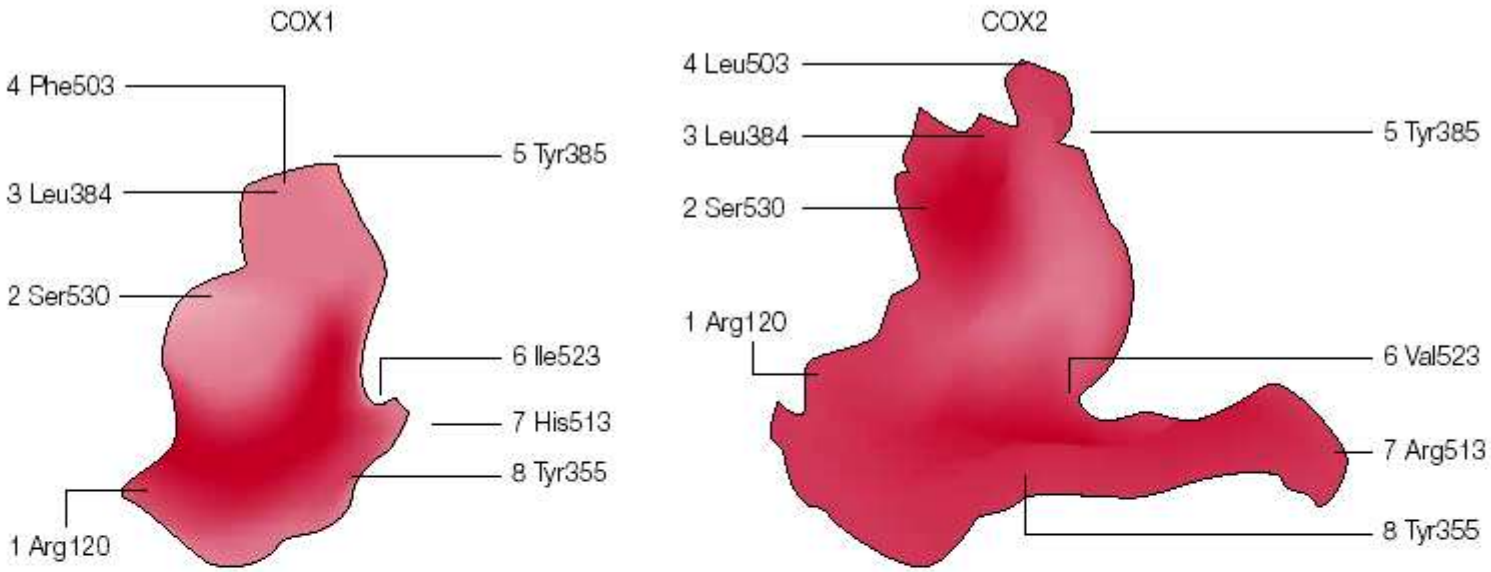


Figure 5 | **Some key residues in COX1 and COX2.** The left-hand panel shows a schematic diagram of the NSAID-binding site in COX1. The highly conserved residues Arg120 and Tyr355 (1 and 8) stabilize the carboxylate group that is present in most NSAIDs, whereas the aromatic ring structures are accommodated within the largely hydrophobic binding channel and often abut onto the highly conserved Tyr385 (5). Tyr385 is close to the peroxidase site that forms a tyrosyl radical that is crucial to the introduction of molecular oxygen into the arachidonic acid substrate. Ser530 (2) is the residue that is acetylated by aspirin. Note the presence of the relatively bulky Ile523 (6) and the presence of Leu384 (3) in proximity to Phe503 (4), and the presence of His513 (7). The right-hand panel depicts the COX2 binding site. The highly conserved residues Arg120 and Tyr355 (1 and 8) are present as before, as is Tyr385 (5) and Ser530 (2). However, residue 4 now becomes Leu503 which, being less bulky, is not packed as tightly. This allows expansion of the available space at the top of the channel. Residue 6 becomes Val523 in COX2, which allows opening of the side pocket. The side pocket can completely accommodate the sulphonamide or analogous group of the COX2 inhibitors that are stabilized by hydrogen bonding with residue 7, which becomes Arg513 in COX2. Overall, the available space in the COX2 binding pocket is more than 25% greater than in the COX1 binding site. This is mainly due to the side pocket and the increase in available space at the top of the channel. Adapted from REFS 72, 143. For clarity, an equivalent numbering system has been used for both COX enzymes. COX, cyclooxygenase; NSAID, non-steroidal anti-inflammatory drug.

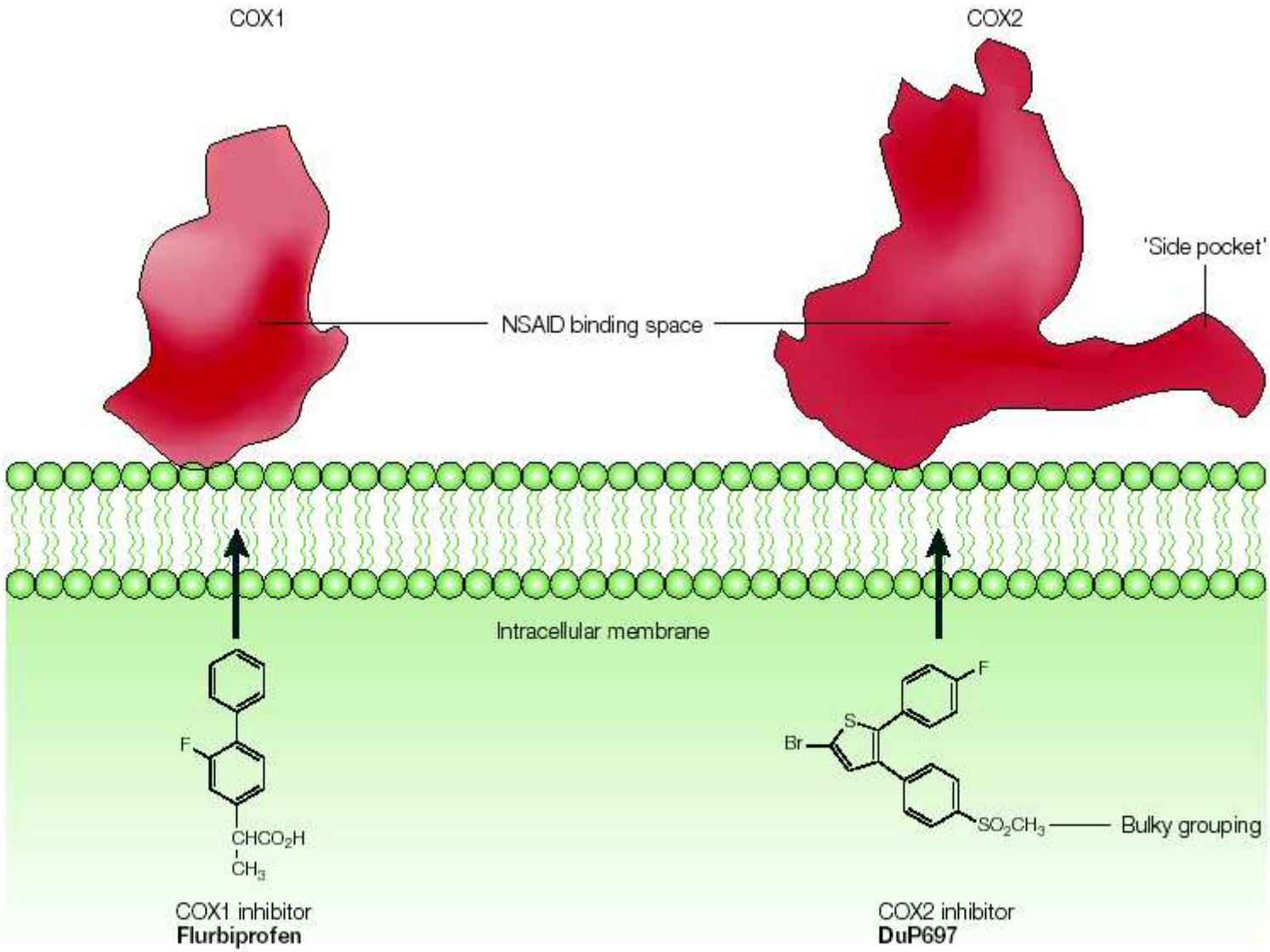


Figure 4 | **Comparison of the NSAID binding sites of COX1 and COX2 after Browner.** Schematic cartoon (modified from REF. 142), showing the differences in the NSAID binding sites of COX1 and COX2. Note that the COX2 binding site is more accommodating and is characterized by a 'side pocket' that can accommodate bulky groups such as the methyl sulphonyl moiety of DuP697. COX, cyclooxygenase; NSAID, non-steroidal anti-inflammatory drug.

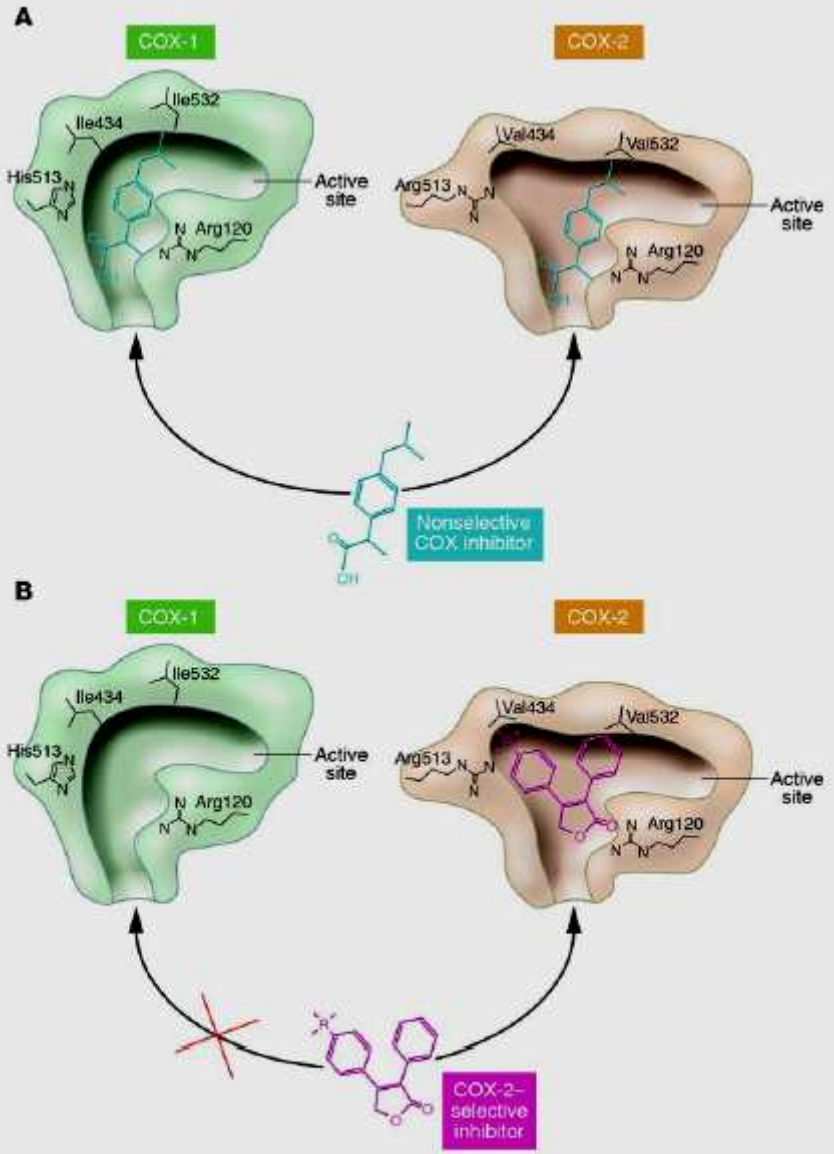
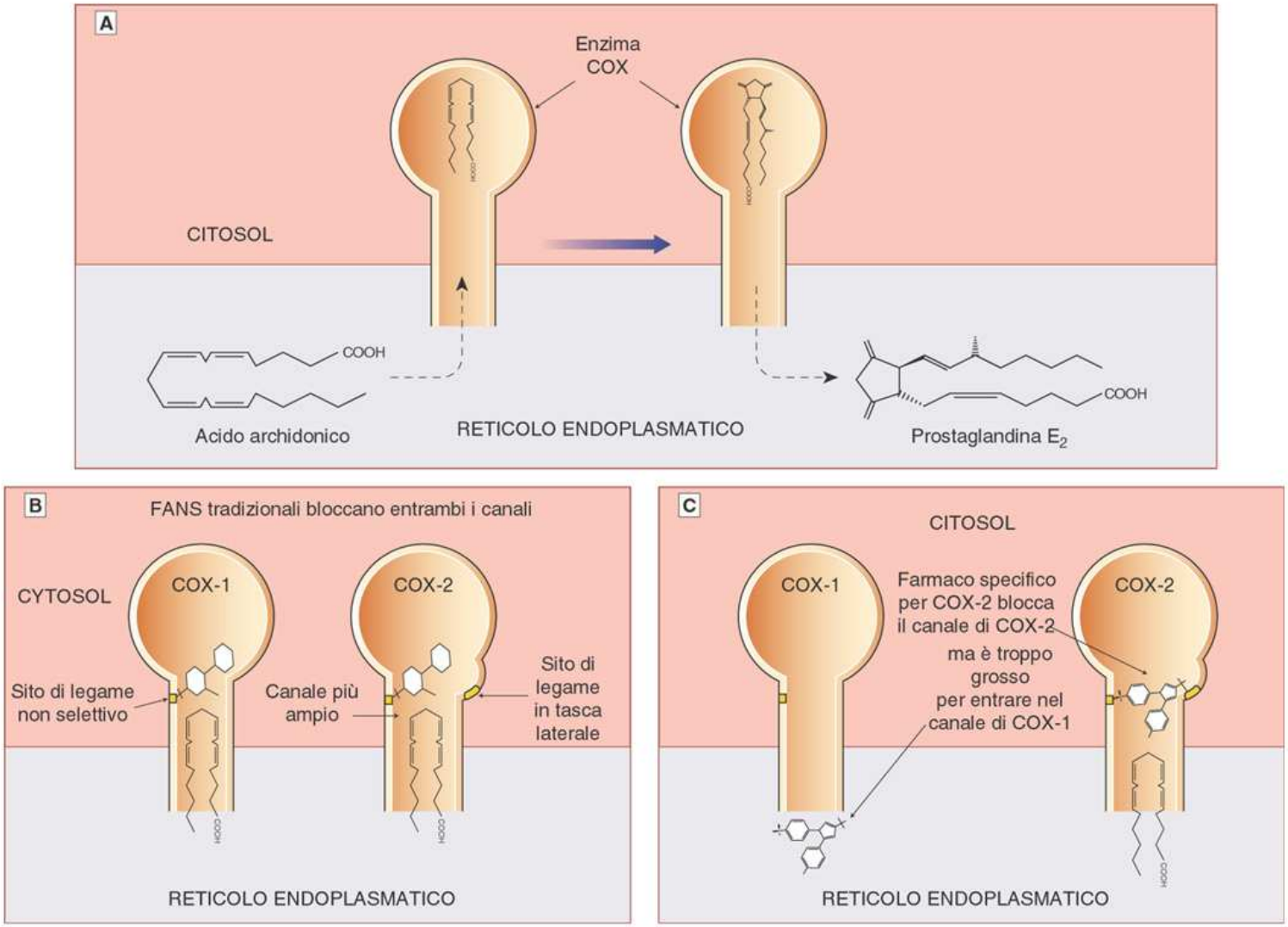
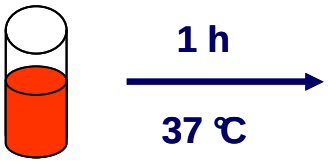


Figure 1
Schematic depiction of the structural differences between the substrate-binding channels of COX-1 and COX-2 that allowed the design of selective inhibitors. The amino acid residues Val434, Arg513, and Val523 form a side pocket in COX-2 that is absent in COX-1. (A) Nonselective inhibitors have access to the binding channels of both isoforms. (B) The more voluminous residues in COX-1, Ile434, His513, and Ile532, obstruct access of the bulky side chains of COX-2 inhibitors. Figure modified with permission from *Nature* from protein structures reported in refs. 18 and 20.


A
Enzima
COX
CITOSOL
COOH
Acido archidonico
RETICOLO ENDOPLASMATICO
COOH
Prostaglandina E_2
B
FANS tradizionali bloccano entrambi i canali
CYTOSOL
COX-1
COX-2
Sito di legame non selettivo
Canale più ampio
Sito di legame in tasca laterale
RETICOLO ENDOPLASMATICO
C
CITOSOL
COX-1
COX-2
Farmaco specifico per COX-2 blocca il canale di COX-2
ma è troppo grosso per entrare nel canale di COX-1
RETICOLO ENDOPLASMATICO

Modello del sangue intero per valutare l'effetto di inibitori della COX sull'attività della COX-1 piastrinica

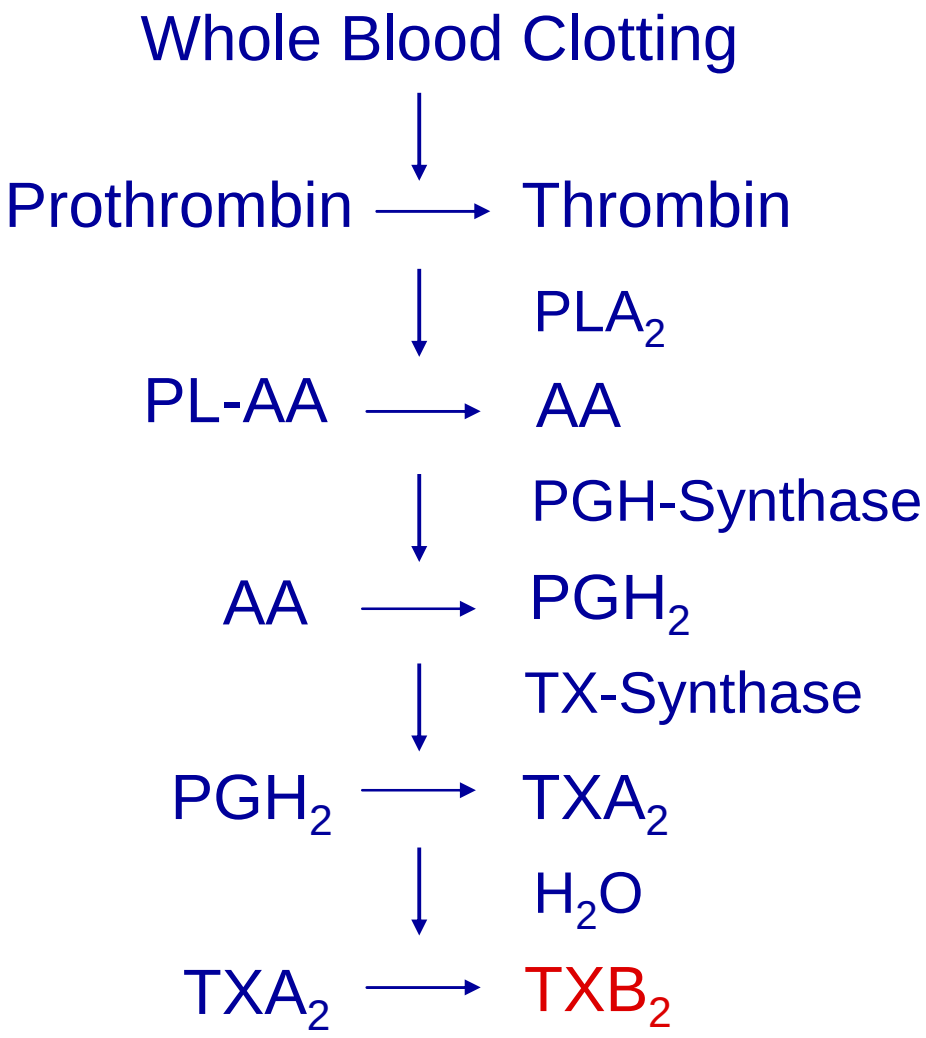


1 h

37 ℃

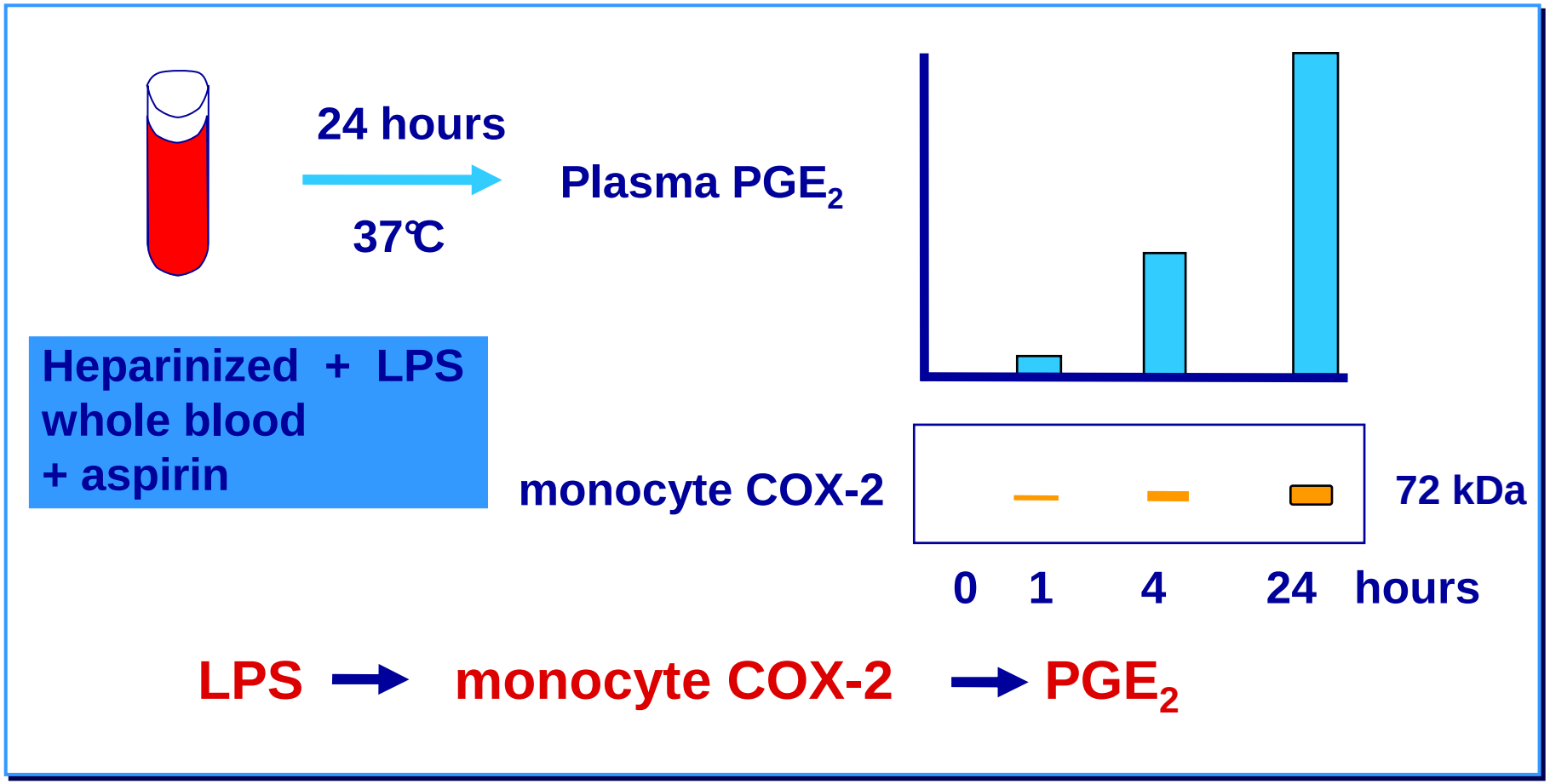
TXB_2 sierico

Sangue intero



Patrono et al, Thromb Res 1980

Whole Blood Assay to Evaluate the Effects of COX-Inhibitors on Monocyte COX-2 Activity *In Vitro* and *Ex Vivo*



Patrignani et al, JPET 1994;271:1705-12

Table 1 Comparison of nonsteroid anti-inflammatory drugs for their selectivity towards COX-1 or COX-2

Drug	IC_{50} COX-1 (μM)	IC_{50} COX-2 (μM)	Ratio IC_{50} COX-2/COX-1	System
Nonselective for COX-2				
Piroxicam	0.0005	0.3	600	Cultured animal cells (110)
Aspirin	1.67	278	166	Cultured animal cells (110)
Indomethacin	0.028	1.68	60	Cultured animal cells (110)
Diclofenac	1.57	1.1	0.7	Cultured animal cells (110)
6-MNA[a]	278	187	0.67	Human whole blood (111)
Selective for COX-2				
Etodolac	34	3.4	0.1	Human whole blood (111)
Meloxicam	4.8	0.43	0.09	Human whole blood (111)
Nimesulide	9.2	0.52	0.06	Human whole blood (111)
SC58125	38.7	0.27	0.007	Human whole blood (111)
NS398	16.8	0.10	0.006	Human whole blood (111)
L-745, 337	369	1.5	0.004	Human whole blood (111)
Celecoxib	15	0.04	0.003	Human enzymes (80)
DFU	>50	0.04	<0.001	Human enzymes (81)

[a]6-MNA, 6-methoxy-2-naphthyl acetic acid, the active metabolite of nabumetone.

Da Vane et al., 1998, Annu. Rev. Pharmacol. Toxicol. 38: 97-120

Table 2 Inhibition of COX-1 and COX-2 by NSAIDs in different systems

System	COX-2/COX-1 Ratio			
	Indomethacin	Nimesulide	Etodolac	Meloxicam
Cultured animal cells	22 (112)	0.05 (122)		0.33 (113)
	60 (110)	0.1 (123)		0.8 (110)
	30 (113)			
	6 (114)			
Human recombinant enzymes	1.3 (115)	0.2 (118)	~0.001 (81)	0.003 (81)
	>75 (116)	0.02 (124)	0.09 (127)	0.01 (118)
	2.3 (14)	0.16 (125)	0.8 (116)	
	9 (117)	0.01 (81)		
	3.5 (118)	0.02 (120)		
Human whole blood cells	0.51 (119)	0.19 (120)	0.1 (127)	0.09 (111)
	12.5 (120)	0.06 (111)		
	2.9 (121)	0.07 (126)		

Da Vane et al., 1998, Annu. Rev. Pharmacol. Toxicol. 38: 97-120

FANS ⇒ Pressocchè equipotenti su COX-1 e COX-2

Inibitori preferenziali COX-2

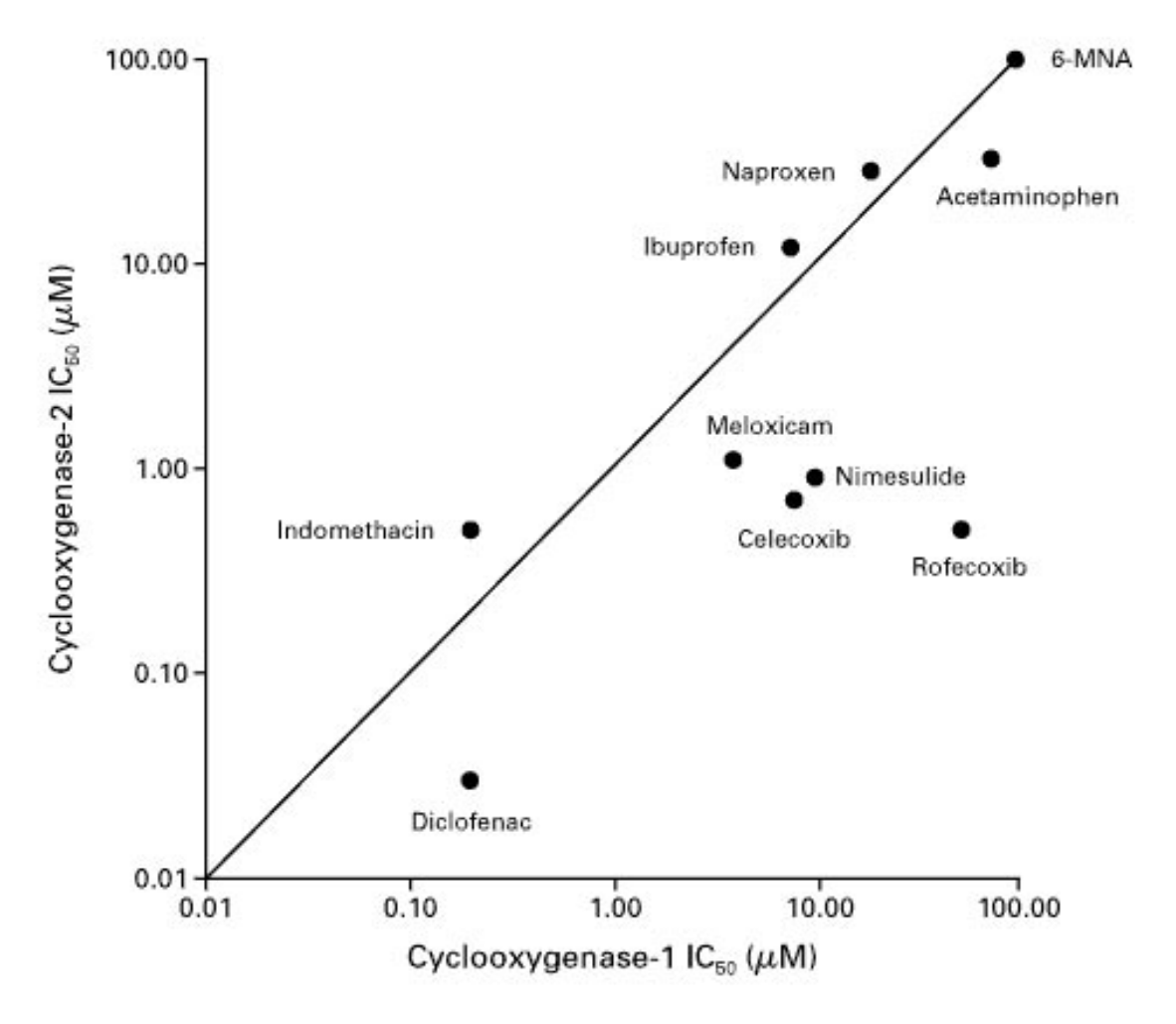
Inibitori selettivi COX-2

Table 6. Differential inhibition of COX-1 and COX-2 (IC_{50}) by various NSAIDs in the human whole blood assay. IC_{50} values are expressed in μmol/l. Ratio describes the ratio of the IC_{50} of COX-2/IC_{50} of COX-1

	Patrignani et al[37]			*Brideau et al*[39]			*Young et al*[38]			*Glaser et al*[28]			*Pairet et al*		
	COX-1	*COX-2*	*Ratio*	*COX-1*	*COX-2*	*Ratio*	*COX-1*	*COX-2*	*Ratio*	*COX-1*	*COX-2*	*Ratio*	*COX-1*	*COX-2*	*Ratio*
ASA							2.8	>167	>59						
Flurbiprofen	0.9	0.9	1.0	0.44	6.42	14	0.55	7	13						
Naproxen	15.6	27.8	1.8	7.76	73.74	9.5	11.0	4.3	0.4	20	23	1.2			
Ibuprofen	9.2	18.3	2.0	4.75	>30	>6	9.2	56	6.1						
Piroxicam	2.86	0.93	0.3	0.76	8.99	12	2.8	7.3	2.6						
Indomethacin	0.53	0.28	0.5	0.16	0.46	2.9	0.13	1.7	13				0.17	0.14	0.8
6-MNA	278	187	0.7	ND	>30		83	301	3.6						
Diclofenac				0.14	0.05	0.4	0.17	0.12	0.7						
Nimesulide	9.2	0.52	0.06				17	3.2	0.2						
Etodolac										34	3.4	0.1			
Meloxicam	4.8	0.43	0.09										3.27	0.25	0.08
Flosulide				32.3	0.75	0.02									
DuP-697				1.18	0.06	0.05									
NS-398	16.8	0.10	0.006	4.81	0.47	0.09	11	0.3	0.03						
L-745,337	369	1.5	0.004	>30	9.67	<0.3									
SC58125	38.7	0.27	0.007	>30	2.25	<0.08							20	<0.48	<0.02

Da Pairet et al., 1998, In: "Selective COX-2 Inhibitors", Vane J., Botting J., Eds., Kluwer Academic Publishers and William Harvey Press; pp. 27-46.

Concentrations of Various Drugs Required to Inhibit the Activity of Cyclooxygenase-1 and Cyclooxygenase-2 by 50 Percent (IC50) in Assays of Whole Blood



FitzGerald, G. A. et al. N Engl J Med 2001;345:433-442

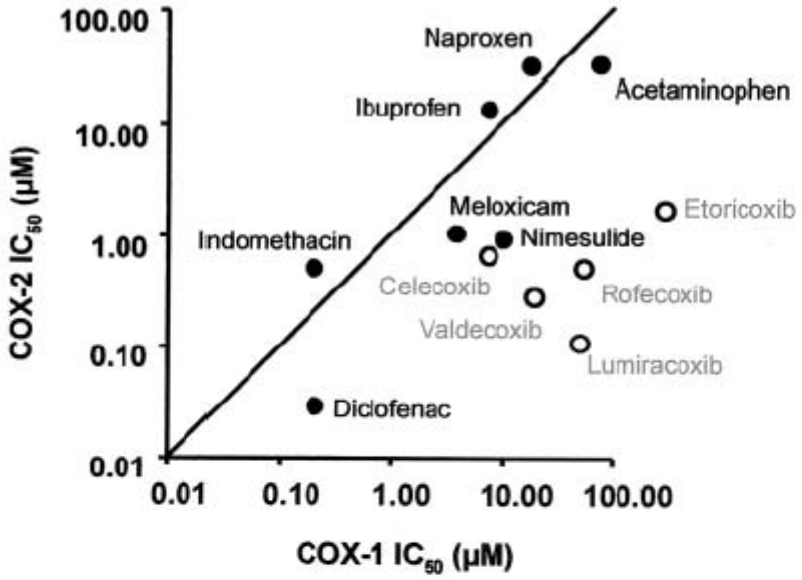
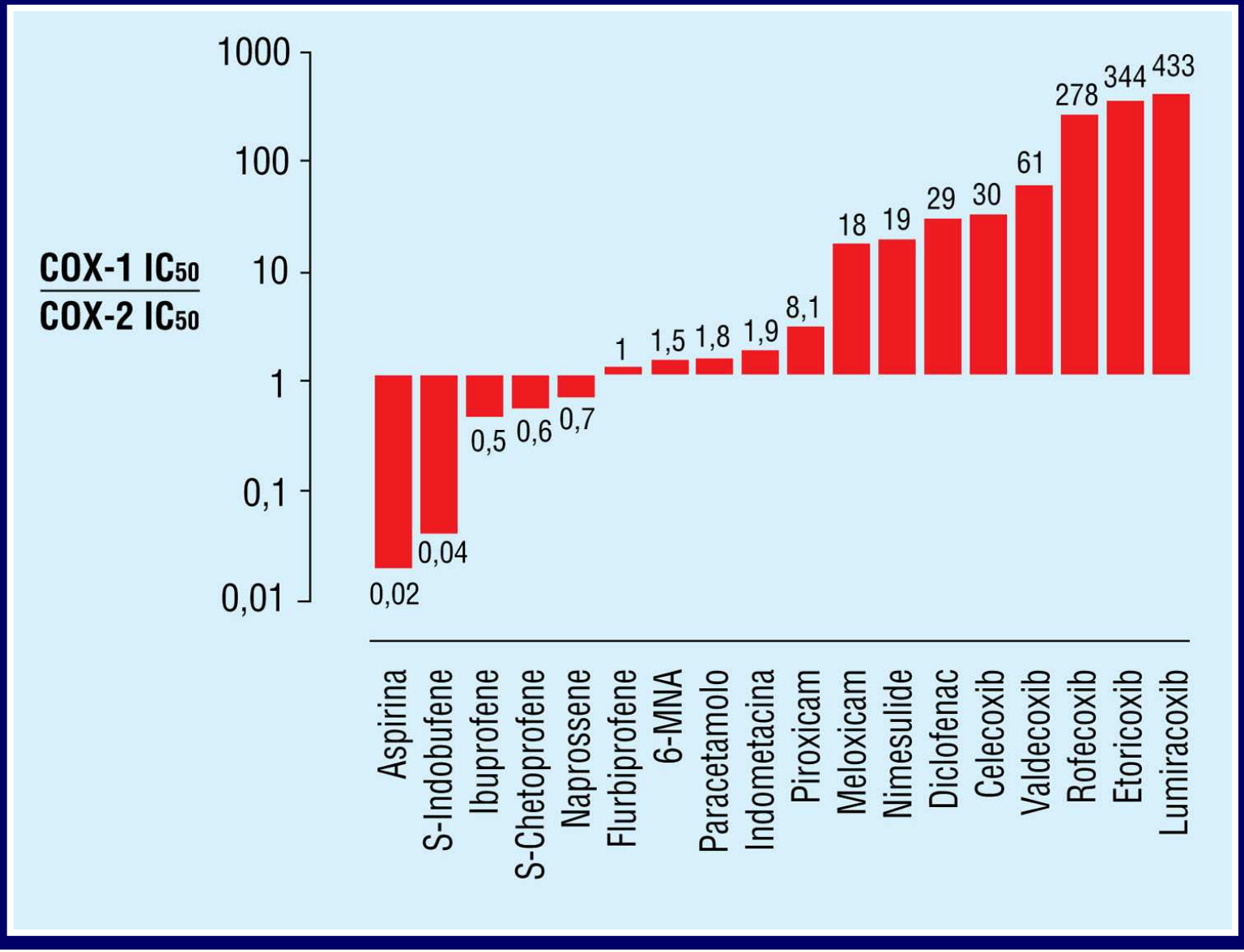


Figure 2. The degrees of cyclooxygenase (COX)-selectivity of various traditional non-steroidal anti-infammatory drugs (tNSAIDs) and coxibs (open circles). The concentrations required to inhibit COX-1 and COX-2 by 50% (IC_{50}) have been measured using whole blood assays of COX-1 and COX-2 activity in vitro.[18] The line indicates equivalent COX-1 and COX-2 inhibition. Drugs plotted below the line are more potent inhibitors of COX-2 than drugs plotted above the line. The distance to the line is a measure of selectivity. Lumiracoxib is the compound with the highest degree of selectivity for COX-2 as its distance to the line is the largest. Celecoxib and diclofenac have similar degrees of COX-2 selectivity, as their distances to the line are similar; however, diclofenac is active at lower concentrations and, thus, located more to the left. (Updated from FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med. 2001;345:433-442.)

Selettività biochimica verso la COX-1 piastrinica e la COX-2 monocitaria di inibitori delle COX valutata in vitro utilizzando il modello del sangue intero.



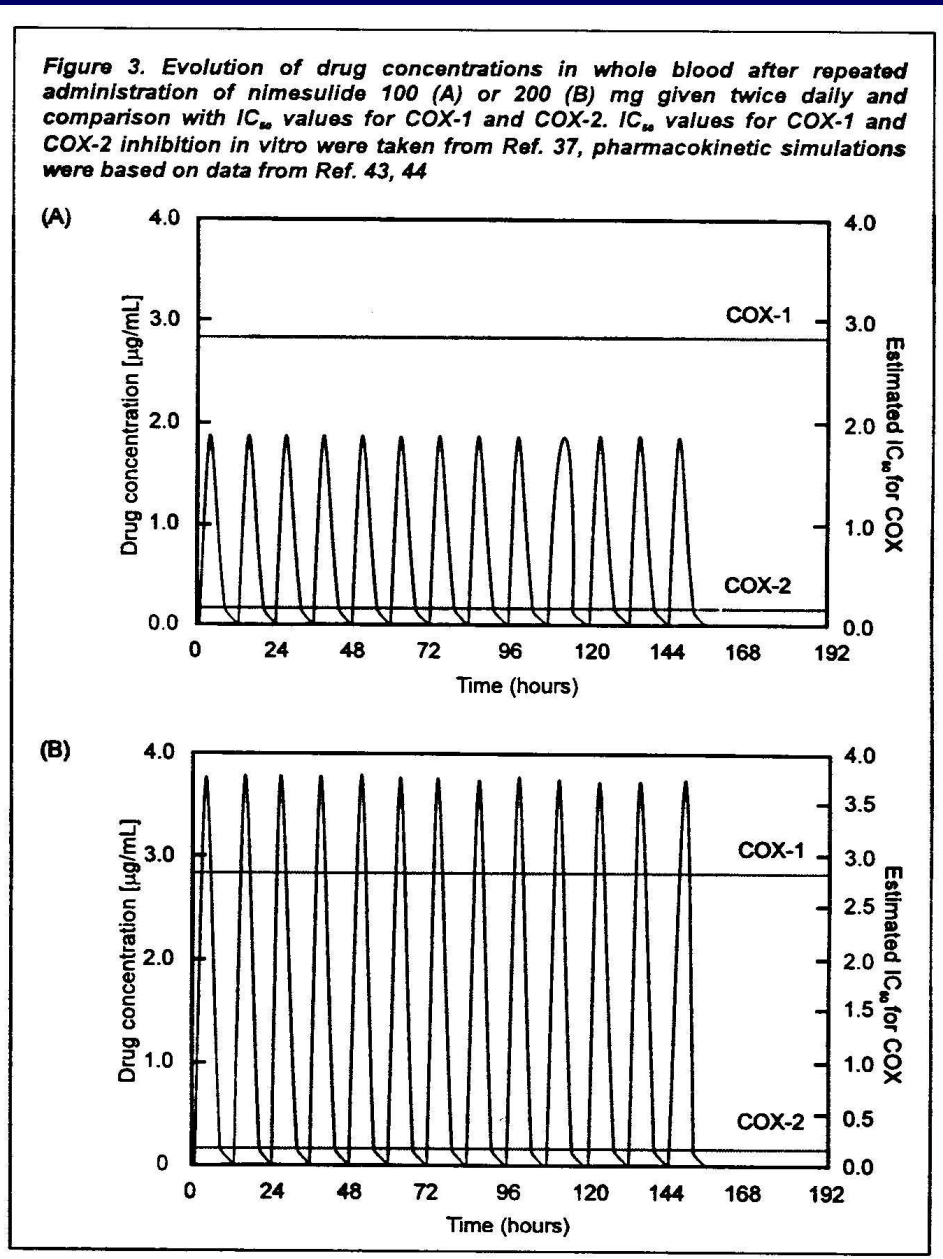
Selettività dei FANS nei confronti di COX-1 e COX-2

- Relativamente selettivi per la COX-1:
  - Aspirina, piroxicam, indometacina
- Pressocchè equipotenti su COX-1 e COX-2:
  - Diclofenac, flurbiprofene, naproxene
- Inibitori preferenziali COX-2:
  - Nimesulide, meloxicam, etodolac
- Inibitori selettivi COX-2:
  - Celecoxib, rofecoxib

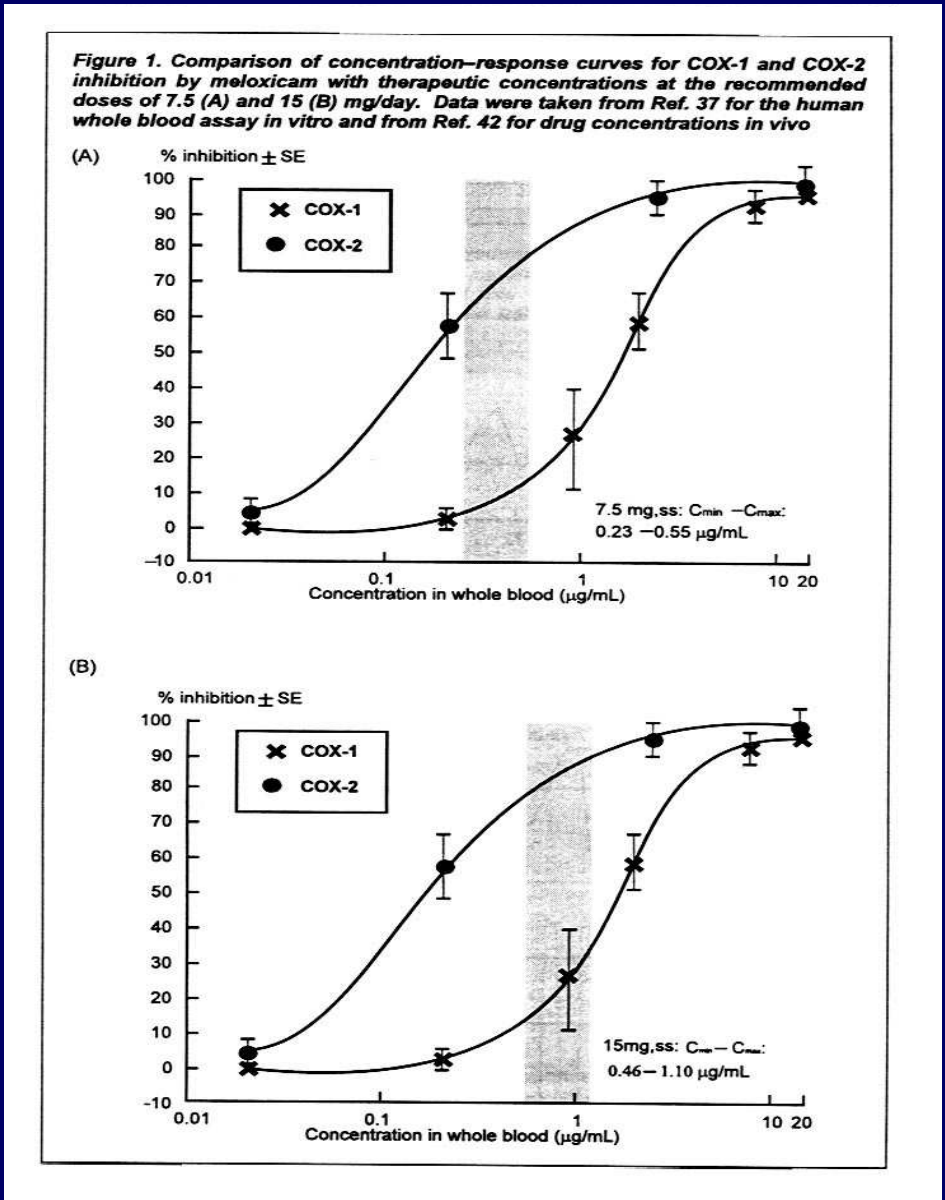
Table 1 | **A classification of NSAIDs according to Warner *et al.*[84]**

Class	Properties	Examples
Group 1	NSAIDs that can completely inhibit both COX1 and COX2 but have little selectivity	Aspirin, diclofenac, fenoprofen, flurbiprofen, indomethacin, ibuprofen, ketoprofen, mefenamic acid, naproxen, piroxicam, sulindac sulphide
Group 2	NSAIDs that inhibit COX2 with a 5–50-fold selectivity	Celecoxib, etodolac, meloxicam
Group 3	NSAIDs that inhibit COX2 with a >50-fold selectivity	Rofecoxib
Group 4	NSAIDs that are weak inhibitors of both isoforms	5-amino salicylic acid, diflunisal, sodium salicylate, nabumetone, sulphasalazine

Adapted from REF. 84. COX, cyclooxygenase; NSAID, non-steroidal anti-inflammatory drug.

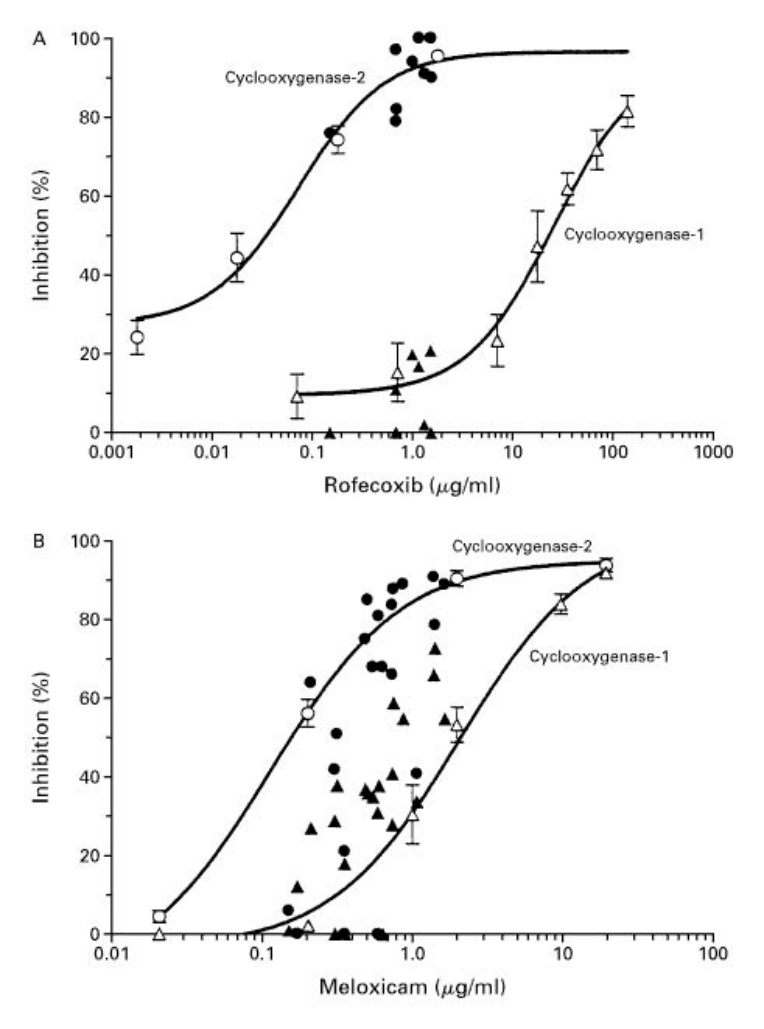


Da Pairet et al., 1998, In: "Selective COX-2 Inhibitors", Vane J., Botting J., Eds., Kluwer Academic Publishers and William Harvey Press; pp. 27-46.



Da Pairet et al., 1998, In: "Selective COX-2 Inhibitors", Vane J., Botting J., Eds., Kluwer Academic Publishers and William Harvey Press; pp. 27-46.

Relations between Mean ({+/-}SE) Steady-State Plasma Concentrations of Rofecoxib (Panel A) and Meloxicam (Panel B) and Inhibition of Cyclooxygenase-1 and Cyclooxygenase-2, as Measured in Vitro



FitzGerald, G. A. et al. N Engl J Med 2001;345:433-442

Pharmacokinetics, Metabolism, and Drug Interactions of Rofecoxib and Celecoxib

TABLE 1. PHARMACOKINETICS, METABOLISM, AND DRUG INTERACTIONS OF ROFECOXIB AND CELECOXIB.*

FEATURE	ROFECOXIB	CELECOXIB
Oral bioavailability (%)	92–93	22–40
Effect of food	Minimal	None
Time to maximal plasma concentration (hr)	2–3	2–4
Elimination half-life (hr)	10–17	Approximately 11
Volume of distribution (liters)	86–91	455±166
Extent of binding to plasma proteins (%)	86	>97
Main pathway of liver metabolism	Cytosolic reduction	Oxidation by cytochrome P-450 2C9, 3A4
Interaction with cytochrome P-450 inhibitors	No	Yes
Interaction with digoxin	No	Not tested
Interaction with warfarin	Causes 10% increase in INR	No
Interaction with methotrexate	At supratherapeutic doses	No
Interaction with antihypertensive drugs	Increases blood pressure	Increases blood pressure
Influence of renal insufficiency	Has little effect	AUC 43% lower
Influence of hepatic impairment	AUC 30–70% higher	AUC 40–180% higher
Approved daily doses (mg)		
For osteoarthritis	12.5–25	100–200
For rheumatoid arthritis	Not approved	200–400
For acute pain	Up to 50	Not approved

*INR denotes international normalized ratio, and AUC area under the curve. Plus–minus value is the mean ±SD.

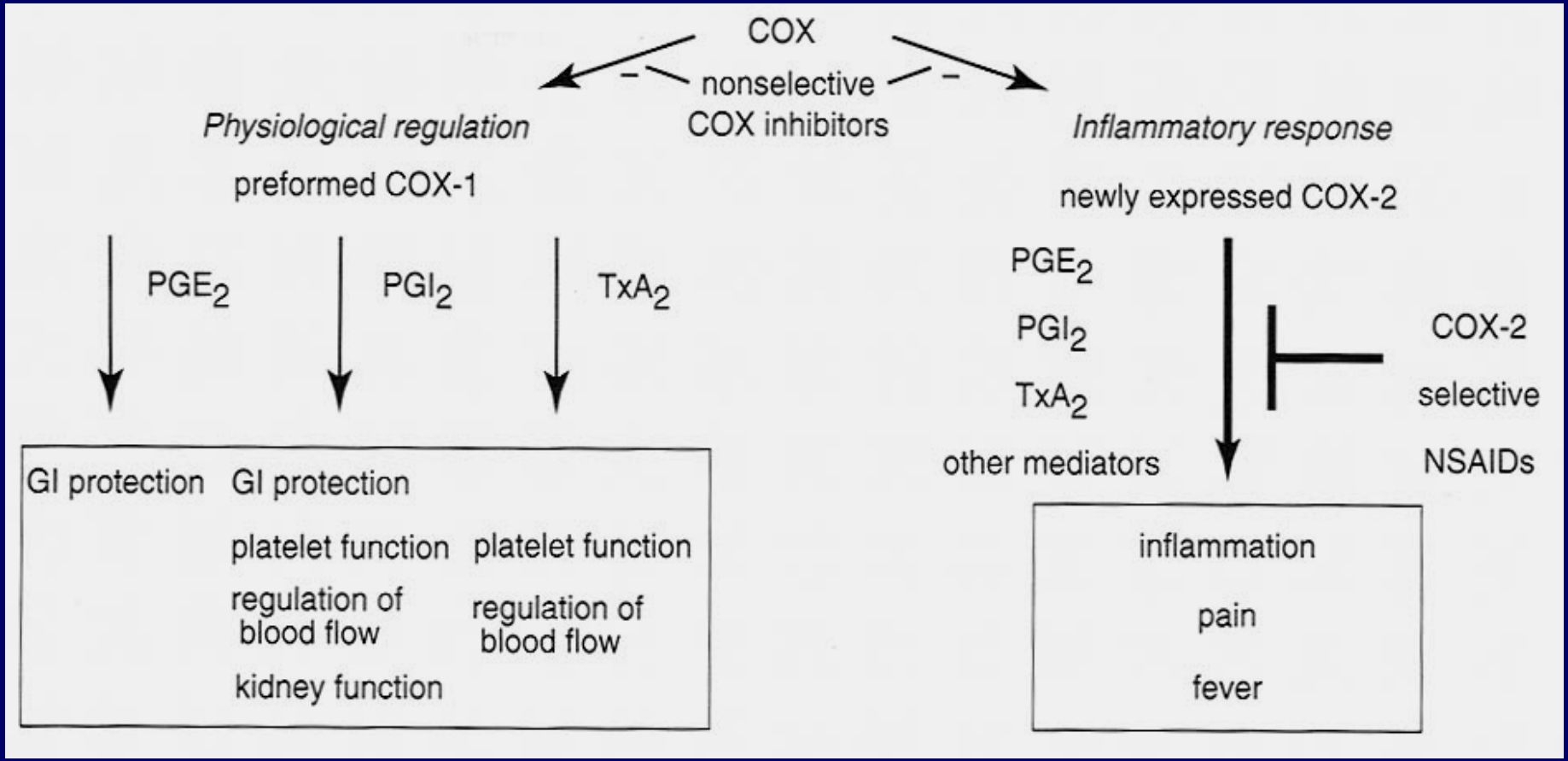
FitzGerald, G. A. et al. N Engl J Med 2001;345:433-442

Table 1 | **Clinical pharmacology of the coxibs**

Drug	Chemistry	COX-1: COX-2 ratio	Pharmacokinetics Oral bioavail-ability (%)	T_{max} (h)	Metabolism Half-life (h)	Volume distribution (l)	Protein binding (%)	Metabolism	Urinary excretion (%)
Celecoxib	Sulphonamide	30	22–40	2–4	11	455	97	Oxidation, CYP450 (2C9, 3A4)	29
Rofecoxib	Sulphonyl	276	92–93	2–3	10–17	86–91	87	Cytoslic reduction	72
Valdecoxib	Sulphonamide	261	83	2.3	8–11	86	98	Oxidation, CYP450 (2C9, 3A4)	70
Etoricoxib	Sulphonyl	344	100	1	22	120	92	Oxidation, CYP450 (3A4)	60
Lumiracoxib	Phenyl acetic acid	433	74	2–3	3–6	9	98	Oxidation, CYP450 (2C9)	54

metaboliti

metaboliti

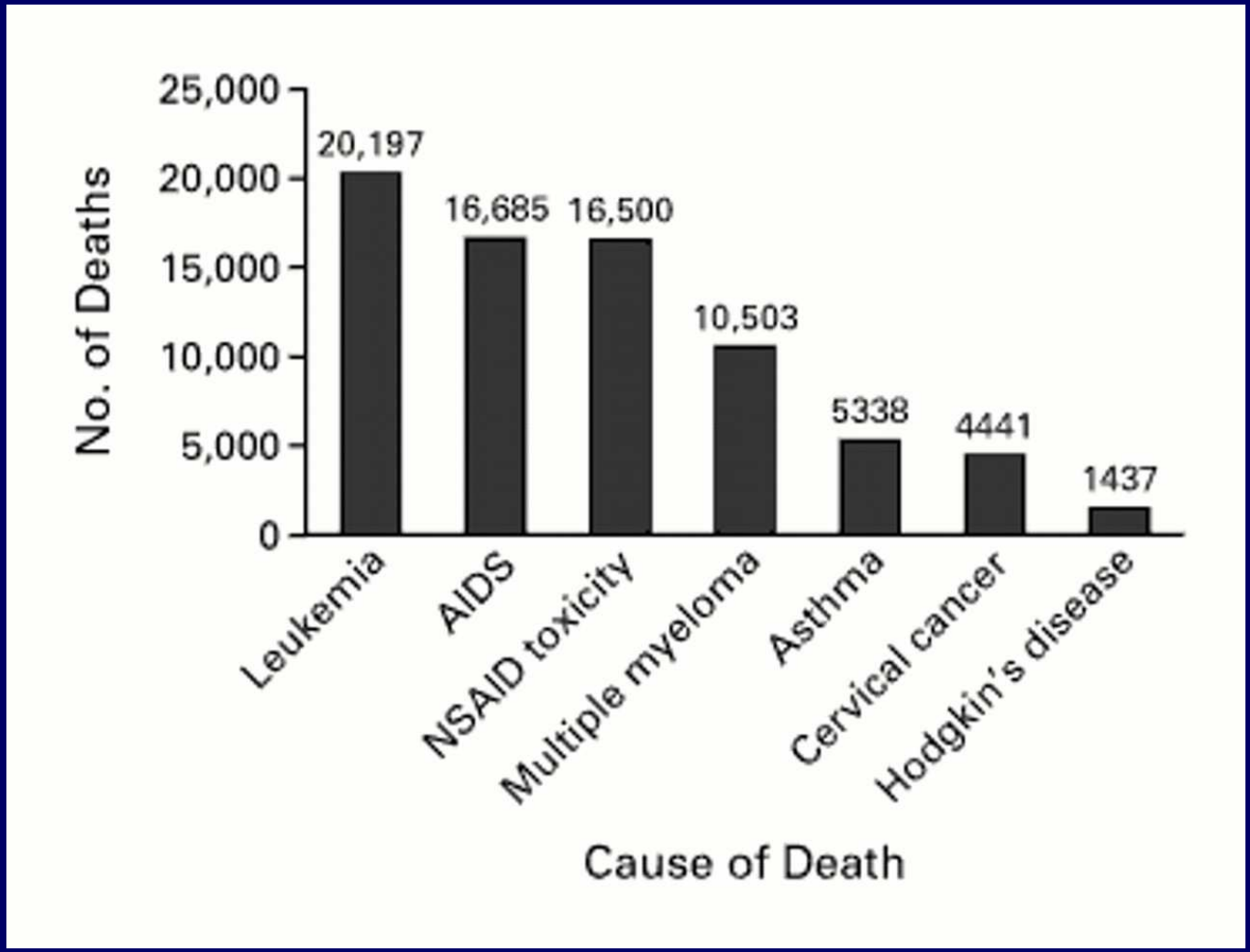


- L'inibizione non selettiva della COX riduce la biosintesi dei mediatori dell'infiammazione ma anche dei prostanoidi coinvolti in importanti funzioni fisiologiche

FANS: effetti collaterali comuni

- Intolleranza ed ulcere gastrointestinali
- Inibizione della funzionalità renale mediata dalle PGs
- Alterazioni delle funzioni delle piastrine
- Prolungamento della gestazione o del travaglio spontaneo
- Reazioni da ipersensibilità

U.S. Mortality Data for Seven Selected Disorders in 1997



Wolfe, M. M. et al. N Engl J Med 1999;340:1888-1899

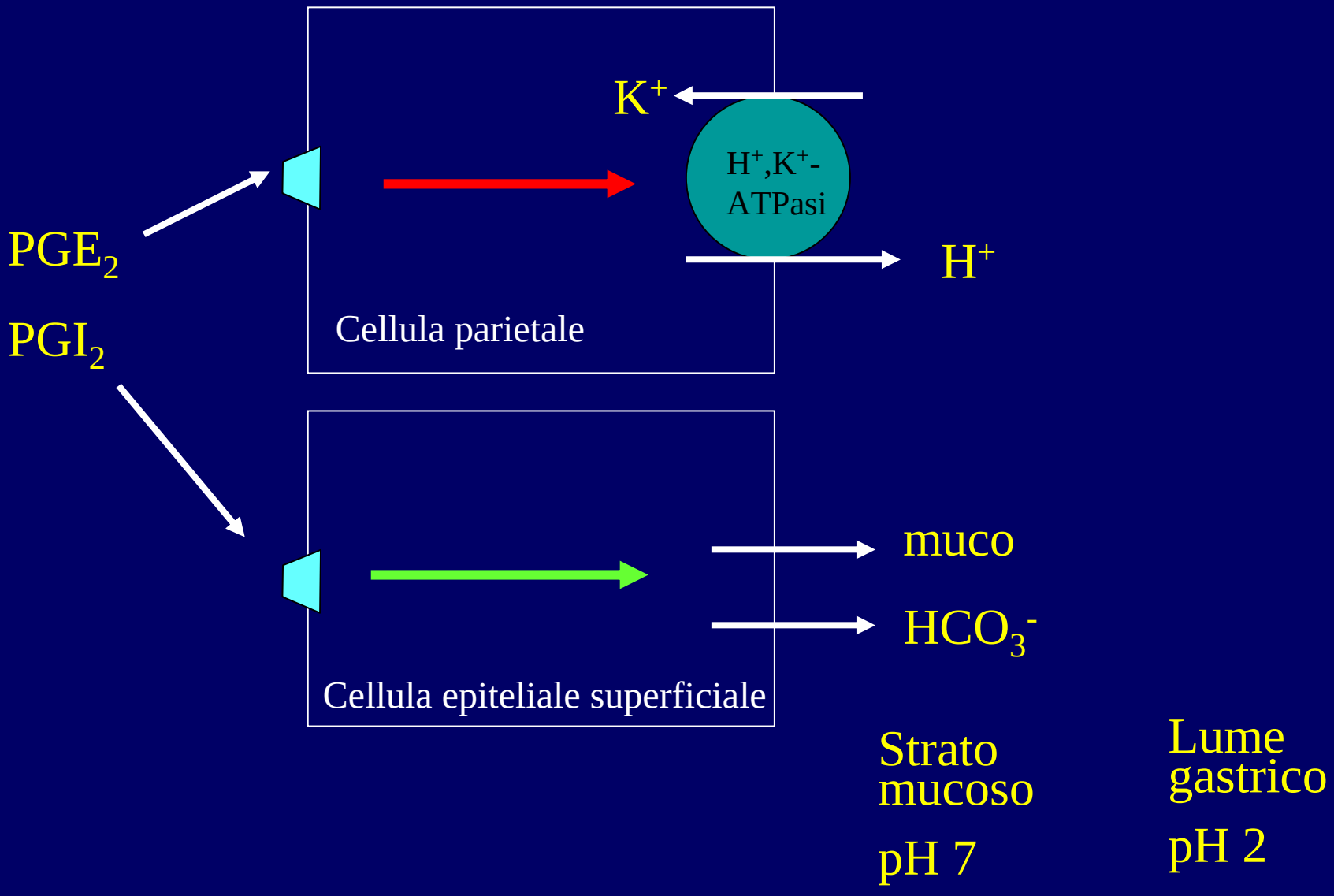
FANS: effetti collaterali GI

- Tendenza a provocare ulcere gastriche ed intestinali
- Si va da lieve dispepsia con pirosi ad ulcere GI, anche fatali

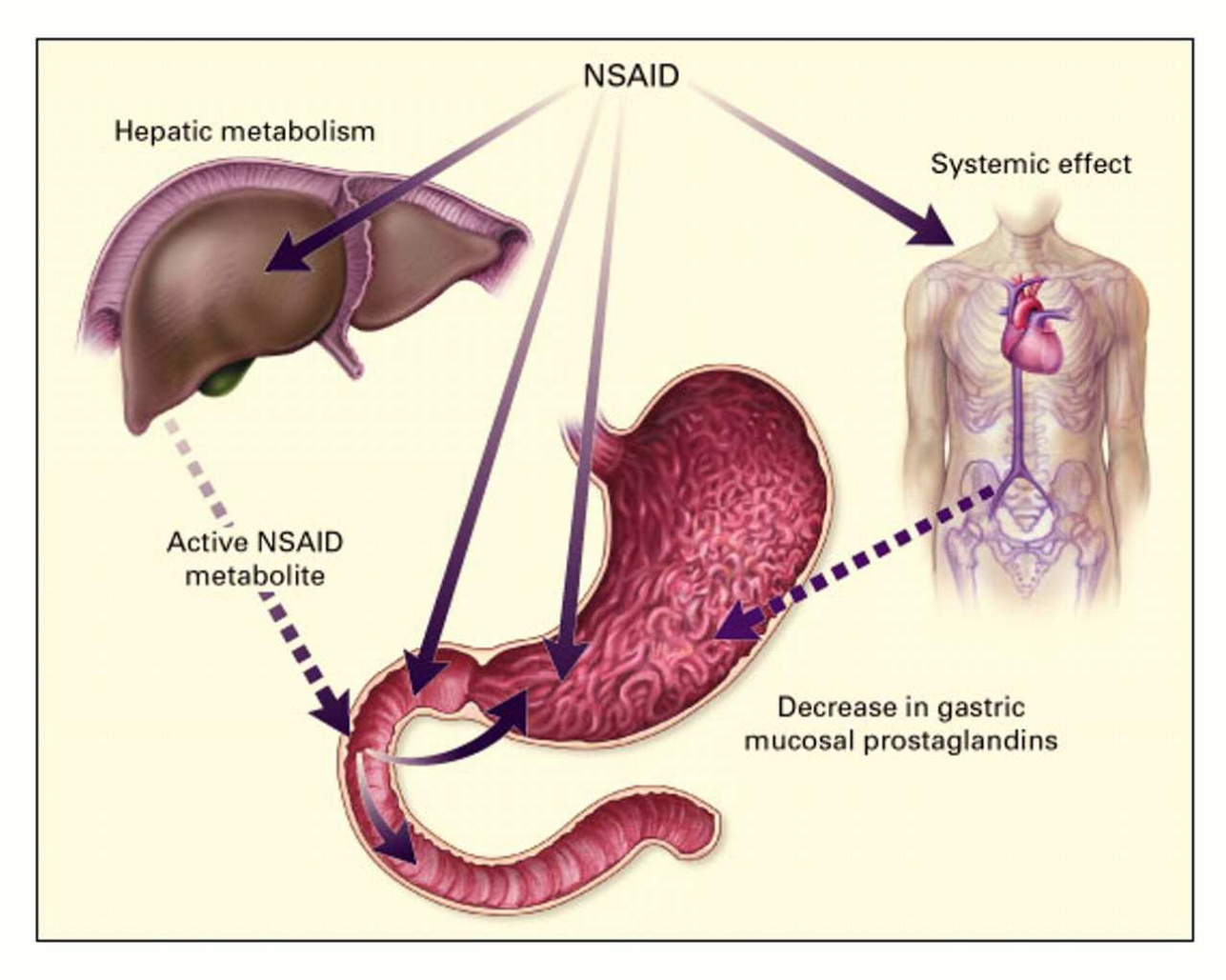
Meccanismi:

- Lesività diretta
- Inibizione sintesi PGs citoprotettive

Prostaglandine: agenti citoprotettivi della mucosa gastrica



Mechanisms by Which NSAIDs Induce Gastroduodenal Mucosal Injury



Wolfe, M. M. et al. N Engl J Med 1999;340:1888-1899

Topical Injury

Mucosal injury is initiated topically by the acidic properties of aspirin and many other NSAIDs.

Because of a low dissociation constant, which varies according to the particular agent, these weak acids remain in their nonionized lipophilic form in the highly acidic gastric lumen. Such conditions favor migration through the gastric mucus across plasma membranes and into surface epithelial cells, where NSAIDs are dissociated into the ionized form, resulting in trapping of hydrogen ions.

NSAIDs can also cause topical mucosal damage by diminishing the hydrophobicity of gastric mucus, thereby allowing endogenous gastric acid and pepsin to injure the surface epithelium.

In addition, topical mucosal injury may occur as a result of indirect mechanisms, mediated through the biliary excretion and subsequent duodenogastric reflux of active NSAID metabolites.

For example, although sulindac is administered as a nontoxic prodrug, its active etabolite, sulindac sulfide, is excreted into the bile. On entry into the duodenum, sulindac sulfide causes topical injury to the mucosa by virtue of its acidic properties.

FANS: Alterazione della funzione delle piastrine

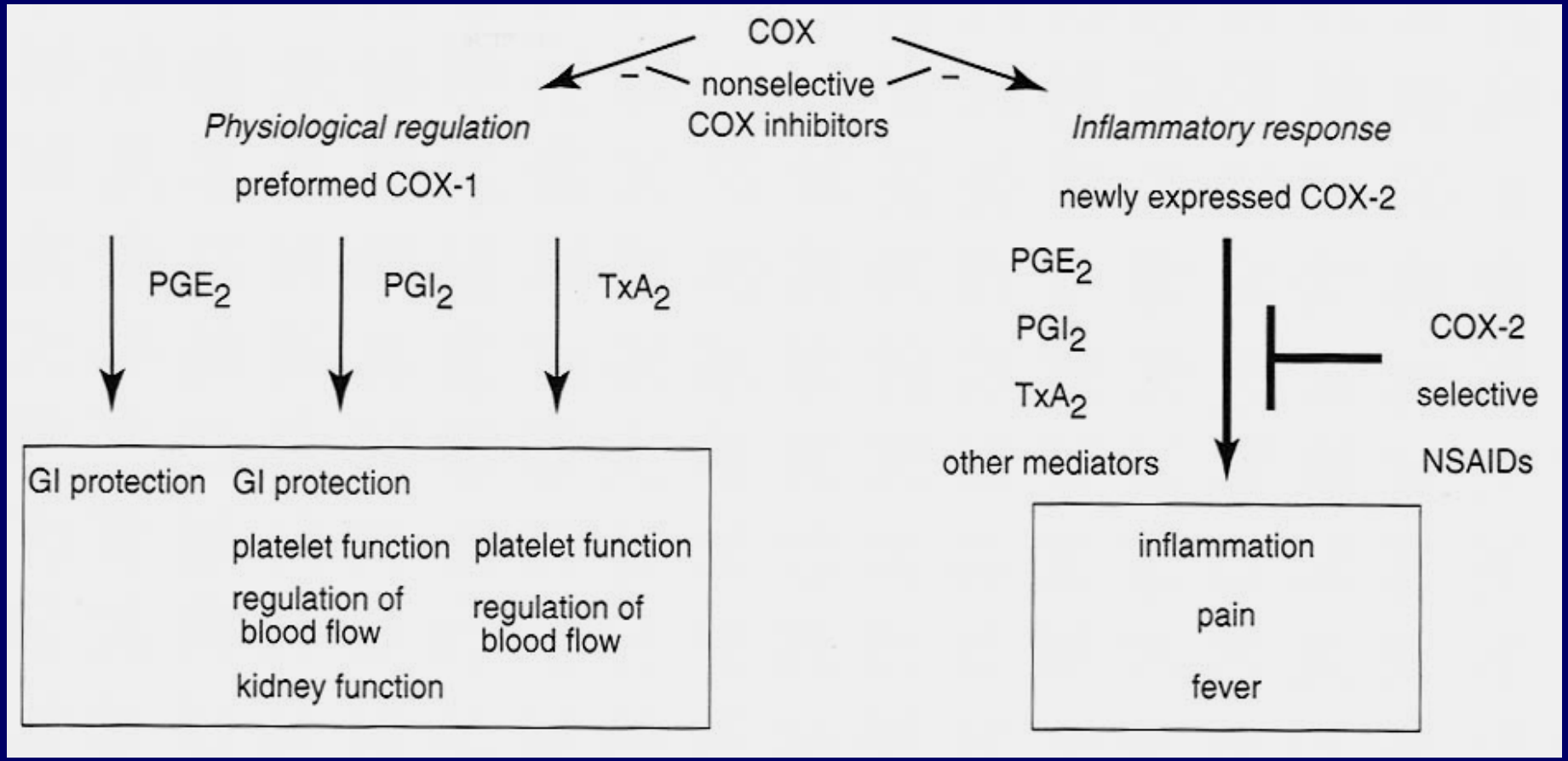
- Per inibizione sintesi piastrinica TXA2
  - Con tendenza ad allungare il tempo di emorragia
- Effetto particolarmente evidente con aspirina:
  - Sfruttato per il trattamento profilattico dei disordini tromboembolici

FANS: alterazioni renali

Prostaglandine sono implicate in:

- ❖ Controllo della secrezione di renina
- ❖ Regolazione del tono vascolare
- ❖ Controllo della funzione tubulare
  - – Aumentano il flusso ematico renale
  - – Attraverso la dilatazione dei vasi renali antagonizzano gli effetti vasocostrittori di NA ed angiotensina II

- I FANS riducono il flusso ematico renale e la filtrazione glomerulare in patologie nelle quali la perfusione renale è > dipendente dalle PGs:
  - – Insufficienza cardiaca congestizia
  - – Cirrosi epatica con ascite
  - – Nefropatia cronica o ipovolemia



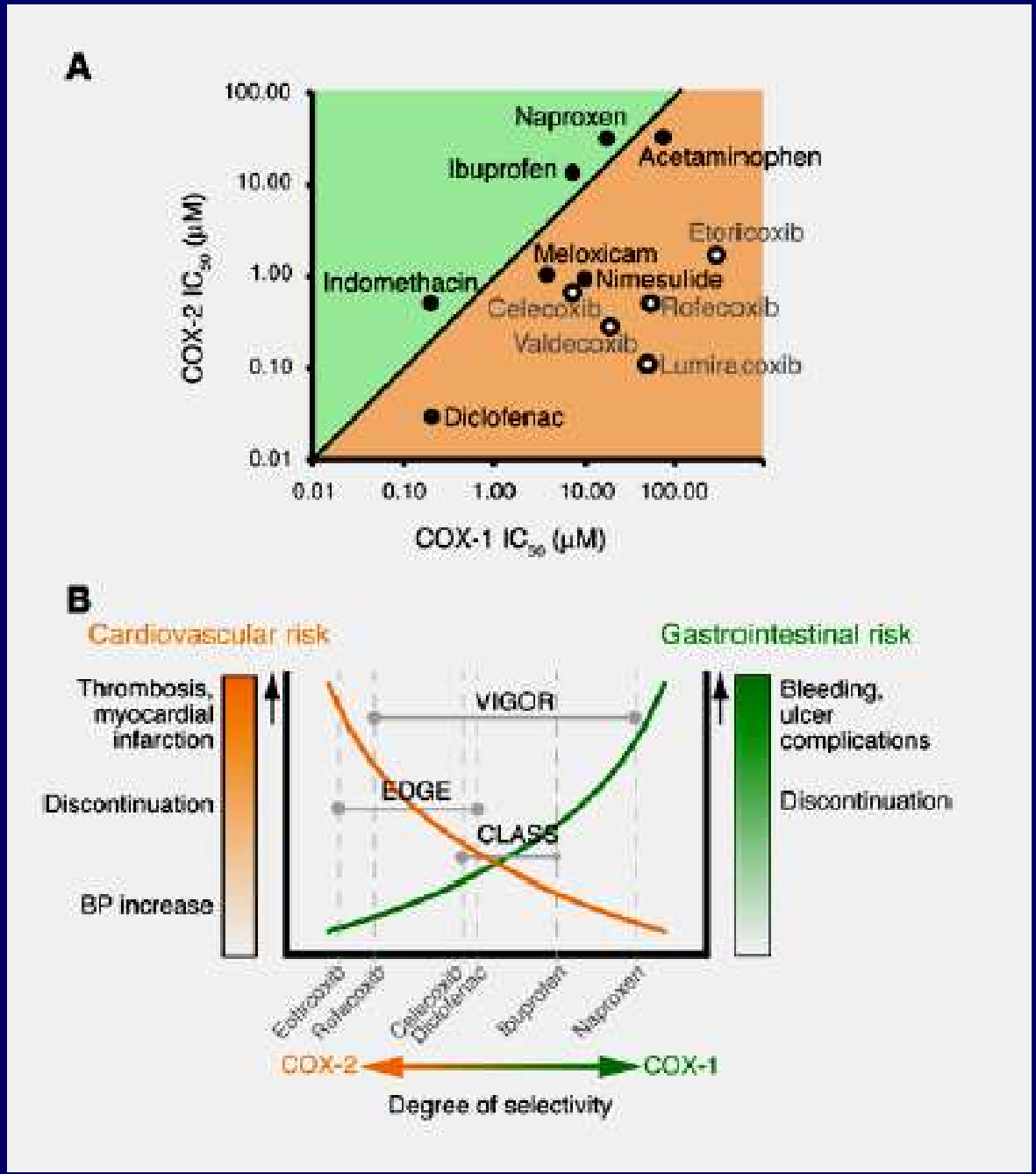
L'inibizione selettiva della COX-2 riduce solo la biosintesi dei prostanoidi flogogeni

?

Table 1 | **Comparison of NSAIDs and selective COX2 inhibitors**

Drug	COX2/COX1 selectivity ratio	Effectiveness	Serious GI risk	Serious CV risk
Naproxen	Inhibits both COX1 and COX2, has little selectivity	N/A	High in at risk patients*	Low
Diclofenac, fenoprofen, flurbiprofen, indomethacin, ibuprofen, ketoprofen, mefenamic acid, piroxicam, sulindac sulphide	Inhibit both COX1 and COX2, have little selectivity	N/A	High in at risk patients*	Moderate
Celecoxib (Celebrex; Pfizer)	30	Similar to NSAIDs	Uncertain‡	Moderate
Rofecoxib (Vioxx; Merck)	276	Similar to NSAIDs	Low compared with NSAIDs	High
Valdecoxib (Bextra; Pfizer)	261	Similar to NSAIDs	Uncertain‡	High
Etoricoxib (Arcoxia; Merck)	344	Similar to NSAIDs	Uncertain‡	High
Lumiracoxib (Prexige; Novartis)	433	Similar to NSAIDs	Low compared with NSAIDs	Moderate at 1 year (no long-term data available)

*Factors that include risk of GI bleeding include older age, history of peptic ulcers, upper GI bleeding, prior GI side effects, high-dose or multiple NSAIDs use, concurrent use of prednisone, H2 antagonists/antacids. ‡Reduce the incidence of GI ulcers visualized at endoscopy compared with certain non-selective NSAIDs. COX, cyclooxygenase; CV, cardiovascular; GI, gastrointestinal; NSAID, non-steroidal anti-inflammatory drug.


A
100.00
10.00
1.00
0.10
0.01
COX-2 IC50 (μM)
0.01
0.10
1.00
10.00
100.00
COX-1 IC50 (μM)
Naproxen
Acetaminophen
Ibuprofen
Etoricoxib
Meloxicam
Nimesulide
Indomethacin
Celecoxib
Rofecoxib
Valdecoxib
Lumiracoxib
Diclofenac
B
Cardiovascular risk
Gastrointestinal risk
Thrombosis, myocardial infarction
Discontinuation
BP increase
Bleeding, ulcer complications
Discontinuation
VIGOR
EDGE
CLASS
Etoricoxib
Rofecoxib
Celecoxib
Diclofenac
Ibuprofen
Naproxen
COX-2
COX-1
Degree of selectivity

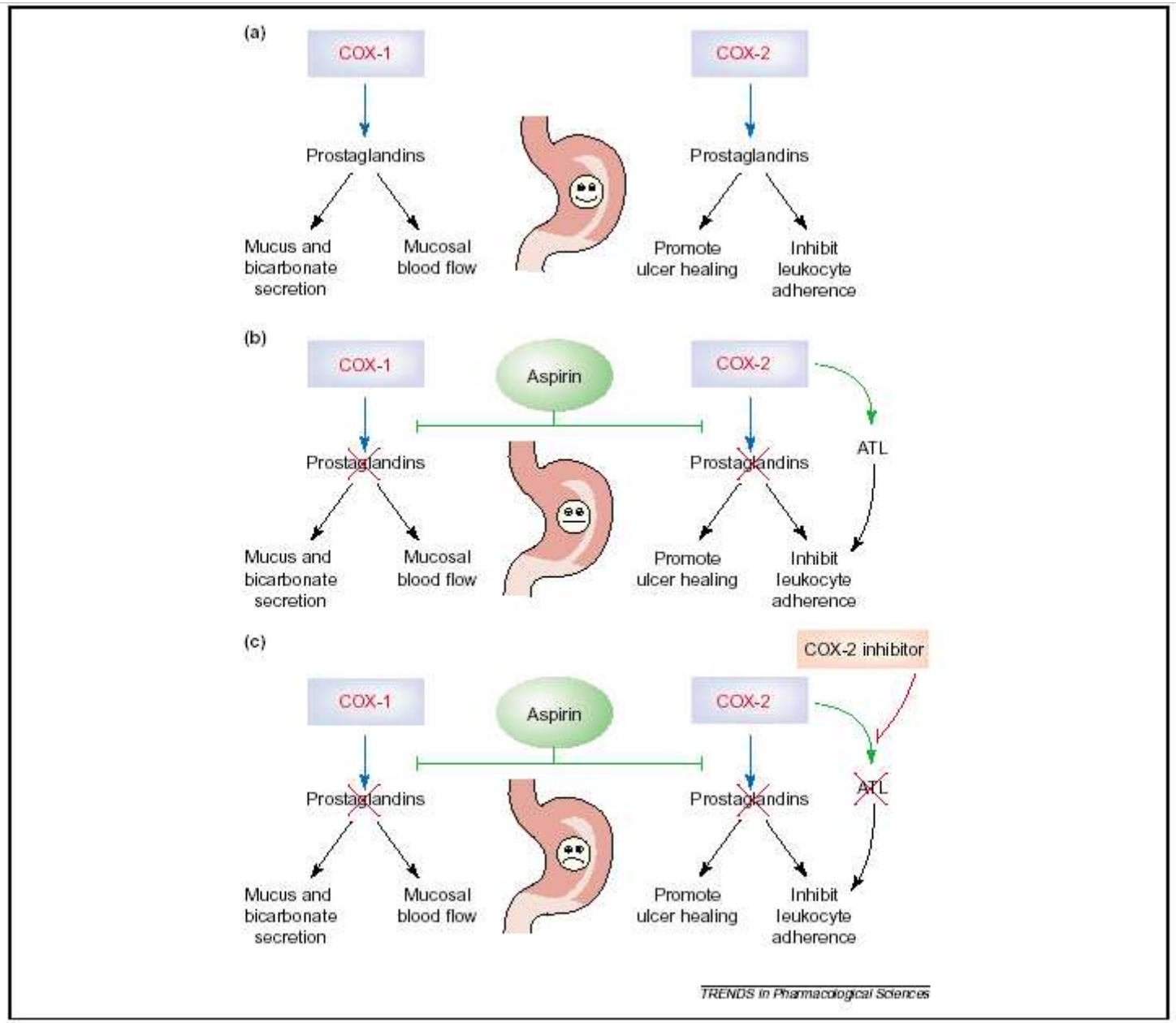


Fig. 1. The protective role of prostaglandins in the stomach and the effects of aspirin and selective cyclooxygenase 2 (COX-2) inhibitors. (a) Under normal circumstances prostaglandins produced by COX-1 and COX-2 contribute to many aspects of mucosal defence. Note that the prostaglandins produced by the two isoforms of COX regulate different aspects of mucosal defence, as has been demonstrated in studies using selective inhibitors of each COX isoform [3,10]. Selective inhibition of COX-1 or COX-2 does not, in itself, result in significant gastric damage [3]. (b) Aspirin suppresses prostaglandin synthesis via both COX-1 and COX-2, thereby impairing mucosal defence and leading to hemorrhagic erosion formation. However, aspirin also triggers the generation of lipoxin (ATL), which partially counteracts the detrimental effects of prostaglandin suppression. ATL is produced via COX-2, but not via COX-1. (c) Inhibition of COX-2 activity by a selective COX-2 inhibitor or a conventional non-steroidal anti-inflammatory drug (NSAID) removes the formation of ATL by aspirin. In the absence of the protective effects of ATL, the extent of gastric damage is increased.

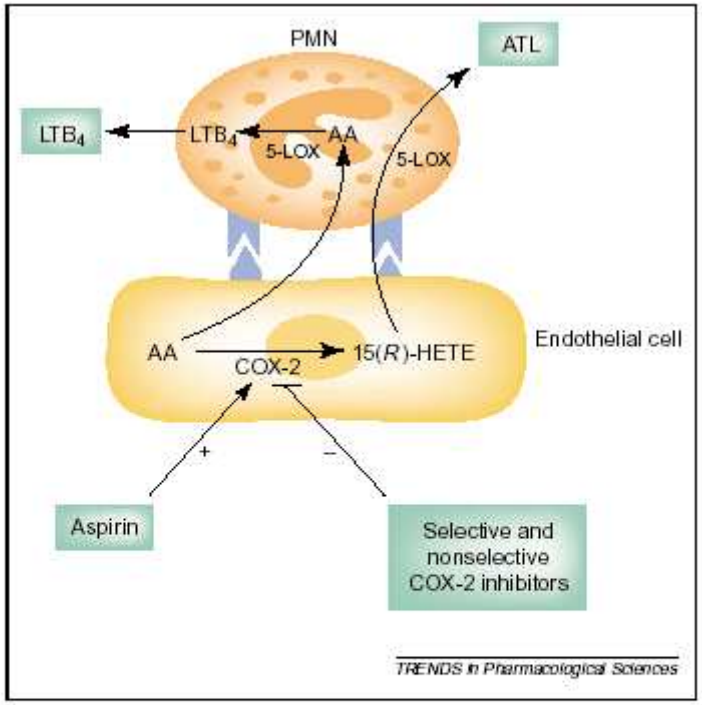
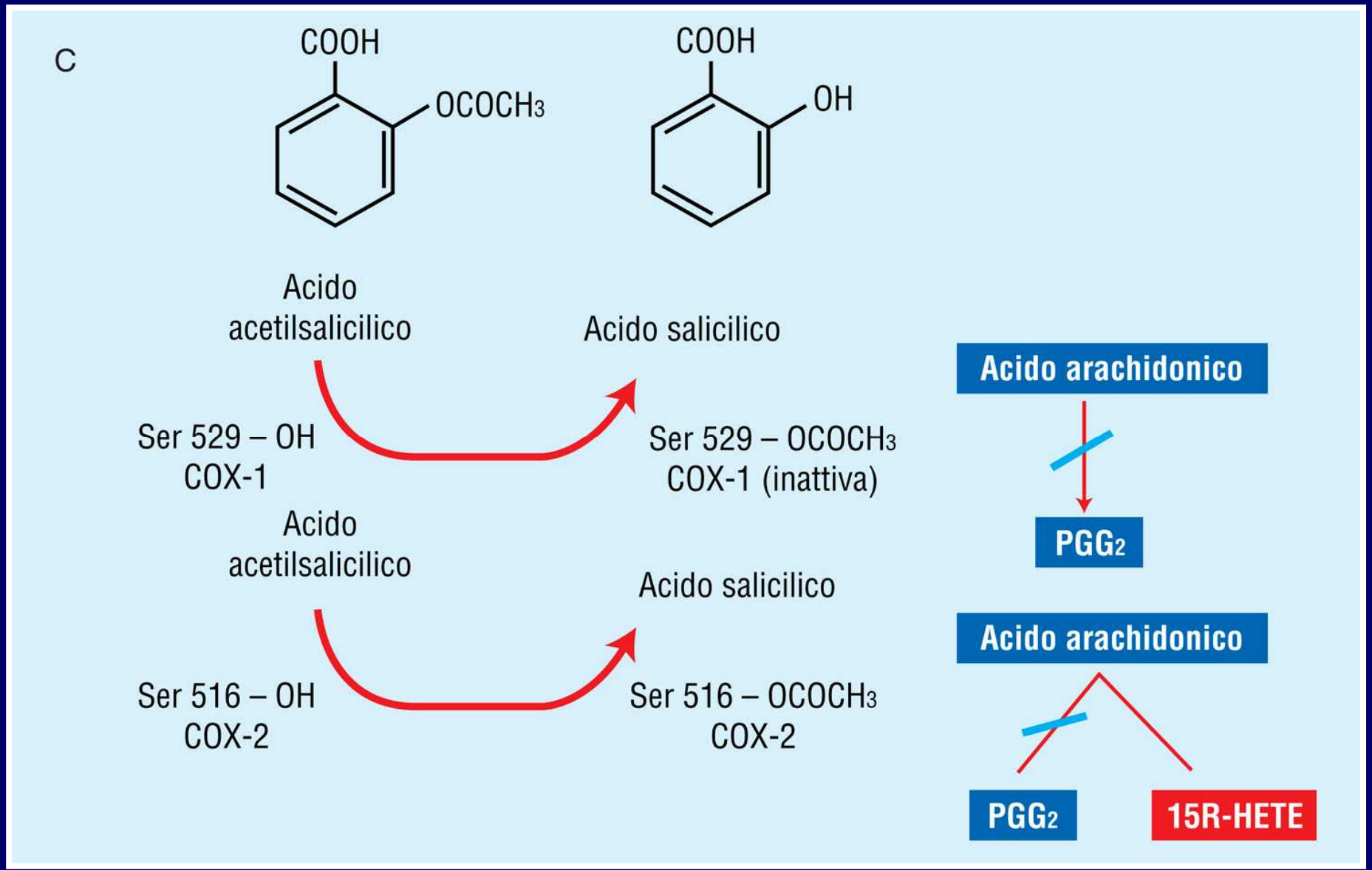


Fig. 3. Aspirin-triggered lipoxin [ATL; 15(*R*)-epi-lipoxin A_4] synthesis occurs via a transcellular pathway. Acetylation of endothelial cyclooxygenase 2 (COX-2) by aspirin blocks prostanoid synthesis but allows the production of 15(*R*)-hydroxyeicosatetraenoic acid [15(*R*)-HETE] via the metabolism of arachidonic acid (AA) by COX-2. Selective COX-2 inhibitors and conventional non-steroidal anti-inflammatory drugs (NSAIDs) can inhibit the generation of 15(*R*)-HETE. 15(*R*)-HETE can diffuse out of the endothelial cell and be metabolized by 5-lipoxygenase (5-LOX) in polymorphonuclear neutrophils (PMNs) to yield ATL. Leukotriene B_4 (LTB_4) generated by neutrophils via 5-LOX will promote adhesion of neutrophils to the endothelium, whereas ATL inhibits these adhesive interactions. The transcellular synthesis of ATL is described in more detail in [6].

Meccanismo di azione dell'aspirina: Cambiamento attività catalitica COX-2



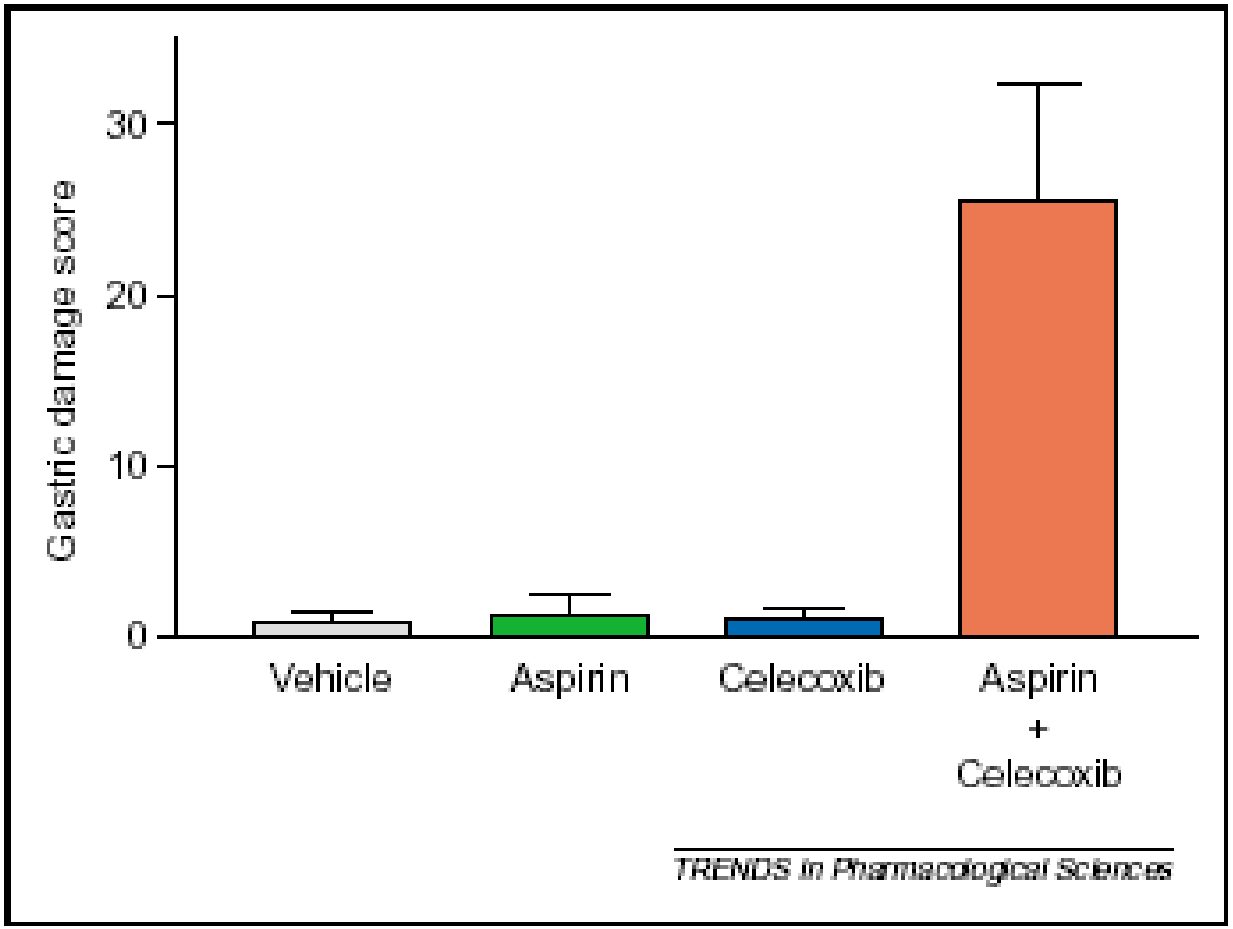
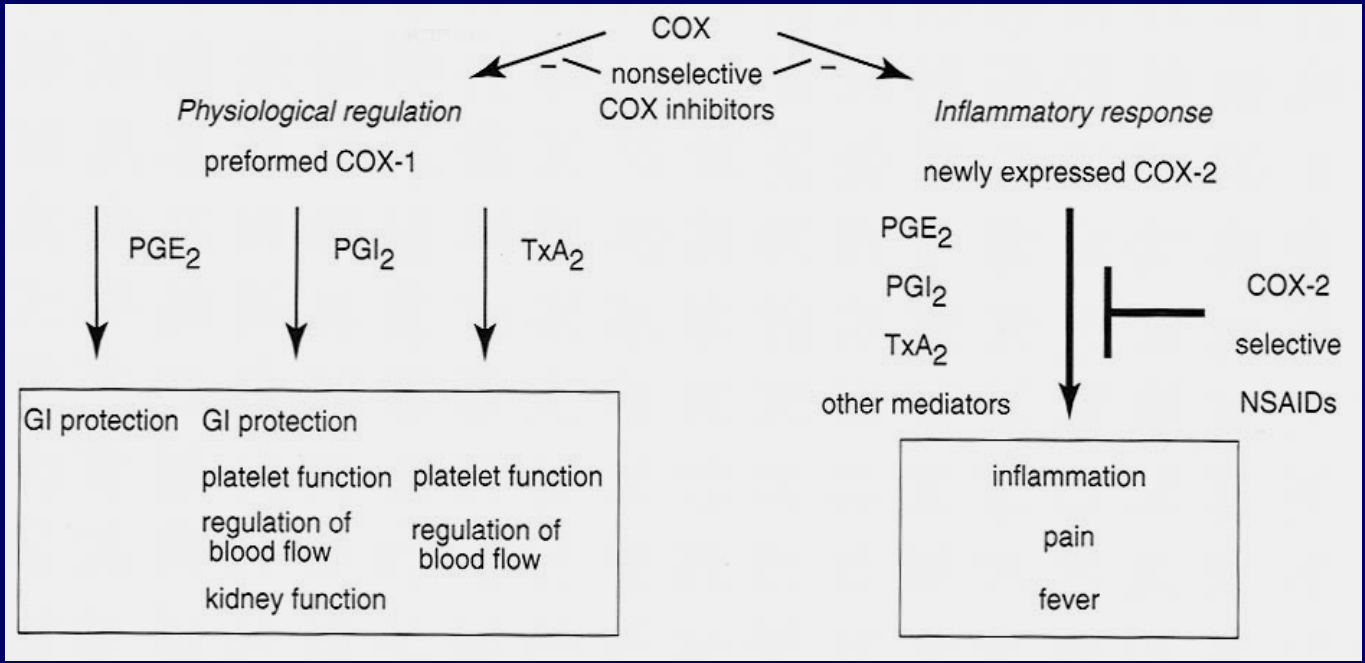


Fig. 2. Administration of aspirin (orally) together with a selective cyclooxygenase 2 (COX-2) inhibitor (celecoxib, by intraperitoneal injection) results in substantially more gastric damage in the rat stomach that either drug alone. Both drugs were administered at a dose of 10 mg kg^{-1} 3 h before blind measurement of the length of hemorrhagic lesions in the stomach (in mm). The sum of the lengths of all lesions represents the 'gastric damage score'. Complete dose–response studies are reported in [3].



Inibizione COX-2

Interferenza con:

- Ruoli fisiologici della COX-2 (es. a livello renale)
- Processi di riparazione tessutale
- Processi di trasformazione neoplastica e crescita tumorale

Distribuzione delle isoforme della COX nel rene

COX-1

- Vasi
- Dotti collettori
- Tratto ascendente sottile dell'ansa di Henle

COX-2

- Vasi
- Macula densa
- Cellule interstiziali midollari

Prostaglandine e controllo della secrezione di renina

VIA DELLA MACULA DENSA

Riassorbimento di NaCl a livello della macula densa

↓ flusso di NaCl
⇓
rilascio PGs
⇓
stimolazione secrezione renina

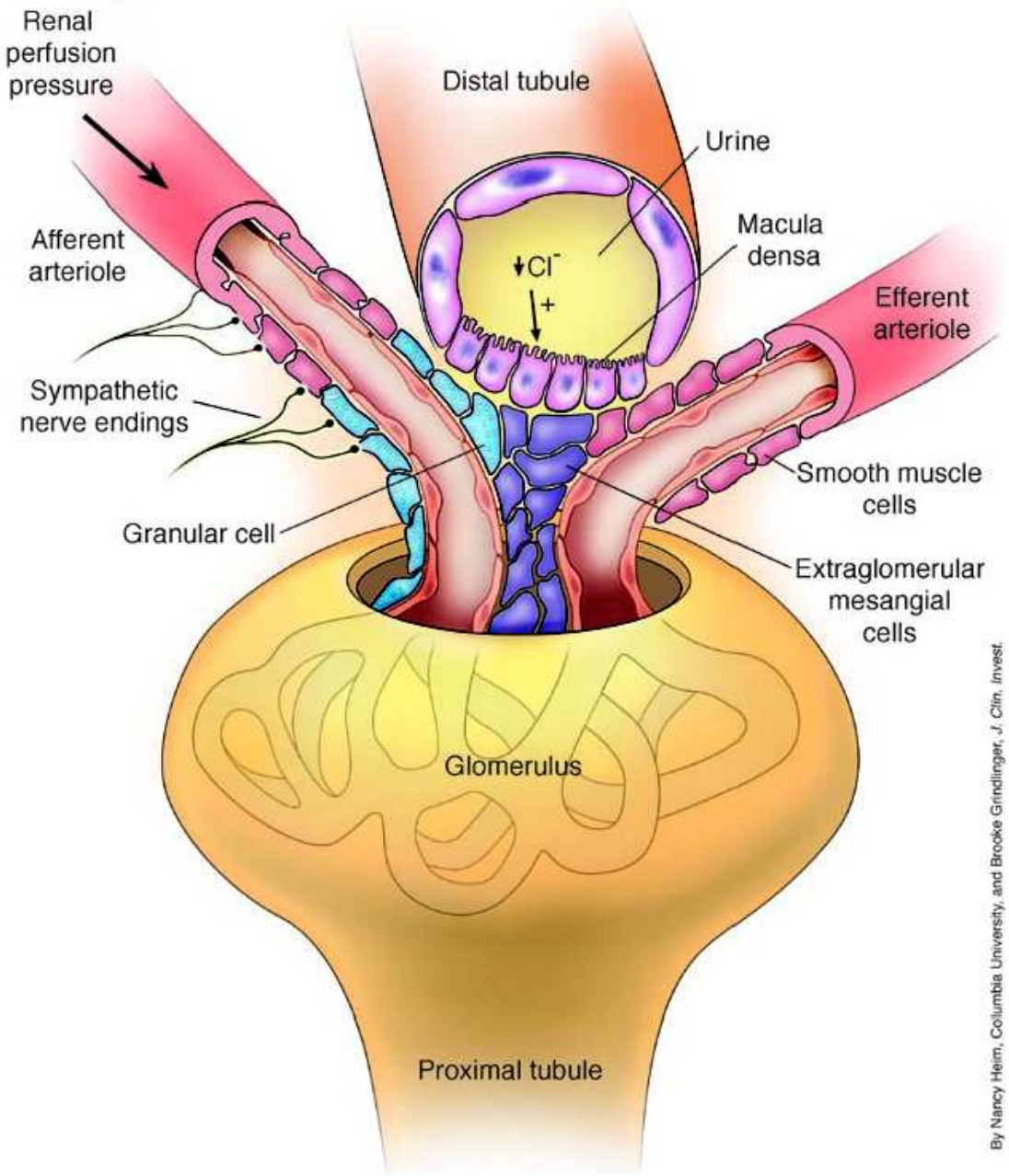
La COX-2 è l'isoforma espressa nella macula densa dei mammiferi e la sua espressione:

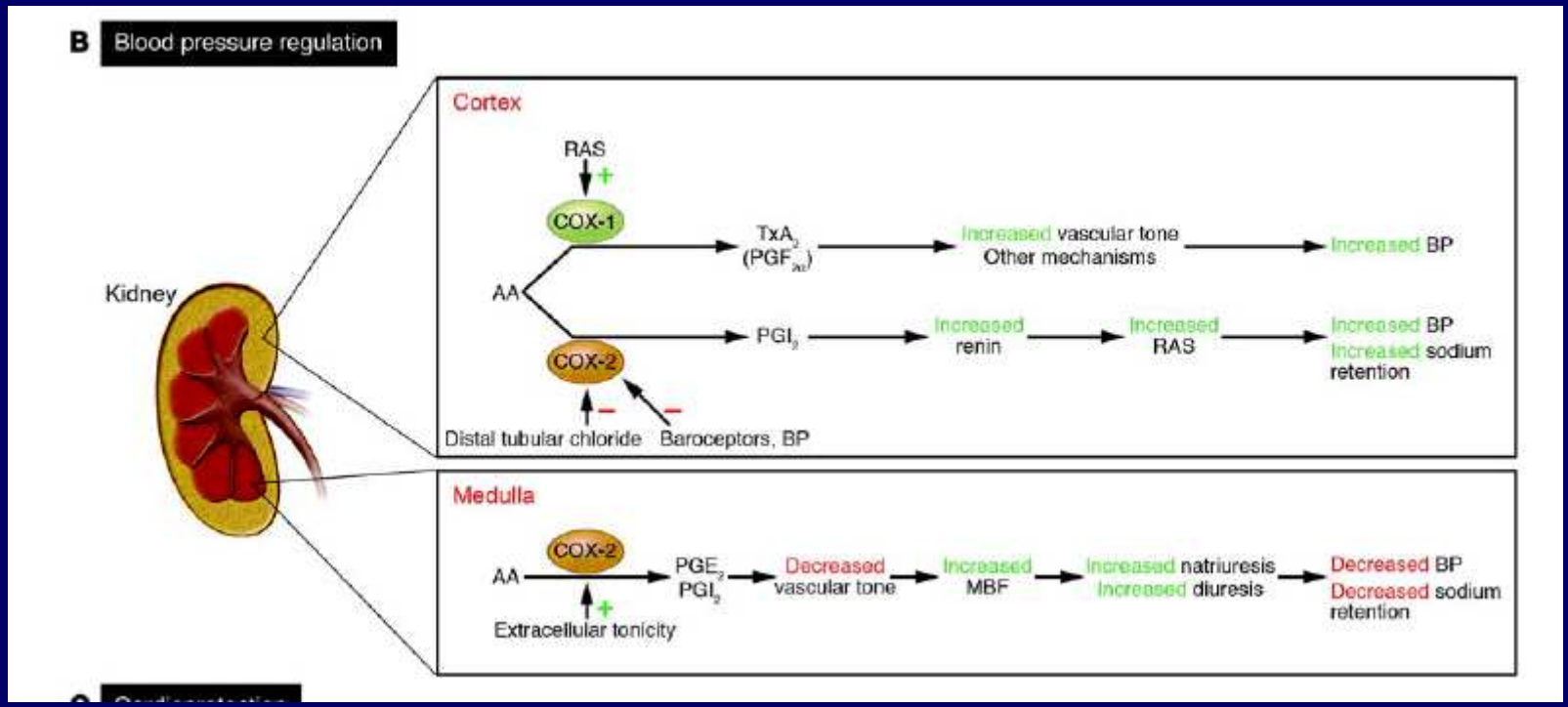
- aumenta in ratti sottoposti a restrizione di Na
- è ridotta dall'angiotensina II

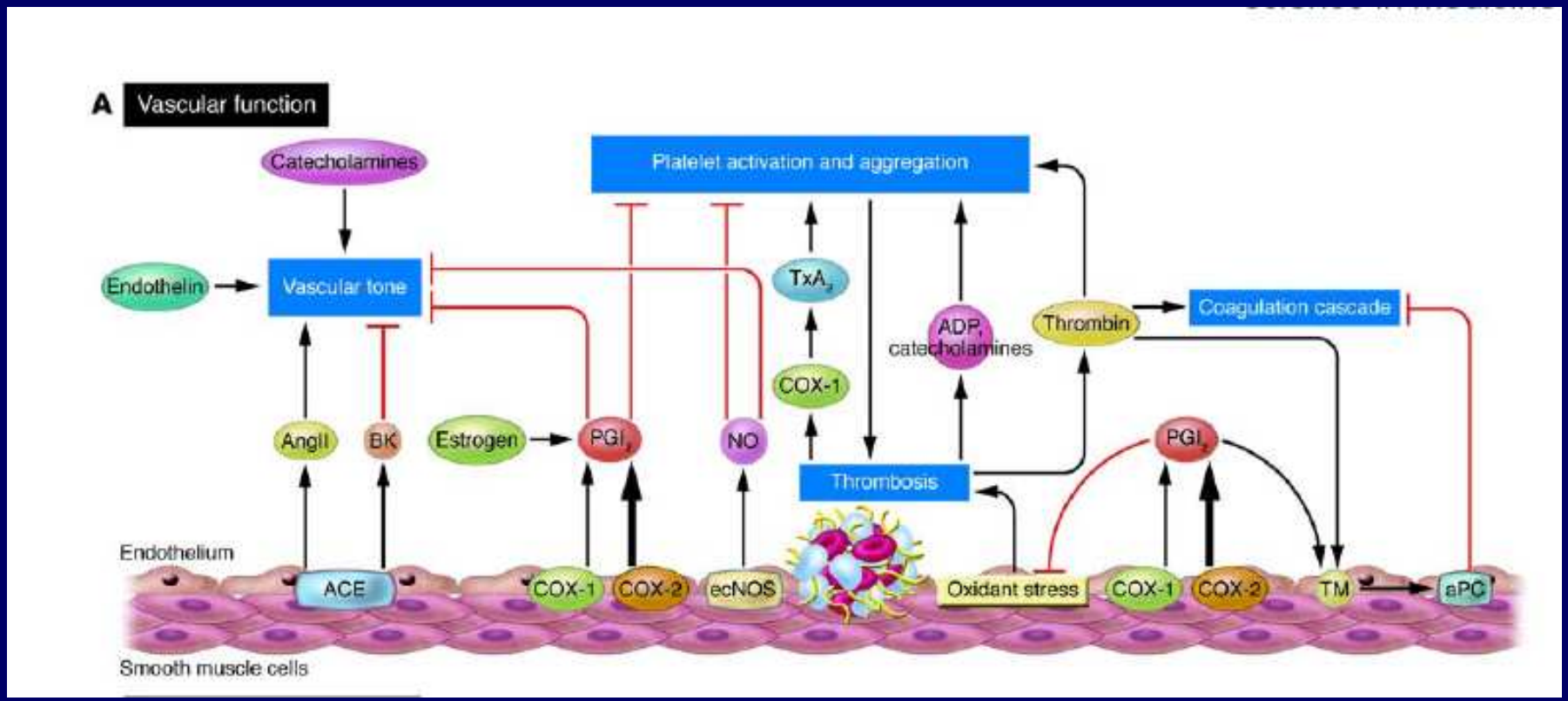
VIA RENALE DEI BAROCETTORI

Pressione nei vasi preglomerulari

↓ pressione perfusione renale
⇓
rilascio PGs
⇓
stimolazione secrezione renina


Renal perfusion pressure
Distal tubule
Urine
Macula densa
Afferent arteriole
$\downarrow Cl^-$
+
Efferent arteriole
Sympathetic nerve endings
Smooth muscle cells
Granular cell
Extraglomerular mesangial cells
Glomerulus
Proximal tubule
By Nancy Heim, Columbia University, and Brooke Grindlinger, J. Clin. Invest.


B Blood pressure regulation
Kidney
Cortex
RAS
+
COX-1
AA
TxA2
(PGF2α)
Increased vascular tone
Other mechanisms
Increased BP
PGI2
Increased
renin
Increased
RAS
Increased BP
Increased sodium
retention
COX-2
–
–
Distal tubular chloride
Baroceptors, BP
Medulla
COX-2
AA
PGE2
PGI2
Decreased
vascular tone
Increased
MBF
Increased natriuresis
Increased diuresis
Decreased BP
Decreased sodium
retention
+
Extracellular tonicity



Roles of the COX isozymes in cardiovascular (**A** and **C**) and renal (**B**) biology. ACE, angiotensin-converting enzyme; ADP, adenosine diphosphate; aPC, activated protein C; BK, bradykinin; ecNOS, endothelial cell NOS; MBF, medullary blood flow; RAS, renin-angiotensin system; TM, thrombomodulin.