Nitric oxide donors and inhibitors, vasodilators, pharmacology of vasoactive peptides

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Nitric oxide (NO)

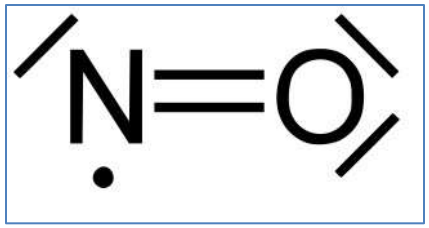
donors and inhibitors

- **therapeutic significance ??? donors ↔ inhibitors**
  - great (nitrates) ↔ under intensive research

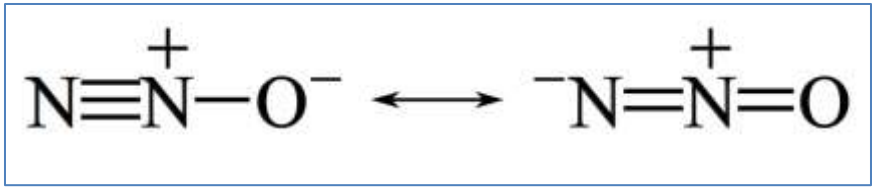
- Background
  - physico-chemical properties
  - physiology - biological role
  - pathophysiology - role of NO in diseases
- NO in therapy
  - direct / NO donors
  - (inhibitors)

Nitric oxide (NO)

- nitric oxide (NO) ≠ nitrous oxide (N_2O)
- both are **gases !**
- NO: **signaling molecule** – N_2O anesthetic gas („laughing gas")
- free diffusion through lipid membranes
- **lability / reactivity** (free radical)
  - only a few seconds lifetime
  - quick reaction with metals and oxigen radicals



NO

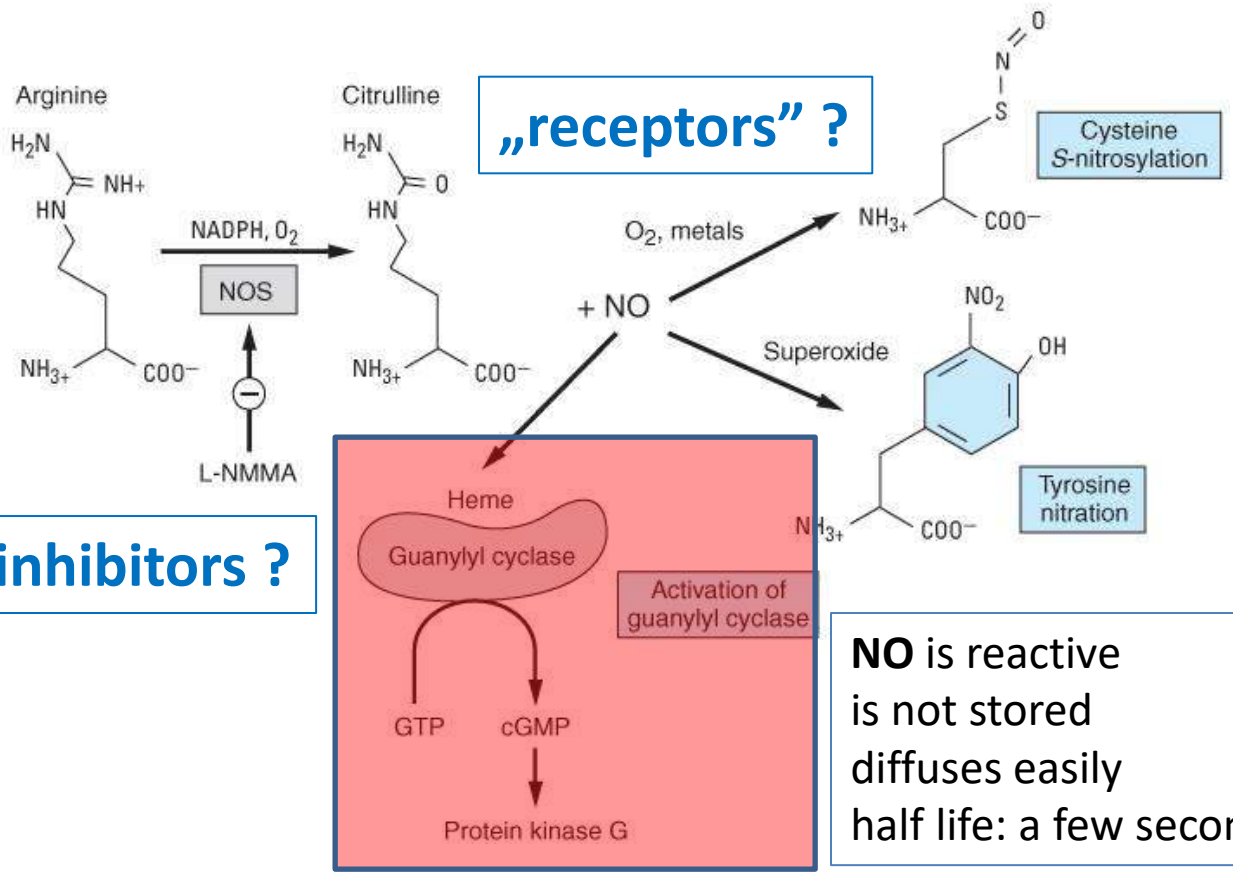


N_2O

Discovery of biological role of NO

- endotoxin exposure
  - *in vitro* **nitrite**s (RONO) /**nitrate**s ($RONO_2$) accumulation
  - *in vivo* urinary levels of nitrites/nitrates ↑
- ACh ⟶ rabbit aorta vasodilation BUT only if endothelium intact
  - EDRF – endothelium derived relaxing factor = NO
    - Robert Furchgott (1979) → Furchgott – Ignarro (1988)
    - 1998 Nobel prize (Furchgott, Murad, Ignarro)
    - “"for their discoveries concerning nitric oxide as a signalling molecule in the cardiovascular system"

Endogenous synthesis and effects of NO



selective inhibitors ?

NO is reactive
is not stored
diffuses easily
half life: a few seconds

NO synthase (NOS) isoforms – nNOS, iNOS, eNOS
BUT the expression is not strictly tissue specific

Endogenous synthesis and effects of NO 2.
Isoforms of NO synthase

Names	NOS-1	NOS-2	NOS-3
Other names	nNOS (neuronal NOS)	iNOS (inducible NOS)	eNOS (endothelial NOS)
Tissue	neurons epithelial cells	macrophages smooth muscle cells	endothelial cells
Expression	Constitutive	Transcriptional induction	Constitutive
Calcium regulation	Yes	No	Yes
Chromosome	12	17	7
Approximate mass	150-160 kDa	125-135 kDa	133 kDa

iNOS: **higher amount of NO** / irreversible calmodulin binding

Endogenous regulation of NOS activity

ADMA: asymmetric dimethylarginine
endogenous NOS inhibitor

ADMA plasma concentration is ↑

in chronic renal insuff. ~ mortality
hypercholesterolemia

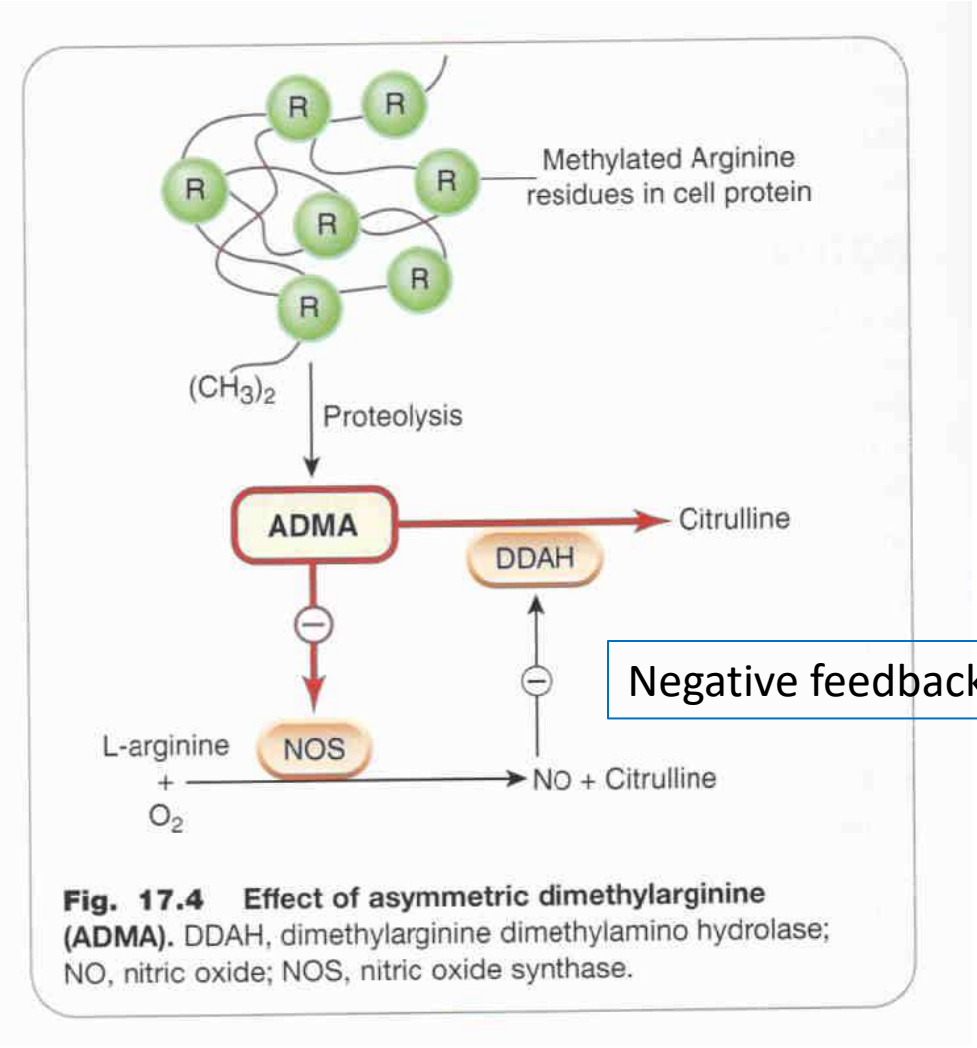


Fig. 17.4 Effect of asymmetric dimethylarginine (ADMA). DDAH, dimethylarginine dimethylamino hydrolase; NO, nitric oxide; NOS, nitric oxide synthase.

Effects of NO **at molecular level**

- covalent modification of proteins
  - metalloproteins
    - **soluble guanylyl cyclase – heme containing → activation**
    - cytochrom oxidase – inhibition
    - heme containing cP450 enzymes – inhibition – e.g. inflamm. liver dis.
  - cystein S-nitrosylation
    - thiol → nitrosothiol (*specific reversible posttranslational modif.*)

    → alteration of function, stability, or localization of target proteins
      - e.g. H-ras – activation
      - e.g. glyceraldehyde-3-phosphate-dehydrogenase – inhibition
    - nitrosoglutathion / nitroso Hgb – *endogenous transporter ?*
  - tyrosine nitration
    - **peroxynitrite** (O_2^-+NO → $ONOO^-$) formation → irreversible nitration of tyrosine → modified protein function
    - ↑oxidative stress → ↑nitrotyrosine
    - mitigated by ic. glutathione (scavanger)

Effects of NO **at cellular level**

- **smooth muscle relaxation**
  - vasodilation – arterioles (basal tone) / erectile function
    - eNOS stimulus: mediators (ACh, bradykinin, …) / shear force
  - other smooth muscles (GI / bronchial / genitourinary)
- **cell adhesion**
  - ↓thrombocyte aggregation, ↓neutrophil adhesion
- **inflammation** ↑ (quantity!)
- **nervous system**
  - peripheral (autonomic): NANC neurotransmission
  - central
    - synaptic plasticity (retrograde transmitter, LTP – e.g. memory)
    - NMDA excitotoxicity (nNOS stimulus: NMDA receptor activation)

Acetylcholine evoked vasodilation

1. Binding to **non-innervated** M_3 ACh receptors on **endothelial cells**
2. activation of G_q-PLC-IP_3 pathway
3. **Ca^{2+} mobilization**
4. Ca^{2+}-calmodulin dependent activation of endothelial NO synthase
5. NO production – diffusion to **smooth muscle cells**
6. stimulation of soluble guanylyl cyclase – cGMP ↑
7. activation of cGMP dependent **protein kinase** (PKG)
   a) Ca^{2+} influx ↓
   b) phosphorylation of myosin light chain phosphatase (MLCP) → dephosphorylation of myosin light chain
   c) phosphorylation of myosin light chain kinase (MLCK) → MLCK inhibition ?
   d) membrane K^+ channel opening → hyperpolarisation
8. **smooth muscle relaxation**

NO in diseases 1.

- **vascular system**
  - critical role in maintenance and repair
    - endothelial dysfunction → decreased production of NO
  - **not enough NO**
    - **high blood pressure**
    - **thrombosis – atherosclerosis /** atherogenesis
      - ↓ inhibition of smooth muscle cell proliferation
      - ↓ inhibition of thrombocyte aggregation
      - ↓ inhibition of monocyte / leukocyte adhesion
    - pulmonary hypertension
      - beneficial in **respiratory insufficiency** associated with pulmonary hypertension in newborns
  - **too much NO** (non-endothelial origin)
    - **septic shock**
      - endotoxin, cytokines → iNOS (excess production of NO) → shock

NO in diseases 2.

- **inflammatory diseases**
  - role in inflammatory / immune response
  - BUT too much or for too long is harmful (excess production of NO)
- **nervous system**
  - central nervous system
    - overproduction → **excitotoxicity, neurodegenerative diseases** (e.g. ischaemic stroke)
  - peripheral nervous system
    - NANC neurons – neurotransmitter
      - gastric emptying – **hypertrophic pyloric stenosis** (↓ production)
      - erection – corpora cavernosa, smooth muscle relaxation
        - » dysfunction → **impotency** (↓ production)

NO related therapeutic modalities 1.

- **inhibition of NO synthesis** – currently no clinical use
  - non-isoform selective arginin analogs – eNOS is inhibited too
  - in development – **isoform selective inhibitors**
    - minimal differences in binding sites / inhibition of dimerisation
    - inflammation, sepsis – iNOS – BBS-2 ?
    - neurodegenerative diseases – nNOS – 7-nitroindazole
- **prevent decreased NO synthesis (endotheldysfunction)**
  - antioxidants, statins, L-arginine (?)
  - **nebivolol** – β_1 antagonist + vasodilator (by NO)

NO related therapeutic modalities 2.

- **NO donors**
  - **nitrates** / nitrites
    - organic nitrates
      - **nitroglycerin**
      - isosorbid dinitrate / isosorbid-5-mononitrate
    - organic nitrites – amylnitrite
    - Na-nitroprusside / molsidomine
  - hybrid NO donors – in development
    - incorporation of NO in CV drugs (aspirin, captopril, nitro-statins)
  - inhalation of NO gas
    - primary pulmonary hypertension in newborns
- **modification of NO „downstream" signaling pathways – PDE inhibitors**
    - erectile dysfunction – e.g. sildenafil (Viagra®)

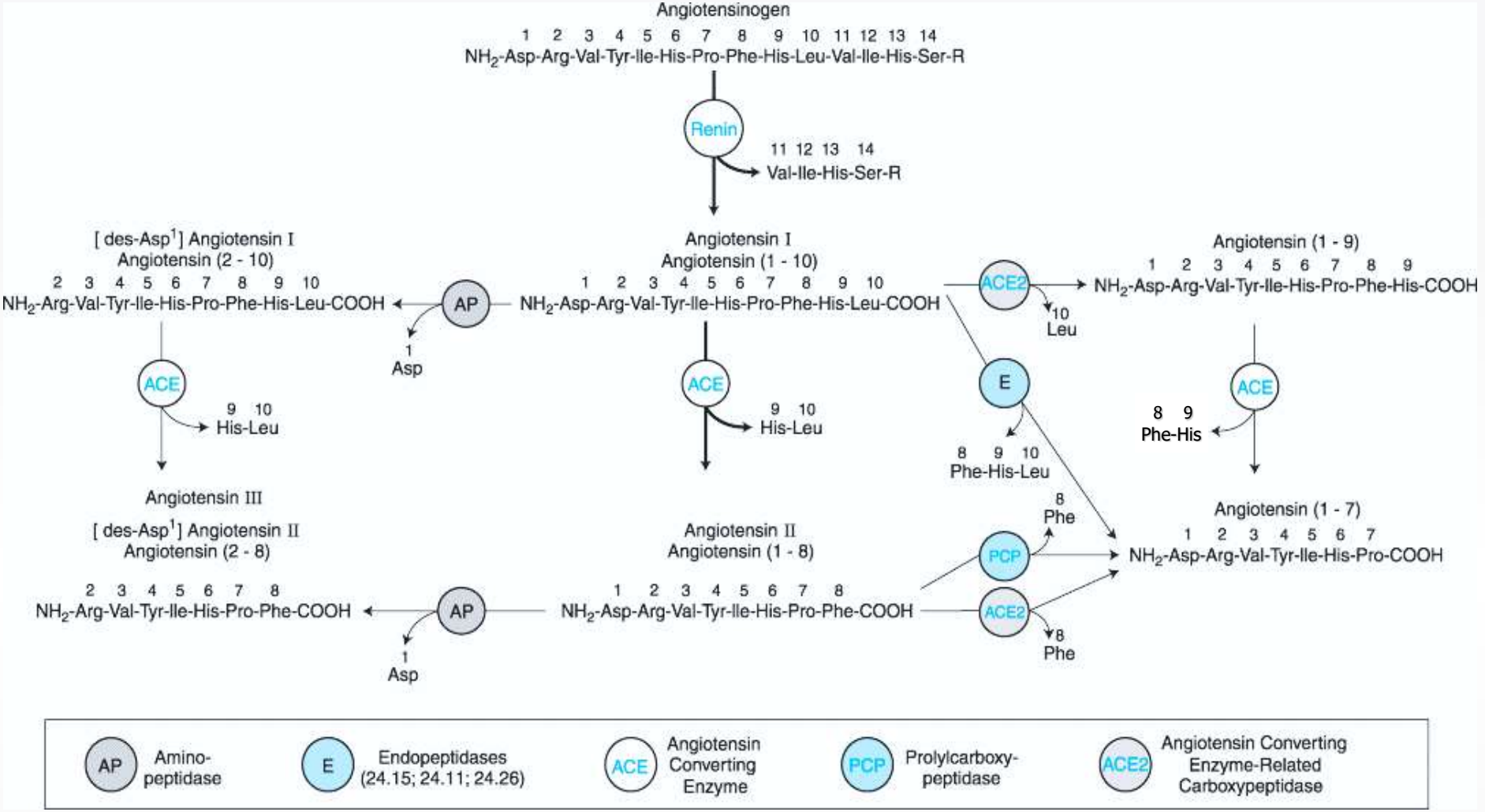
Inhaled NO gas

- in high dose toxic (pulmonary edema, methemoglobinemia)
- selective dilation of pulmonary vasculature (< 50 ppm)
- ↓ pulmonary pressure, ↑ oxygenation
- FDA: newborns with severe persistent pulmonary hypertension
  - ↑ oxygenation
  - ↓ need for extracorporeal membrane oxygenation
- questions – long term outcome???
  - ARDS
  - primary pulmonary hypertension
  - pulmonary embolism
  - lung transplantation

The main vasoactive peptides

- Vasoconstrictors
  - Angiotensin II
  - Vasopressin
  - Endothelins
  - Neuropeptide Y
  - Urotensin II
- Vasodilators
  - Kinins (bradykinin and tachykinins)
  - Natriuretic peptides
  - Vasoactive intestinal peptide
  - Substance P
  - Neurotensin
  - Calcitonin gene-related peptide
  - Adrenomedullin

Angiotensin II



Effects of angiotensin II

Through AT_1 and AT_2 receptor agonism (especially via AT_1 receptor stimulation):

- Vasoconstriction (especially on arterioles; it is one of the strongest agent)
- Increase of aldosterone synthesis (hence increase of renal Na^+ reabsorption)
- Dipsogenic effect (thirst)
- Increase of plasma vasopressin level
- Increase of plasma ACTH level
- Enhancement of cell (especially smooth muscle cell) proliferation

Inhibition of RAAS I.

(**r**enin-**a**ngiotensin-**a**ldosterone **s**ystem)

- Inhibitors of renin secretion:
  - α_2 agonists (**clonidine**)
  - β_1 antagonists (**bisoprolol**)
- (Pro)renin receptor antagonists:
  - complex effects; under investigation

Inhibition of RAAS II.
(**r**enin-**a**ngiotensin-**a**ldosterone **s**ystem)

- Renin inhibitors:
  - **aliskiren**, **remikiren**, **enalkiren**
- ACE (angiotensin converting enzyme) inhibitors:
  - „-pril" drugs (**captopril**, **enalapril**, **ramipril**, **perindopril**)
- ARB-s (AT_1 receptor blockers):
  - „-sartan" drugs (**losartan**, **valsartan**, **telmisartan**, **irbesartan**)

Inhibition of RAAS III.
(**r**enin-**a**ngiotensin-**a**ldosterone **s**ystem)

- Aldosterone (mineralocorticoid) receptor antagonists:
  - **spironolactone** (non-selective), **eplerenone** (selective)
- Epithelial sodium channel blockers:
  - action on the renal collecting tubule; **triamterene**, **amiloride**

Natriuretic peptides

ANP: atrial („A-type") natriuretic peptide (produced mainly by atrium)

BNP: brain („B-type") natriuretic peptide (despite its name, produced mainly by ventricle)

CNP: C-type („complementing") natriuretic peptide (produced mainly by endothelium)

By stimulating NPR_A, NPR_B and NPR_C receptors, they:

- decrease the renin (+ angiotensin II, aldosterone) and vasopressin production
- increase the renal Na^+ excretion (diuretic effect)
- decrease blood pressure

Inactivation: neprilysin (neutral endopeptidase)

Synthetic (recombinant) BNP: **nesiritide**; may be useful in acute decompensation of congestive heart failure

Endopeptidase inhibitors
(other than renin and trypsin inhibitors)

Omapatrilat, **sampatrilat**, **fasidotrilat** („vasopeptidase inhibitors”):

- Inhibitors of two metalloproteinase enzymes, neprilysin (neutral endopeptidase; NEP-24.11) and ACE, and thereby increase the natriuretic peptide levels and decrease the angiotensin II level – no clinical use

In addition to the natriuretic peptide levels, inhibition of neprilysin affects the concentration of several other bioactive compounds eliminated by neprilysin, e.g. glucagon, enkephalins, substance P, neurotensin, oxytocin and bradykinin. Moreover, neprilysin participates in the elimination of beta amyloid peptide too.

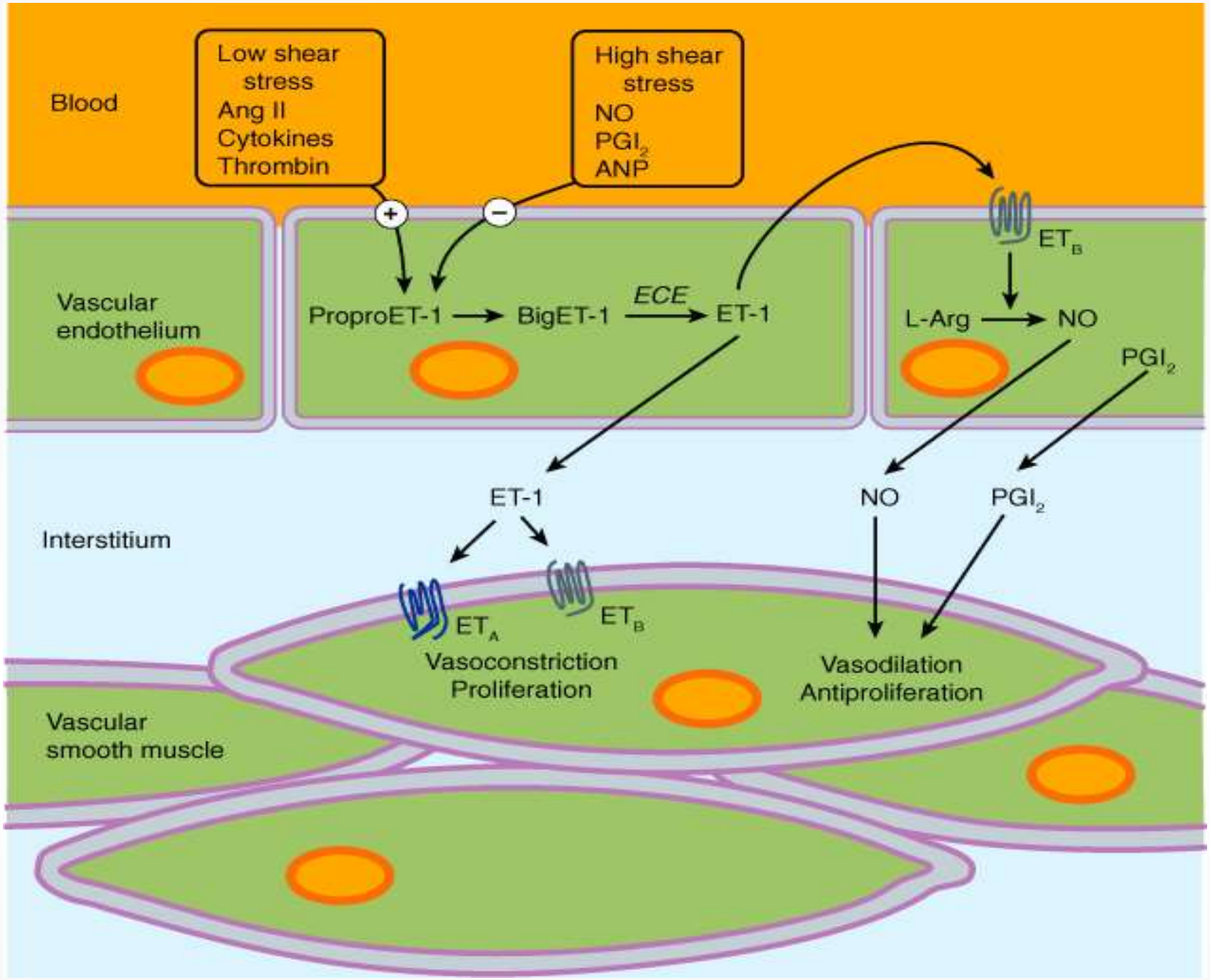
Sacubitril (active metabolite: sacubitrilat):

- selective neprilysin inhibitor, used (in combination with valsartan) to treat chronic heart failure (with reduced ejection fraction)

Endothelins

The 3 isoforms (ET-1, ET-2, ET-3), by stimulating ET_A and ET_B receptors, evoke:

- vasoconstriction (e.g. coronary arteries, renal arteries)
- bronchoconstriction
- increase in concentration of renin (and thus angiotensin II and aldosterone), vasopressin and atrial natriuretic peptide
- smooth muscle proliferation
- but: via endothelial ETB stimulation, they cause NO and prostacyclin (PGI_2) production, which latter ones have vasodilatory and antiproliferative actions


Blood
Low shear stress
Ang II
Cytokines
Thrombin
High shear stress
NO
PGI2
ANP
+
−
Vascular endothelium
ProproET-1
BigET-1
ECE
ET-1
ETB
L-Arg
NO
PGI2
Interstitium
ET-1
NO
PGI2
ETA
ETB
Vasoconstriction
Proliferation
Vasodilation
Antiproliferation
Vascular smooth muscle

Inhibition of endothelin system

- Endothelin receptor antagonists
  - non-selective: **bosentan** (to treat severe pulmonary arterial hypertension; orally active; hepatotoxicity reported)
  - ET_A selective: **sitaxentan** (hepatotoxicity reported) – no clinical use; **ambrisentan** (fetal harm reported) – to treat exclusively pulmonary arterial hypertension (orphan drug *)
- Endothelin converting enzyme inhibitors
  - **phosphoramidon** (additional inhibition of neprilysin) – no clinical use

(* orphan drug – a drug to treat exclusively one rare medical condition)