Bradykinin System

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Lecture outline:

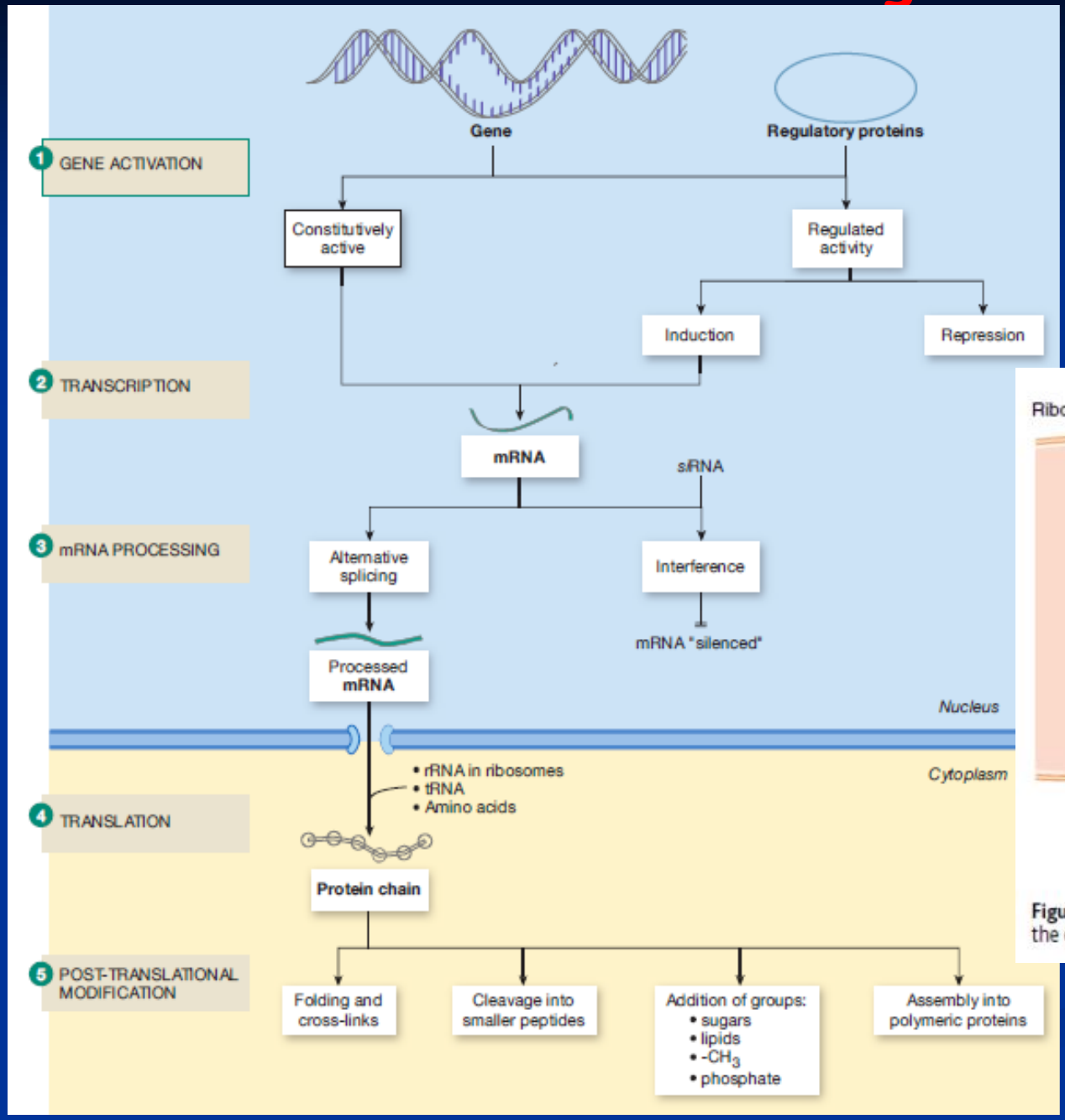
1. Introduction to bioactive peptides, enzyme systems, synthesis and degradation
2. Physiology of kinins.
3. Pharmacology of kinins: receptors and signaling.
4. Therapeutic targets and current trends.

1: Introduction

Synthesized and processed according to **central dogma**

- Post-translational processing from large precursor proteins into 3-36 amino acid active peptides into 3-36 amino acid active peptides
- Serve as neurotransmitters/hormones e.g. endorphins, dynorphins, substance P, neuropeptide Y, ANP, CCK, vasopressin, oxytocin, etc.
- OR autocrine/paracrine/endocrine physiological signaling molecules or mediators e.g. bradykinin, kallidin, angiotensin, vasopressin, oxytocin, ANP/F etc.
- Large molecules compared to small molecules transmitters- e.g. ACh, dopamine, norepinephrine, NO etc

1: Introduction: Central dogma



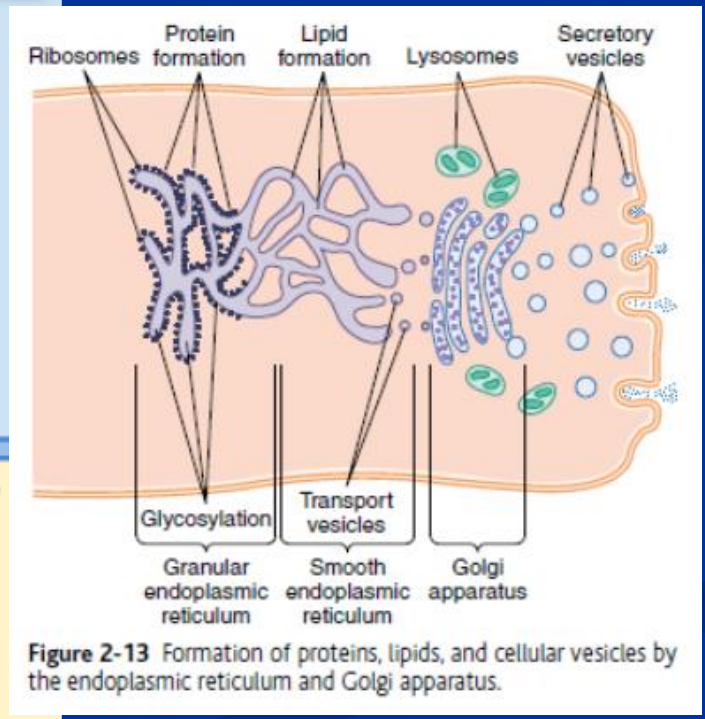
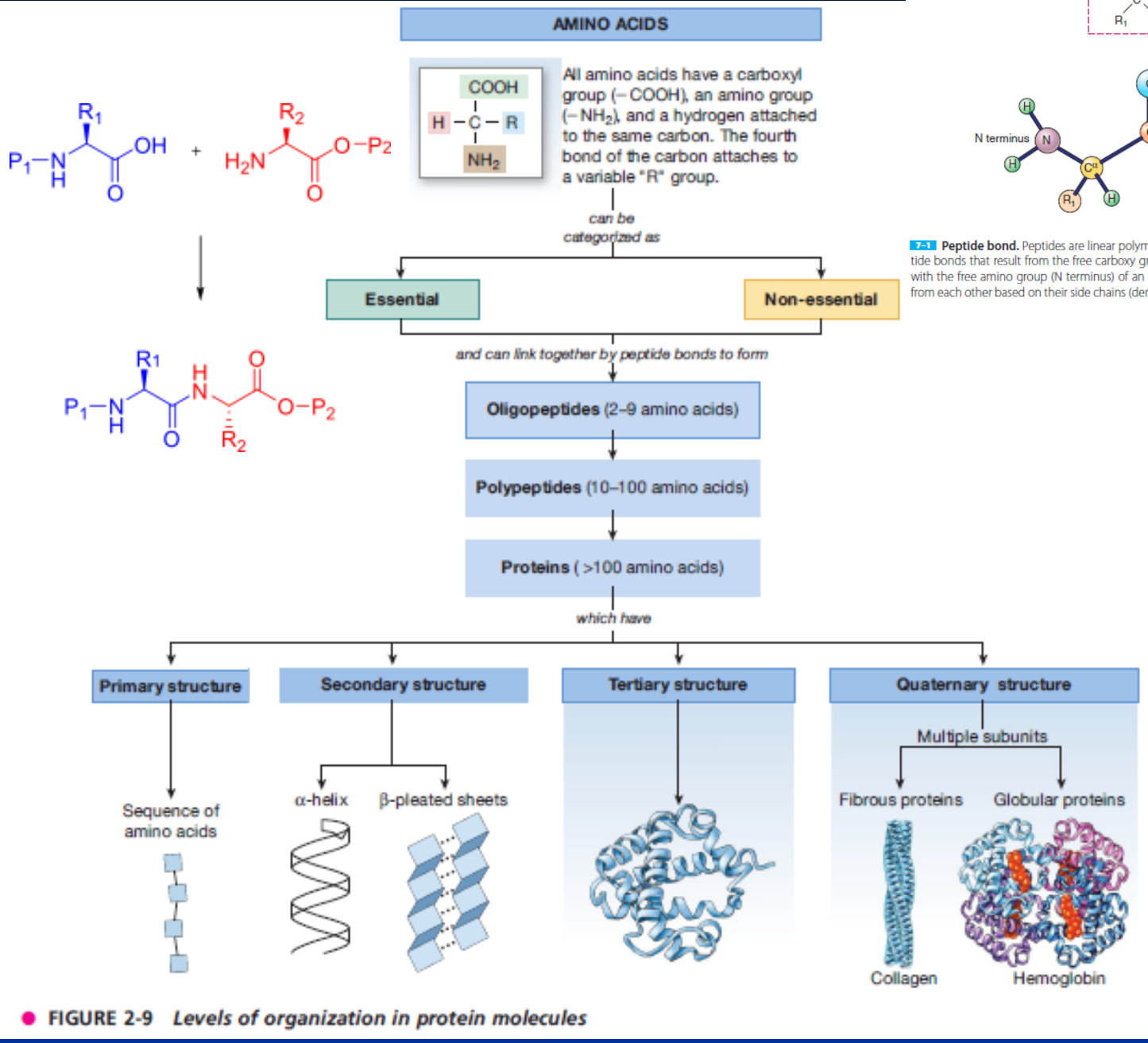
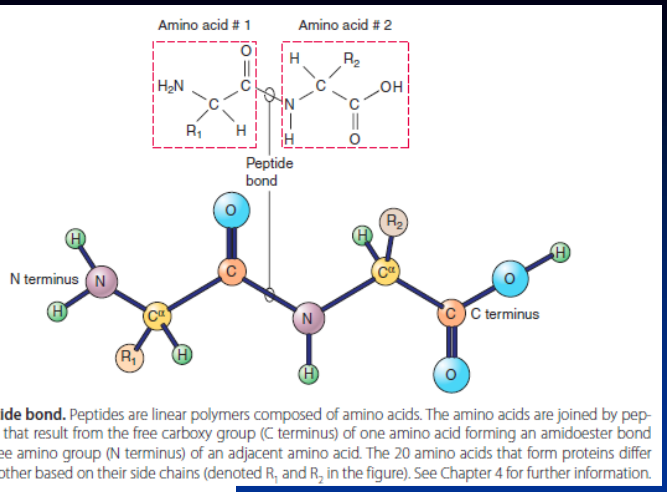


Figure 2-13 Formation of proteins, lipids, and cellular vesicles by the endoplasmic reticulum and Golgi apparatus.

1: Introduction

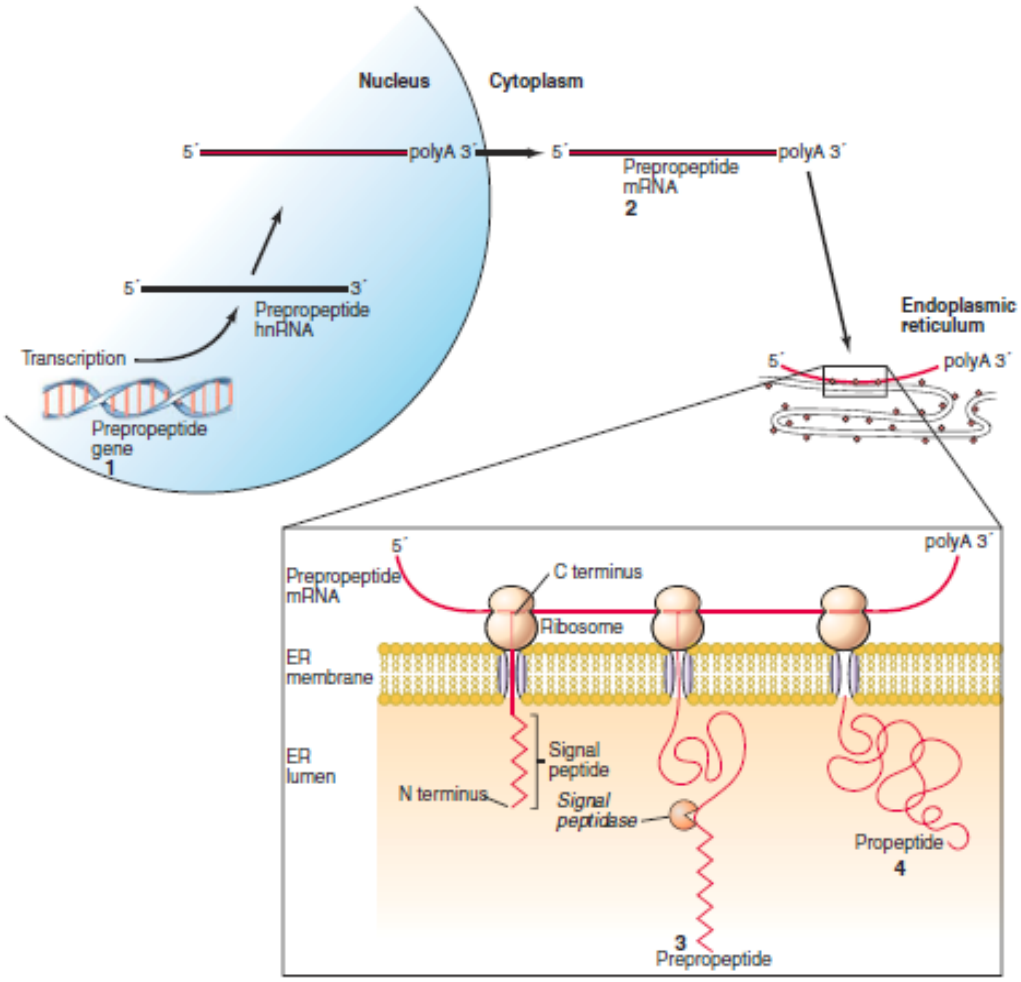


● FIGURE 2-9 *Levels of organization in protein molecules*



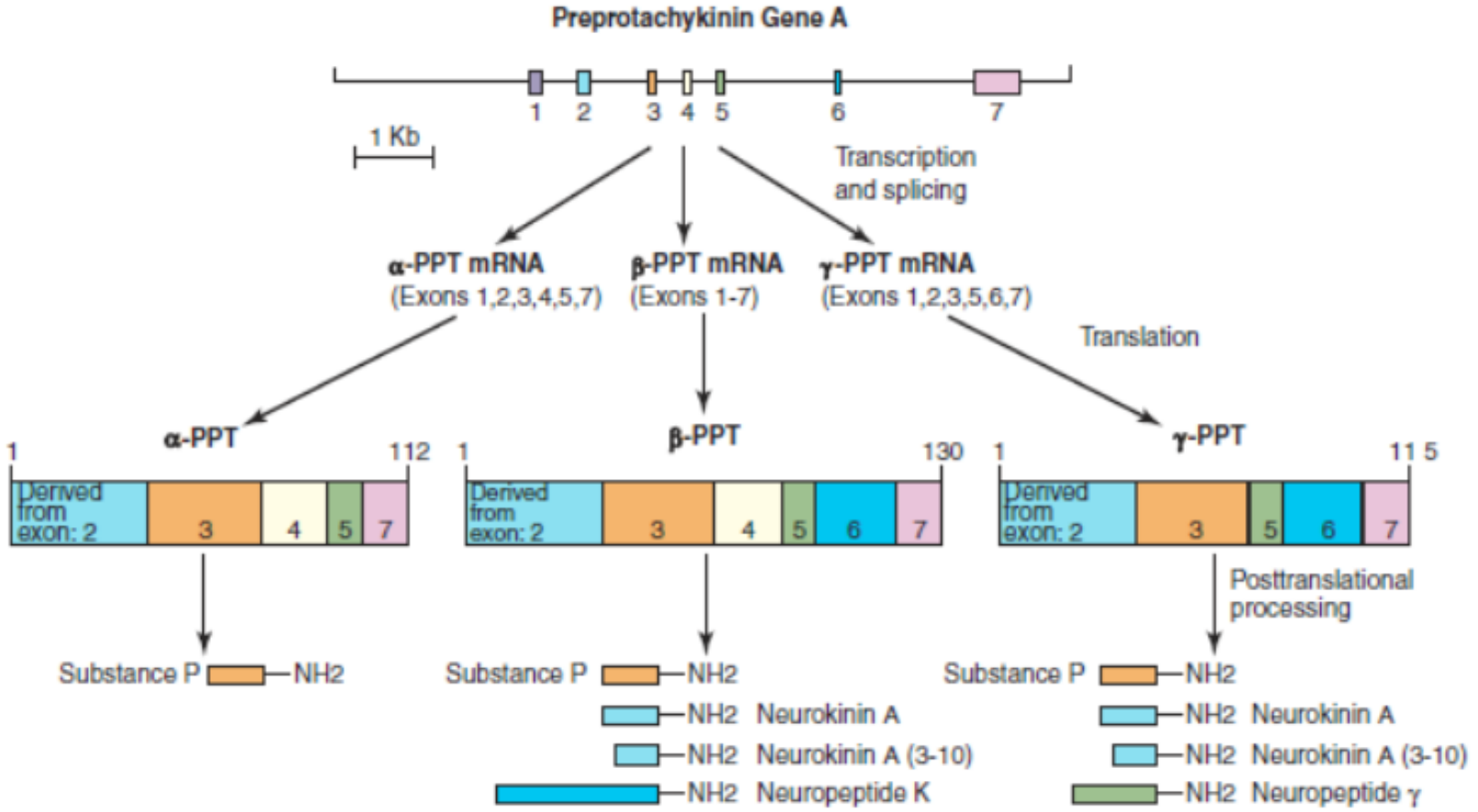
7-1 **Peptide bond.** Peptides are linear polymers composed of amino acids. The amino acids are joined by peptide bonds that result from the free carboxy group (C terminus) of one amino acid forming an amidoester bond with the free amino group (N terminus) of an adjacent amino acid. The 20 amino acids that form proteins differ from each other based on their side chains (denoted R_1 and R_2 in the figure). See Chapter 4 for further information.

1: Introduction



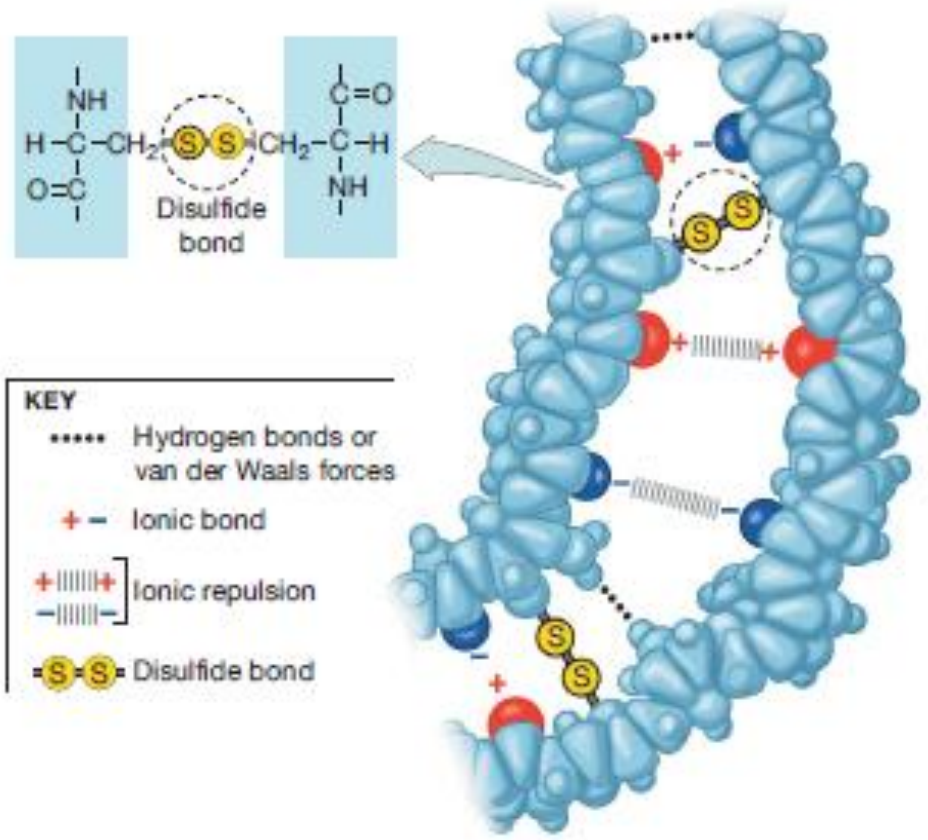
7-2 Synthesis of a neuropeptide. Neuropeptide synthesis is a multistep process that begins with (1) nuclear transcription of the gene that encodes one or more prepropeptides in the nucleus, splicing of the resulting primary RNA transcript to produce a messenger RNA (mRNA; described in detail in 7-5), and (2) transport of the mRNA into the cytoplasm where it is translated by ribosomes in the rough (ribosome studded) endoplasmic reticulum (ER). (3) The N terminus of the growing prepropeptide is translated first, and contains a signal sequence that targets the growing peptide to the lumen of the ER and thence to the regulated secretory compartment. The signal sequence is cleaved by a signal peptidase even before the entire peptide is translated to yield (4) a propeptide that must undergo further enzymatic modification as required to produce active peptides.

1: Introduction

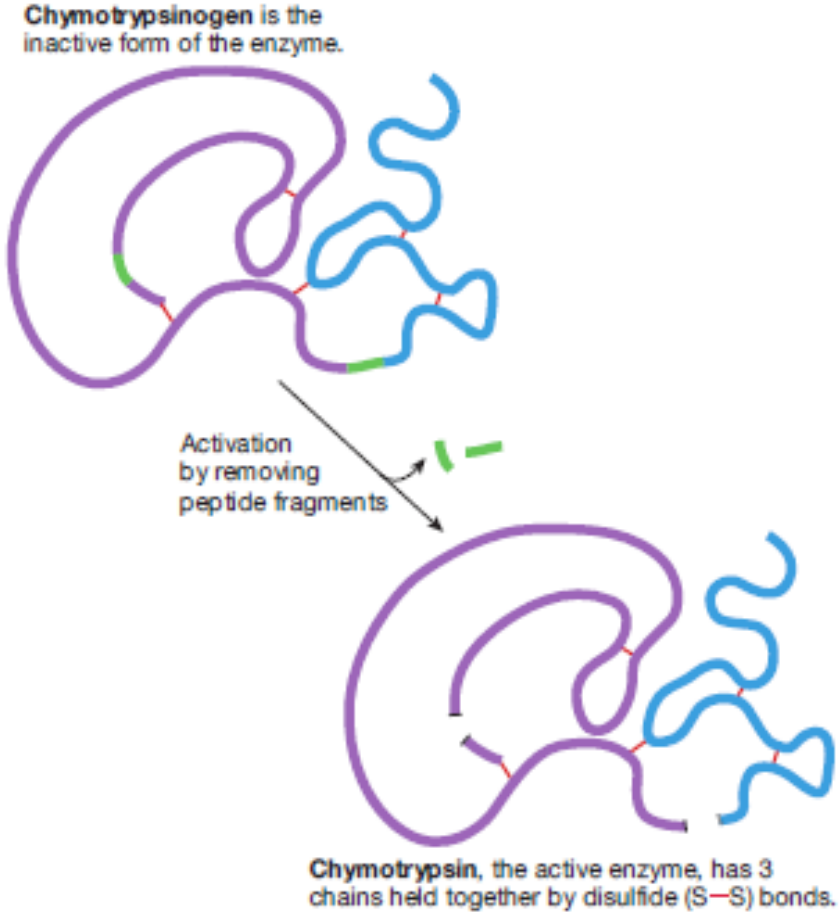


7-3 Alternative splicing of the preprotachykinin-A (PPT-A) gene. This gene, also called PPT-I gene or substance P–neurokinin A gene, contains seven exons (numbered boxes), which are alternatively spliced into three prepropeptides (α, β, and γ PPT). The number shown above each PPT splice variant represents its amino acid length after translation. After translation and proteolytic processing, all three PPT splice variants liberate substance P, which is encoded in exon 3. Neuropeptide K is encoded in exons 3–6 and thus is derived only from β-PPT. Neuropeptide γ is encoded in exons 3, 5, and 6, which occur together only in γ-PPT. Neurokinin A and the neurokinin A fragment (3-10) can be synthesized from either β- or γ-PPT. (Adapted with permission from Helke CJ, Krause JE, Mantyh PW, et al. *FASEB J.* 1990;4:1608.)

1: Introduction



● **FIGURE 2-10** ***Noncovalent bonds hold molecules or parts of molecules together.*** Hydrogen bonds, disulfide bonds, van der Waals forces, and ionic bonds help create the tertiary structure of proteins. (Figure adapted from *Molecular Cell Biology 5/e* by H. Lodish *et al.*)



● **FIGURE 2-17** ***Proteolytic activation of the enzyme chymotrypsin***

1: Introduction: Methods used in determinations of proteins

Non-specific methods that detect total protein only:

- Absorbance (100 µg/mL to 1 mg/mL).
- Bradford protein assay: Detection in the range of ~1 mg/mL.
- Lowry Protein assay: Detection in the range of 0.01–1.0 mg/mL

Specific methods to detect amount of a single protein

- High-performance liquid chromatography (HPLC)
- Liquid chromatography–mass spectrometry (LC/MS)
- Protein immunoprecipitation
- Immunoelectrophoresis
- Western blot: couples gel electrophoresis and incubation with antibodies to detect specific proteins

Protein sequencing: The two major direct methods are **mass spectrometry** and **Edman degradation** using a protein sequenator (sequencer).

2. Physiology of Kinins

- In the 1920-30s, Frey, Kraut, and Werle characterized a hypotensive substance in urine, which was also found in other fluids and tissues, and especially in pancreas and named it kallikrein (a Greek synonym for the pancreas).
- Later, kallikrein shown to produce active substance-polypeptide (kallidin) from inactive precursor (globulin) in plasma.
- Trypsin and some snake venoms act on plasmaglobulin to produce substance that lowered BP & cause slowly developing contraction of gut.
- They called it "bradykinin" (In Greek "bradys"- slow and "kinein"-to move). KININS are polypeptides like BRADYKININ and KALLIDIN

2. Physiology of Kinins

- **Bradykinin** (9 aa nanopeptide) and **Kallidin** (10 aa decapeptide, essentially bradykinin with an additional Lys aa in N-terminal) were isolated and synthesized in 1960s.

- The decapeptide kallidin is about as active as the nonapeptide bradykinin, even without conversion to bradykinin.

Kallidin	Lys^{1}-	Arg^{2}-Pro^{3}-Pro^{4}-Gly^{5}-Phe^{6}-Ser^{7}-Pro^{8}-Phe^{9}-Arg^{10}
Bradykinin		Arg^{1}-Pro^{2}-Pro^{3}-Gly^{4}-Phe^{5}-Ser^{6}-Pro^{7}-Phe^{8}-Arg^{9}

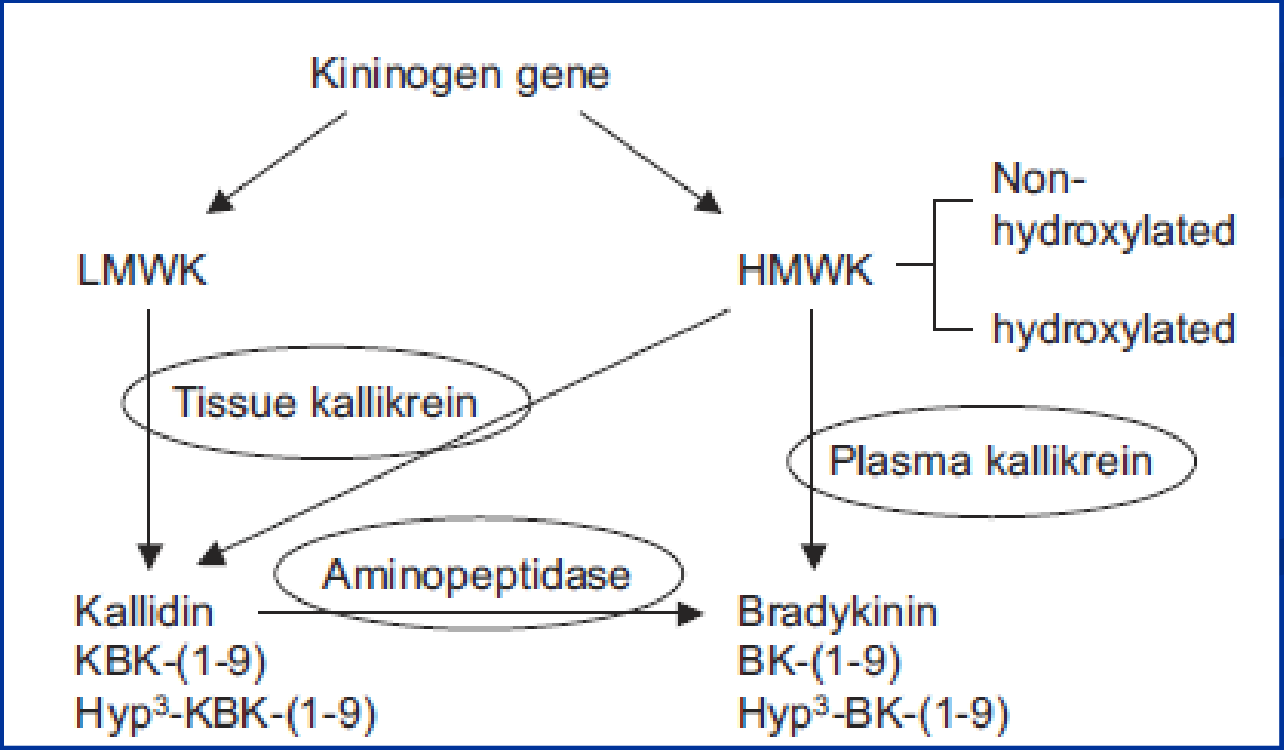
- They are products of enzymatic cleavage of precursor a2 -globulins (proteins) called **kininogens**

- There are **two kininogens**: HMW (120 kDa) kininogen (**HMWK**) and LMW (66 kDa) kininogen (**LMWK**). Both are acidic glycoproteins consisting of single polypeptide chain.

2. Physiology of Kinins

- Two specific serine proteases that release bradykinin and kallidin from the kininogens are termed **kallikreins**.

- **Two** distinct kallikreins: are **Plasma** and **Tissue** kallikreins. About 15-20% of the total plasma kininogen is in HMW form. It is thought that LMWK crosses capillary walls and serves as substrate for tissue kallikrein, while HMWK is confined to bloodstream and acts as substrate for plasma kallikrein



2. Physiology of Kinins:

- **Plasma kallikrein** derived from plasma **prekallikrein**, an 88 kDA inactive protein.

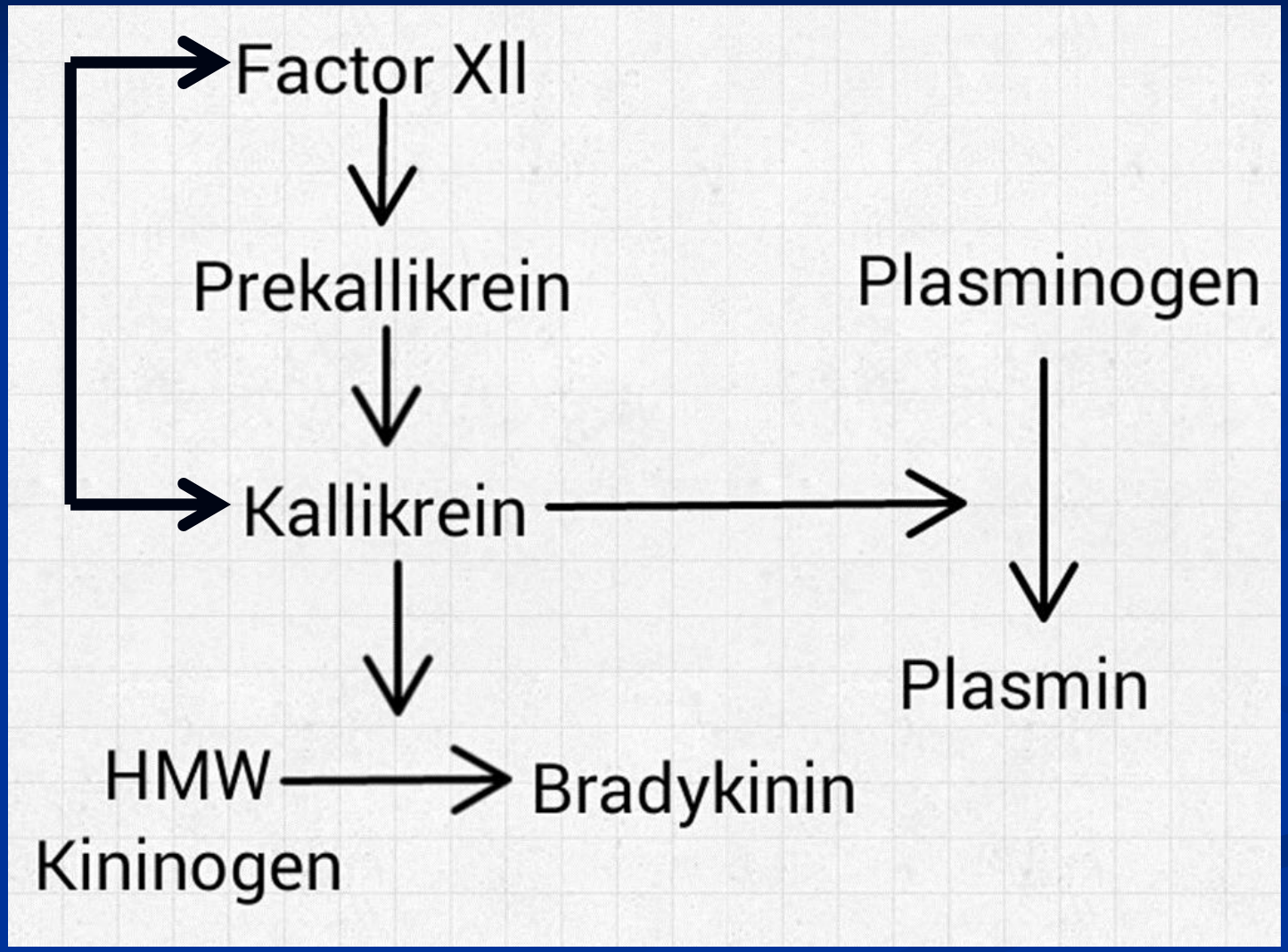
- Under physiological conditions, Prekallikrein forms complex with HMWK. This complex is inhibited by plasma protease inhibitors first component of complement (C1) and a2 macroglobulin.

- Hageman factor (clotting factor XII) a protease, binds to PreK+HMWK complex to activate PreK $\longrightarrow$ Kallikrein.

- Kallikrein in turn activates factor XII (positive feedback)

pKallikrein $\longrightarrow$ Kininogen (HMWK & LMWK) $\longrightarrow$ bradykinin & kallidin.

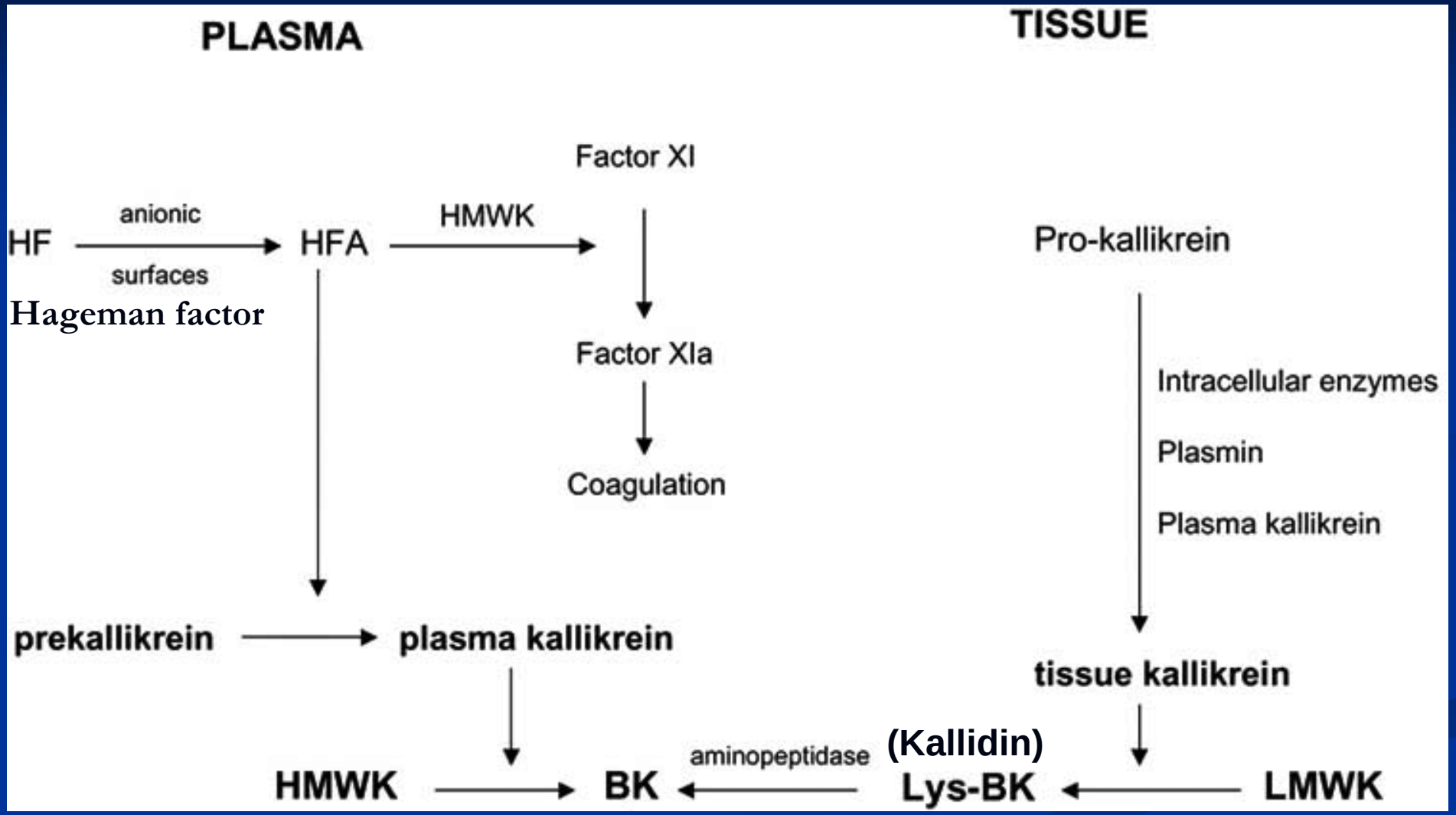
2. Physiology of Kinins:



2. Physiology of Kinins:

- **Tissue Kallikrein** produced from a 29 kDa preproprotein in epithelial cells, secretory cells (e.g. salivary, pancreas, prostate & kidney-distal nephron) & neutrophils.
- Synthesis of tissue prokallikrein controlled by aldosterone & androgens.
- Prokallikrein activated to tissue kallikrein by proteolysis (by plasma kallikrein & serine metalloproteases in vitro) removing 7 amino acid.
- Tissue Kallikrein $\longrightarrow$ LMWK $\longrightarrow$ Kallidin

2. Physiology of Kinins:

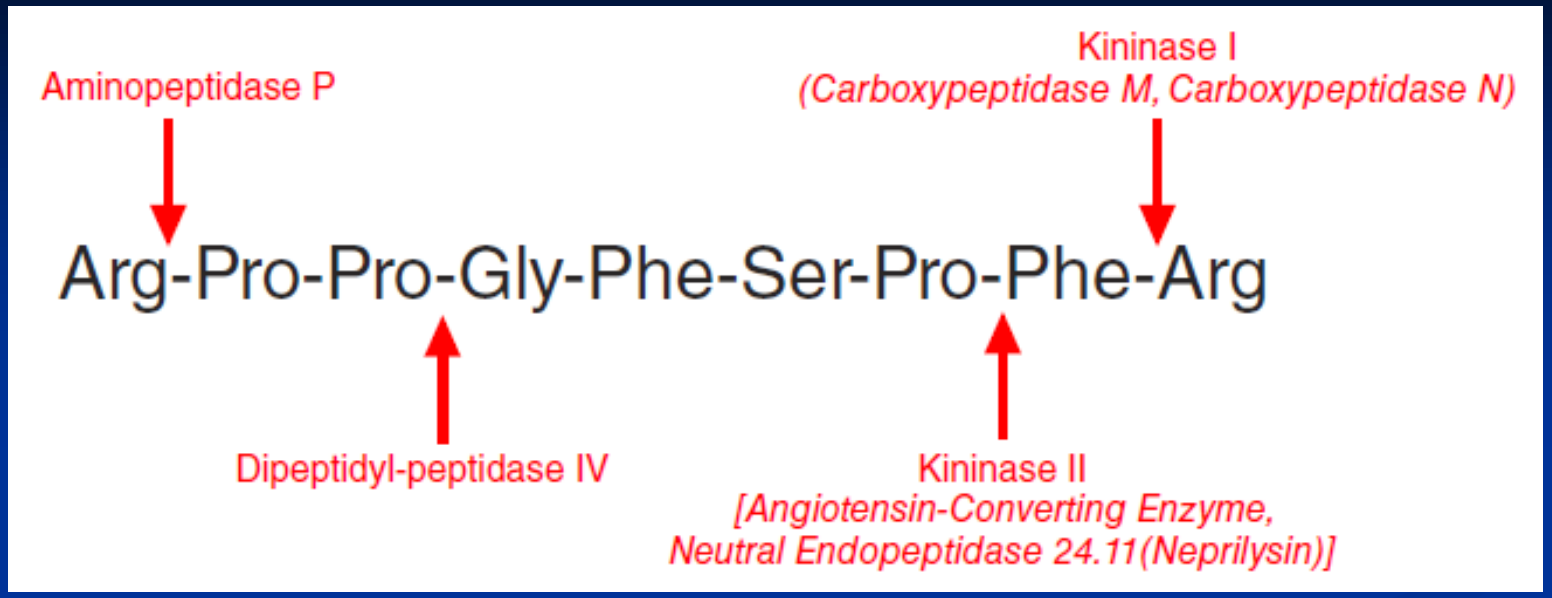


2. Physiology of Kinins: Metabolism of Kinins:

- 80-90% kinins inactivated (metabolized) by single passage through pulmonary vascular bed. $T_{1/2}$ of kinins in plasma is 15-30 seconds.
- Physiological concentrations of bradykinin in blood are in the **picomolar range**. Difficult to measure [plasma]…required adequate inhibition of kininases & kininogenases (eg. kallikrein).
- **Kininases**

1. **Kininase I (carboxypeptidase M & N).**
2. **Kininase II (ACE, neutral endopeptidase-aka NEP/neprilysin).**
3. **Dipeptidyl-peptidase IV (DPP4)**

2. Physiology of Kinins: Metabolism of Kinins



Schematic diagram of the degradation of bradykinin. Arrows denote the primary cleavage sites in bradykinin. Bradykinin and kallidin are inactivated in vivo primarily by kininase II (ACE). Neutral endopeptidase 24.11 (neprilysin) cleaves bradykinin and kallidin at the same Pro-Phe bond as ACE and also is classified as a kininase II–type enzyme. In addition, aminopeptidase P can inactivate bradykinin by hydrolyzing the N-terminal Arg1-Pro2 bond, leaving bradykinin suscepti-ble to further degradation by dipeptidyl peptidase IV. Bradykinin and kallidin are converted to their respective des-Arg9 or des-Arg10 metabolites by kininase I–type carboxypeptidases M and N. Unlike the parent peptides, these kinin metabolites are potent ligands for B1 kinin receptors but not B2 kinin receptors.

2. Physiology of Kinins: Metabolism of Kinins

NAME	STRUCTURE	FUNCTION
Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Agonist, B_2
Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Agonist, B_2
[des-Arg^9]-Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Agonist, B_1
[des-Arg^{10}]-Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Agonist, B_1

2. Physiology of Kinins: Metabolism of Kinins

- **Kininase I** : Carboxypeptidase **M** acts 3x faster than **N**. They (both M and N) cleave C-terminal Arg and produce **[desArg9]-Bradykinin** or [desArg10]-Kallidin. These peptides are biologically active metabolites.
- Rare familial carboxypeptidase N deficiency is associated with angioedema and urticaria

- [desArg9]-Bradykinin is further degraded by **ACE2** to inactive metabolites.

- Carboxypeptidase N is expressed in the liver and secreted into the blood. Other kininases are membrane-anchored peptidase on the surface of endothelial and epithelial cells.

- **Kininase II;** The principal catabolizing enzyme in the lung and other vascular beds is kininase II (aka. ACE) and NEP. They cleave (cut) kinins to produce inactive metabolites (inactive peptides).

2. Physiology of Kinins: Metabolism of Kinins

- Aminopeptidase P (**APN**) cleaves N-terminal Arg of bradykinin and produce inactive metabolites. However, dipeptidyl peptidase IV (DPP4) can further cut these peptides to other inactive metabolites.

- DPP4 also cleaves incretins such Glucagon-like peptide-1 (GLP-1) and the inhibitors of DPP4 are clinically used as oral antidiabetic drugs to prolong the effects of incretins. **Inhibitors of DPP4 are used in treatment of diabetes.**

- Importantly, the **DPP4 is the cell entry receptor for Middle East respiratory syndrome coronavirus (MERS-CoV).**

- Even more importantly, **ACE2** (which degrades [desArg9]-Bradykinin to its inactive metabolites) **is the entry receptor for Severe acute respiratory syndrome coronaviruses (SARS-CoV-1 and SARS-CoV-2) and SARS-CoV-2 is the pathogen virus for COVID-19**

- **APN** is also the entry receptor for other less known coronaviruses such as **human coronaviruses**-229E (HCoV-229E).

2. Physiology of Kinins: Biological effects of Kinins

1. **Cardiovascular:** (Decreases BP, cardio-protective). Potent vasodilators through NO release. Many of the beneficial effects of ACE inhibitors are attributable to enhancement of bradykinin effects, such as their antiproliferative activity or ability to increase tissue glucose uptake.

Bradykinin also stimulates tissue plasminogen activator release from the vascular endothelium and may be beneficial for myocardial infarction and stroke

2. **Kidney:** (Promote natriuresis and diuresis). Paracrine fashion regulate urine volume and compositions. Treatment with mineralocorticoids, ACE inhibitors, and neprilysin inhibitors increases renal kallikrein.

2. Physiology of Kinins: Biological effects of Kinins

3. Respiratory: (Induce cough). Contract bronchial smooth muscle. Kinins are implicated in allergic airway disorders such as asthma and rhinitis. Inhalation of kinins causes bronchospasm in asthmatics but not in normal individuals.

4. Inflammation: (Pro-inflammatory). Disrupt inter-endothelial junctions, and increase vascular permeability and edema. Play a role in pathogenesis of hereditary angioedema.

The B1 receptors on inflammatory cells (e.g., macrophages) stimulate inflammatory mediators IL-1 and TNF-a. Kinins play a role in chronic inflammatory disease (gout, IBD, arthritis).

Disruption of BBB and proinflammatory effects are involved in neuroinflammatory disorders such as neuropathic pain, Alzheimer's disease.

2. Physiology of Kinins: Biological effects of Kinins

5. Pain: (Algesic). Excite by releasing pain mediators such as PGs, SP, neurokinin A, and CGRP from sensory neurons and sensitize somatosensory fibers to these mediators.

B2 receptors generally mediate acute bradykinin algesia, whereas the pain of chronic inflammation appears to involve increased numbers and activation of B1 receptors.

6. Cancer: (Pro-cancerous). Lung & gastric cancers. Cause proliferation, progression, and migration of cancer cells.

7. Fetus: promote dilation of fetal pulmonary artery, closure of ductus arteriosus, constriction of umbilical vessels, and facilitates the transition from fetal to neonatal circulation.

3. Pharmacology of Kinins: Biological effects of Kinins are mediated by 2 subtypes of Bradykinin receptors (B1 and B2).

TABLE 39–3 ■ STRUCTURE OF KININ AGONISTS AND ANTAGONISTS

NAME	STRUCTURE	FUNCTION
Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Agonist, B_2
Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	Agonist, B_2
[des-Arg^9]-Bradykinin	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Agonist, B_1
[des-Arg^{10}]-Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	Agonist, B_1
des-Arg^{10}-[Leu^9]-Kallidin	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro Leu	Antagonist, B_1
NPC-349	[D-Arg]-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg	Antagonist, B_2
HOE-140	[D-Arg]-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Oic-Arg	Antagonist, B_2
[des-Arg^{10}]-HOE-140	[D-Arg]-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Oic	Antagonist, B_1
FR173657	Nonpeptides	Antagonist, B_2
FR190997		Agonist, B_2
SSR240612		Antagonist, B_1

Hyp, trans-4-hydroxy-Pro; Thi, β-(2-thienyl)-Ala; Tic, [D]-1,2,3,4-tetrahydroisoquinolin-3-yl-carbonyl; Oic, (3as,7as)-octahydroindol-2-yl-carbonyl.

3. Pharmacology of Kinins:

- The B1 and B2 receptors are G protein-coupled, 7-transmembrane-spanning receptors.
- B1 receptors are normally absent but upregulated by tissue injury and inflammation and by cytokines, endotoxins, and growth factors.
- B2 receptor is expressed in most normal tissues and mediates the effects of bradykinin and kallidin under normal circumstances.
- B2 receptors rapidly desensitize, whereas B1 receptors do not sensitize, unless they heterodimerize with B2 receptor (cross-desensitization).
- **Physiological response depends on receptor distribution, cell type, cell environment and mediators**

3. Pharmacology of Kinins:

- Both B1 and B2 receptors couple through Gq to activate PLC and increase intracellular Ca2+.

- In **normal endothelial cells**, B2 receptors ⟶ Gq ⟶ Ca2+-calmodulin–dependent eNOS and **short-term** generation of NO ⟶ cyclic GMP↑ and relaxation in smooth muscle.

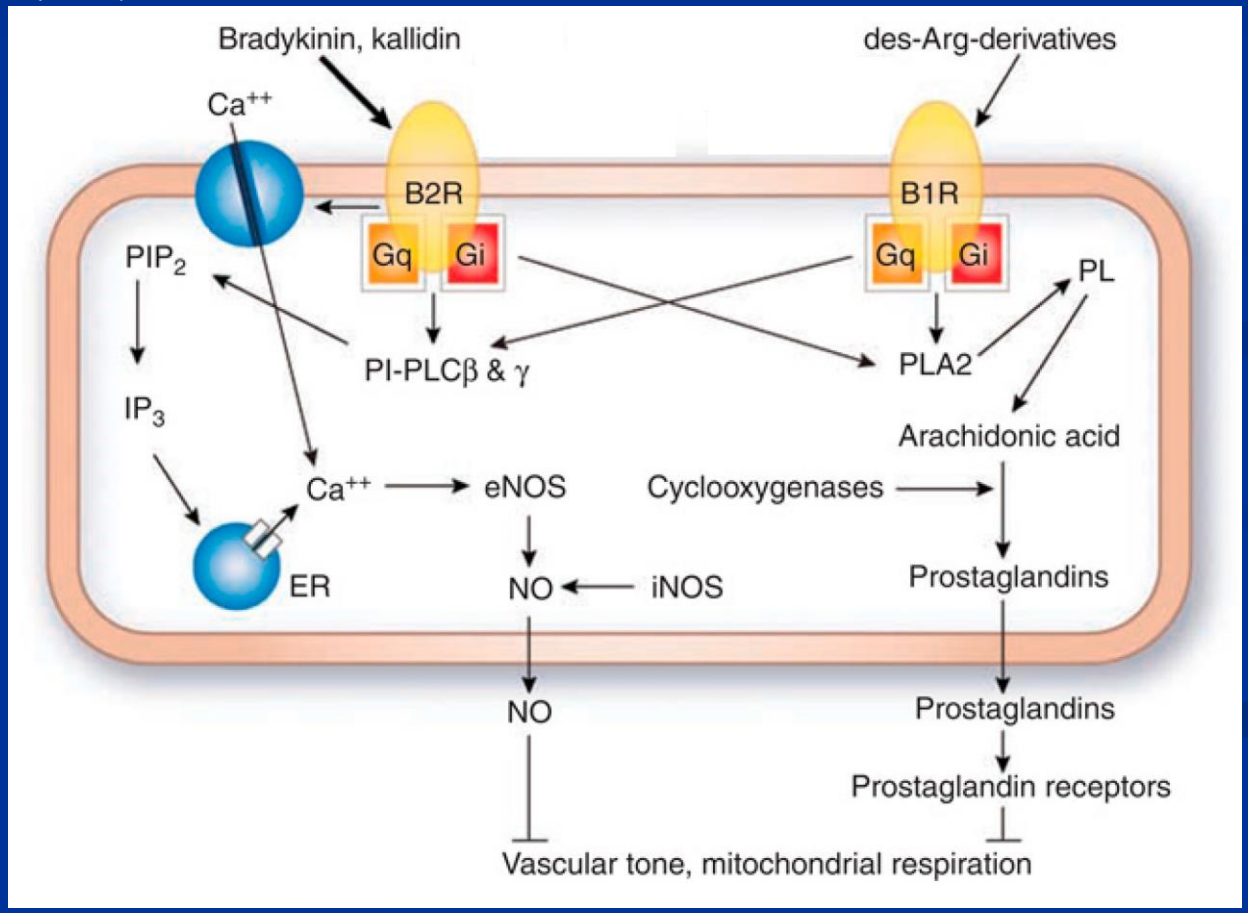
- However, **direct** activation of B1 or B2 receptors on **smooth muscle** cells ⟶ Gq ⟶ increased [Ca2+]i, resulting in contraction.

- **In inflammation**, B2 receptor ⟶Gi⟶ prolonged eNOS-derived NO whereas B1 receptor ⟶ Gi and MAP kinase activation to cause ERK1/2-mediated phosphorylation and activation of iNOS, which generates prolonged, high-output NO

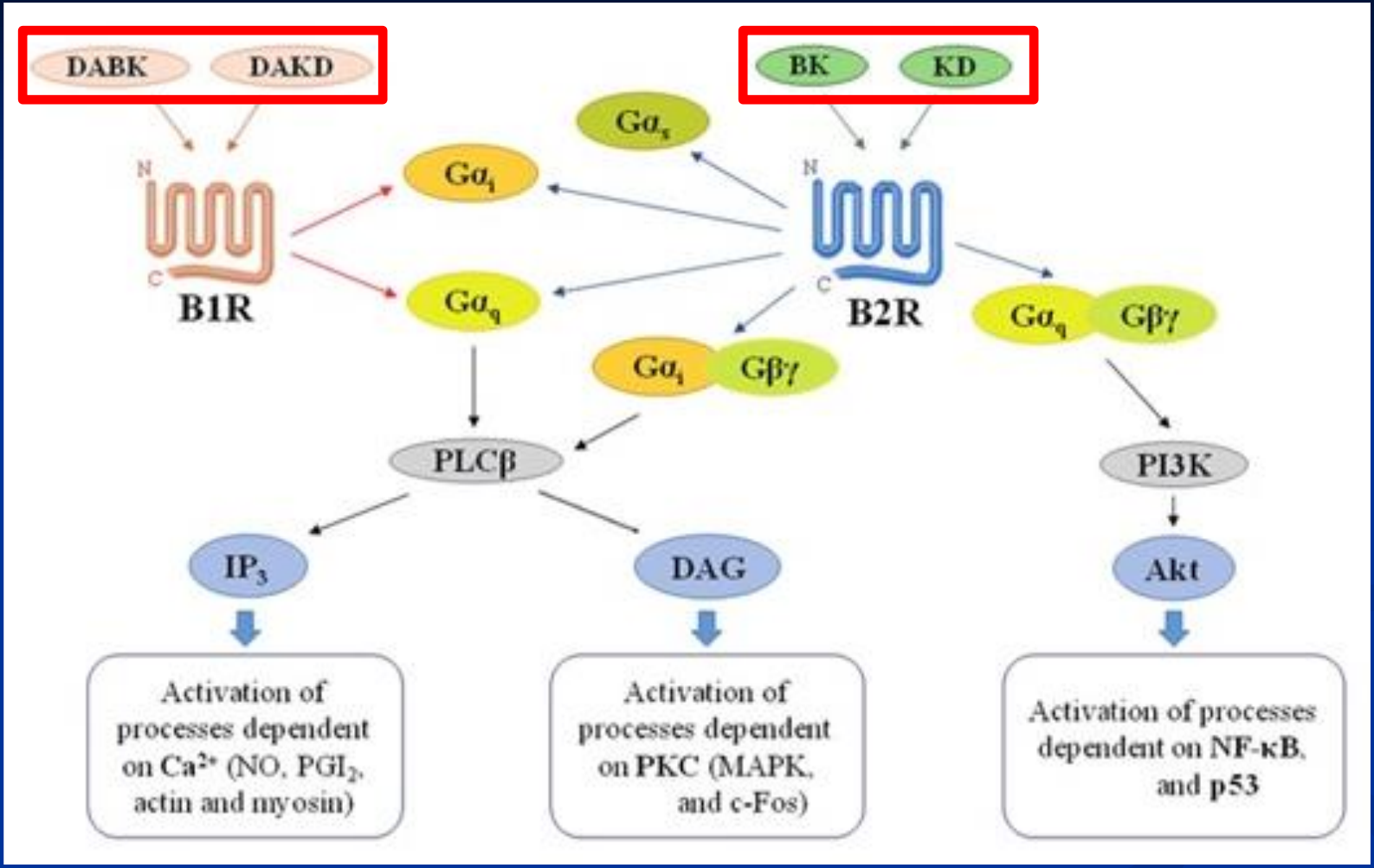
3. Pharmacology of Kinins:

- B1 and B2 receptor → Gαq and βγ subunits → activate the pro-inflammatory transcription factor NF-κB and also activate the MAP kinase pathway.

- B1 and B2 receptors also → Gi → to activate PLA2→ release of arachidonic acid and the local generation of metabolites such as prostaglandins and vasodilator epoxyeicosatrienoic acid.



3. Pharmacology of Kinins:



The B1 receptor interacts with Gaq, and Gai, stimulates many of the same signaling pathways as the B2 receptor, including phosphoinositol hydrolysis, increased [Ca2+]i, arachidonic acid release, eicosanoid production, endothelial NO synthase activation, and NO production.

4. Therapeutic targets

Kallikrein Inhibitors:

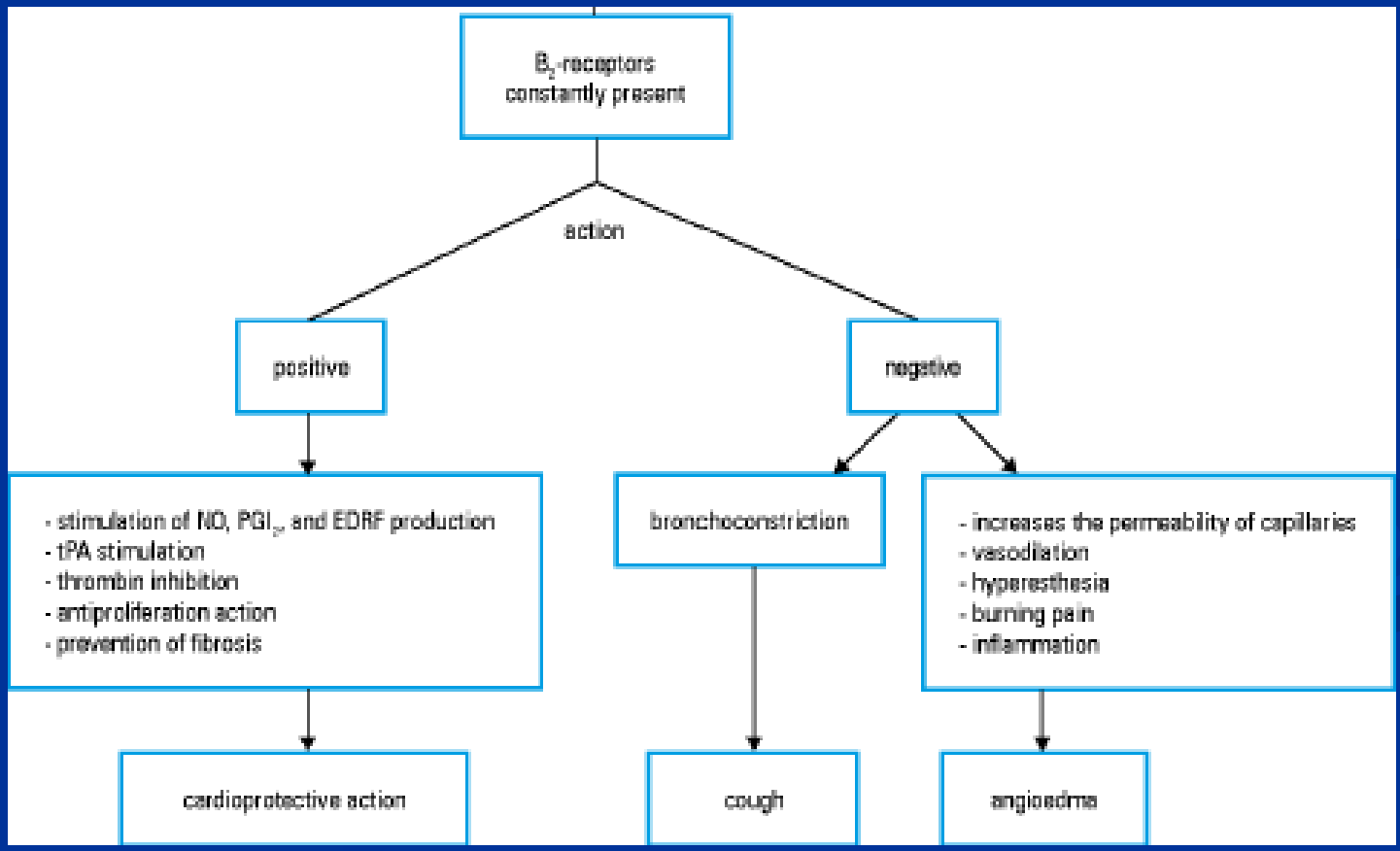
- **Aprotinin:** Natural proteinase inhibitor (serine proteases e.g. kallikrein.. derived from bovine lungs). Also inhibits plasmin resulting in antifibrolysis. It is clinically used to reduce blood loss in surgery but withdrawn due increased mortality.

- **Ecallantide:** Synthetic plasma kallikrein inhibitor. It is used in treatment acute attacks of hereditary angioedema. Approximately 20% of patients develop antibodies to the drug and may be at a higher risk of hypersensitivity reactions (caution anaphylaxis due to drug administration).

4. Therapeutic targets

Bradykinin and the Effects of ACE Inhibitors:

- ACE (kininase II) involved in catabolism of bradykinin and kallidin into inactive products. **ACE Inhibitors (pril-group drugs such as Captopril, Enalapril, Lisinopril) cause increased levels of bradykinin and kallidin and this induces angioedema and cough**



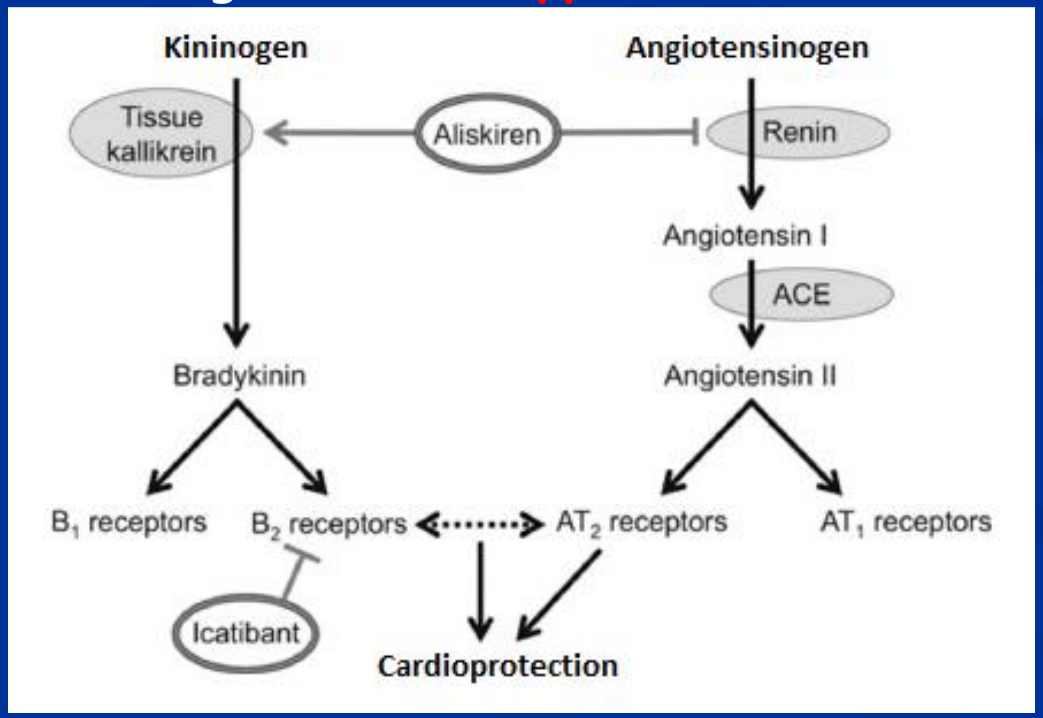
4. Therapeutic targets

Kinin receptor antagonists:

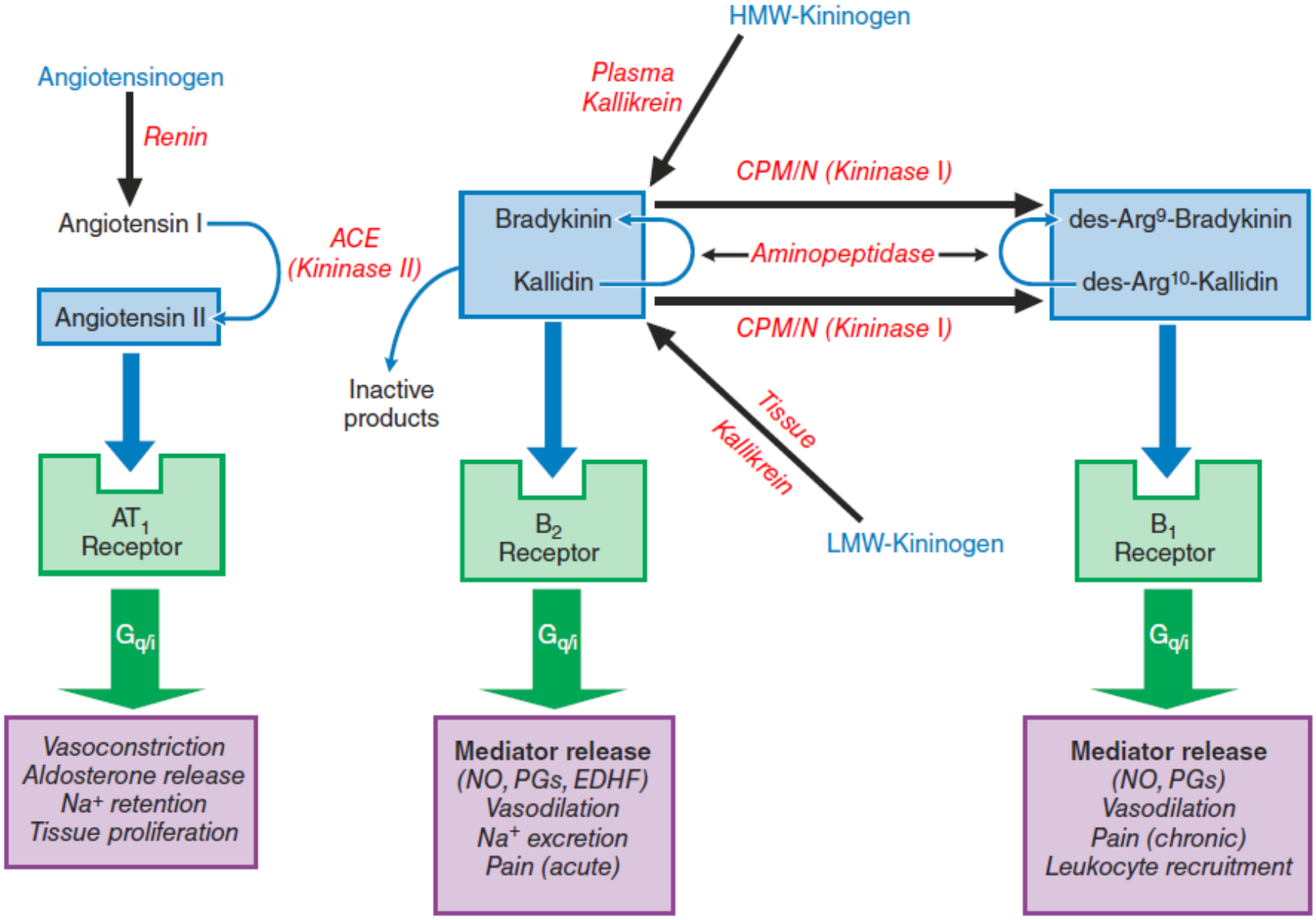
- **Icatibant:** Selective B2 receptor antagonist. It used for acute attacks of hereditary angioedema. It is also being investigated as treatment of ACE inhibitor–induced angioedema

Kallikrein activators:

- **Aliskiren:** Direct renin inhibitor but kallikrein activator. It is used for treating essential hypertension.



BRADYKININ SYSTEM (SUMMARY)



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THANK YOU