

Chapter 37

Nitric Oxide and Vasoactive Peptide Signal Molecules

NITRIC OXIDE

It is one of the oxides of nitrogen with molecular formula $\text{NO}^\bullet$ indicating that it is a free radical with an unpaired electron. Nitric oxide is the smallest endogenous signal molecule and the only one which is a gas at normal temperature. Since its discovery as a biological mediator in 1980, it is now known to perform a wide array of functions.

Furchgott and Zawadzki (1980) demonstrated that ACh caused vascular smooth muscle relaxation only when the endothelial lining was intact, but not when it was denuded by rubbing. They proposed that on activation by ACh, the endothelial cells released a soluble 'endothelium derived relaxing factor' (EDRF) which diffused to the underlying smooth muscle cell causing relaxation. A little earlier Farid Murad (1977) had already shown that GTN caused vasodilatation by releasing NO. Ignarro in 1987 confirmed that EDRF infact was NO. All three, Furchgott, Murad and Ignarro received Nobel Prize in 1998 for these discoveries. Soon a number of other vasorelaxants (histamine, 5-HT, bradykinin, etc.) were also shown to act through EDRF release. It is now established that NO is a key messenger in the cardiovascular and nervous systems, as well as in inflammatory and some other types of cells.

Generation and fate of NO: Nitric oxide is produced in the cells from the amino acid L-arginine by the enzyme nitric oxide synthase (NOS) using molecular oxygen and NADPH

(Fig. 37.1); flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), haeme and tetrahydrobiopterin (H_4B) act as coenzyme to NOS. The rate of NO production depends on the activity of NOS, because the substrate is normally present in excess.

Three isoforms of NOS, viz. neuronal NOS (nNOS or NOS-1), inducible NOS (iNOS or NOS-2) and endothelial NOS (eNOS or NOS-3) have been characterized. Though all

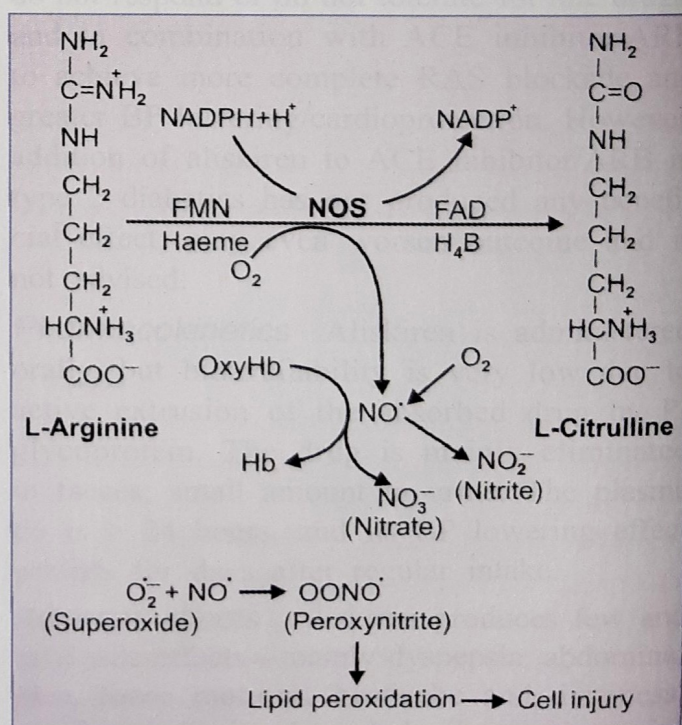


Fig. 37.1: Generation and metabolism of nitric oxide (NO) FMN—Flavin mononucleotide; FAD—Flavin adenine dinucleotide; NADP—Nicotinamide adenine dinucleotide phosphate; NADPH—Reduced form of NADP; H₄B—Tetrahydrobiopterin; NOS—Nitric oxide synthase

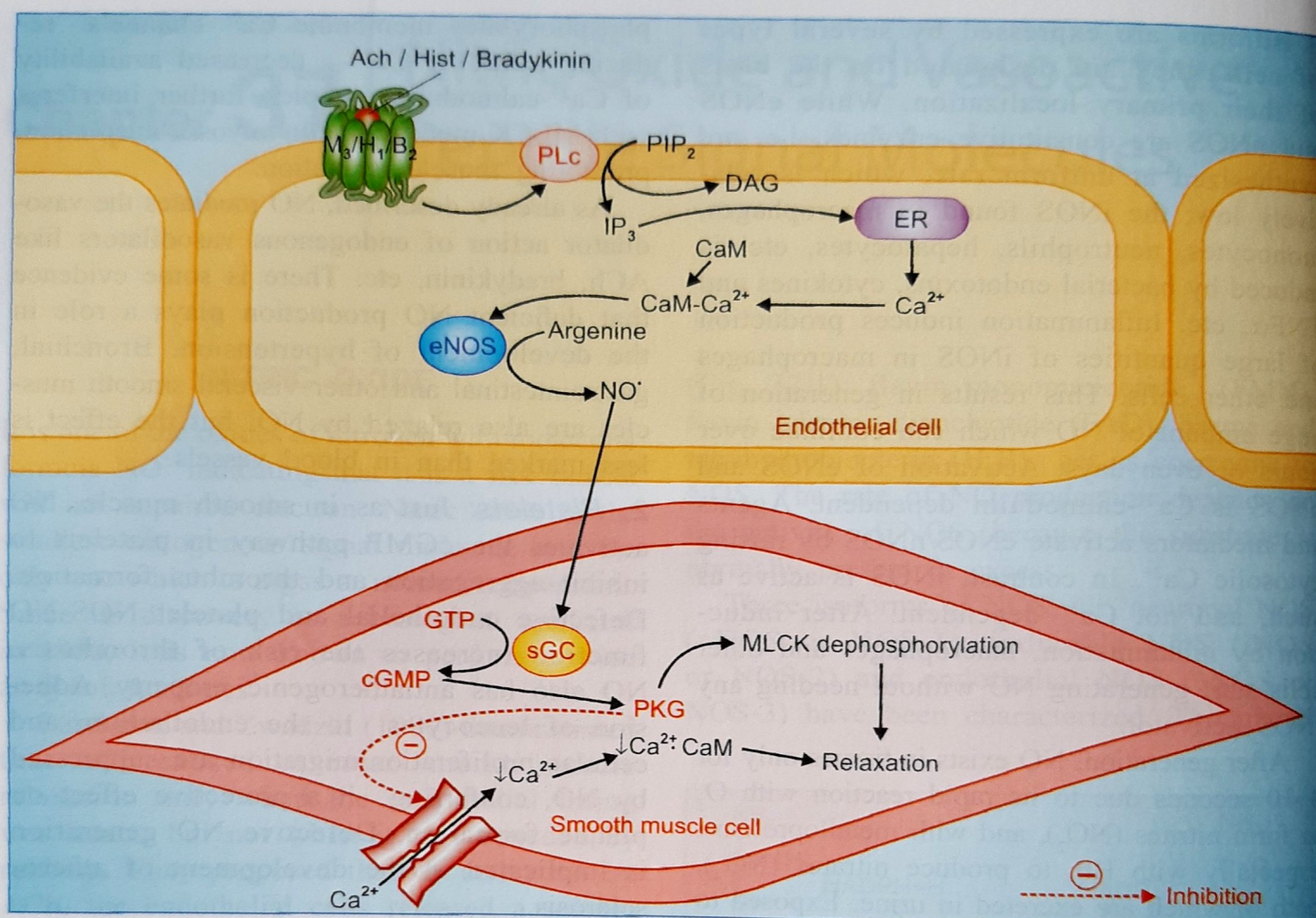


Fig. 37.2: Schematic depiction of major steps in endothelium-dependent vasodilatation and role of nitric oxide (NO). GPCR—G-protein coupled receptor ($M_3/H_1/B_2$); PLC—Phospholipase C; PIP_2 —Phosphatidyl inositol bisphosphate; DAG—Diacylglycerol; IP_3 —Inositol trisphosphate; eNOS—endothelial nitric oxide synthase; sGC—soluble guanylyl cyclase; PKG—Protein kinase G; MLCK—Myosin light chain kinase.

bowel disease (IBD) and in many other chronic inflammatory conditions.

Excessive production of NO has been implicated in causing severe hypotension attending septic shock. Sepsis induces iNOS in inflammatory cells as well as in many other types of cells which produce massive amount of NO over long periods, contributing to the sustained and refractory fall in BP (*see box*). However, use of drugs which check NO production has not yet yielded survival benefit in septic shock.

4. Nervous system: Nitric oxide serves as neurotransmitter/neuromodulator in central

Major actions of Nitric Oxide

- Relaxes vascular and visceral smooth muscle
- Prevents platelet aggregation
- Serves as neurotransmitter in brain and periphery
- Mediates microbicidal action of macrophages
- Plays a role in inflammatory diseases, septic shock, pulmonary arterial hypertension, etc.

as well as in peripheral nervous system. Both nNOS and eNOS are expressed in the central neurones, but unlike other transmitters, there are no preformed stores of NO. It is synthesized on demand and released by diffusion (not exocytosis). NO is released

at many neuronal sites in the brain, the most prominent of which is the excitatory glutamate neurones. Activation of NMDA glutamate receptors causes long-lasting Ca^{2+} influx triggering NO release. Several functions are ascribed to NO in the brain, viz. nociception, increased cerebral blood flow, learning and memory. NO is believed to mediate 'synaptic plasticity' a process by which junctional transmission is strengthened in a use dependent manner. This is achieved by retrograde diffusion of post-junctional NO to enhance prejunctional transmitter release. Excessive production of NO has been implicated in promoting 'excitotoxicity' due to over-stimulation of glutamate neurones. Aberrant NO generation has been ascribed a causative role in degenerative neurological diseases like Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, etc.

In the genitalia and g.i. tract, certain nonadrenergic noncholinergic (NANC) neurones function by releasing NO. It is now established that pelvic nerve stimulation causes penile erection by releasing NO from NANC neurones in the corpora cavernosa. NO relaxes cavernosal smooth muscle, filling the sinusoids with blood and causing penile tumescence (see p. 327). Accordingly, sildenafil and other PDE-5 inhibitors are effective in the treatment of erectile dysfunction, because they inhibit degradation of cGMP which mediates NO action. NO appears to play a role in relaxing pyloric sphincter and facilitating gastric emptying. Defective NO signaling is believed to be involved in pyloric stenosis and gastric dilatation.

5. Lungs: NO appears to play a prominent role in the regulation of pulmonary vascular resistance and the development of pulmonary arterial hypertension (PAH). Sildenafil, the PDE-5 inhibitor which indirectly potentiates the vasodilator action of NO by preventing inactivation of cGMP, causes marked reduction

in pulmonary artery pressure, and is the drug of choice for PAH (see p. 328). Inhalation of diluted NO gas also lowers pulmonary artery pressure in PAH, and is the 1st line therapy in newborns with severe respiratory distress and raised pulmonary artery pressure. Inhaled NO relaxes bronchial smooth muscle as well.

Nitric oxide donors

These are compounds which contain a NO or similar moiety in their structure, that is released in the body, mostly enzymatically, to elicit smooth muscle relaxation. Organic nitrates, nitrites and sodium nitroprusside are the clinically useful NO donors. They are also called 'nitro-dilators'. However, the pattern of vasodilatation produced by different NO donors differs according to the NO species that is released and the mechanisms involved in the release process.

Glyceryl trinitrate (GTN): It is a rapidly acting antianginal drug (see Ch. 40) which mainly dilates veins and coronary arteries. Mitochondrial aldehyde dehydrogenase is the most important enzyme which releases NO from GTN. This enzyme is abundantly expressed in veins, offering an explanation for the preferential venodilator action of GTN.

Isosorbide dinitrate and **Isosorbide mononitrate** generate a related NO species by process/enzymes that are not well characterized. They are slower acting, but the pattern of action is similar to GTN. When administered continuously, organic nitrates produce rapidly developing tolerance (within few hours), which also weans off rapidly. Though the processes involved in rapid nitrate tolerance are incompletely understood, it is believed that the reactive oxygen species generated during metabolism of organic nitrates inhibit mitochondrial aldehyde dehydrogenase and other enzymes which produce the NO species, limiting further action.

Platelets do not have the enzymes to denitrate GTN and other nitrates. Therefore, organic nitrates

do not significantly inhibit platelet aggregation. This is in contrast to NO, which has potent antiaggregatory action.

Amyl nitrite: It is the only organic nitrite that was used for angina pectoris. Inhaled amyl nitrite rapidly dilated both veins and arteries, including coronaries, and produced throbbing headache. Like nitrates, it also needs conversion to a NO species, but the enzymatic mechanism has not been elucidated. Nitrate-like rapid tolerance has not been observed with amyl nitrite.

Sodium nitroprusside: Administered by i.v. infusion, nitroprusside equally dilates arterioles and veins by releasing NO as well as by directly activating guanylyl cyclase. It rapidly lowers BP and the action is titratable. The breakdown of nitroprusside into NO and CN moieties is both enzymatic and non-enzymatic; the latter by reaction with glutathione (*see* Ch. 41). Not only endothelial cells, but RBCs and some other cells can split nitroprusside. However, the enzymes involved have not been identified. Nitrate-like tolerance is absent even after continuous infusion. The released CN moiety is detoxified by forming thiocyanate, which is excreted slowly. Toxic amount of thiocyanate may accumulate if nitroprusside infusion is prolonged.

Nitric oxide as a therapeutic agent: Apart from being an important signal molecule, exogenously administered NO gas has limited therapeutic application as well. Inhaled NO is indicated in severe respiratory distress in neonates with elevated pulmonary arterial pressure. It dilates pulmonary vessels, particularly in areas of the lung that are better ventilated, reducing the ventilation-perfusion mismatch and improving gaseous exchange. The elevated pulmonary artery pressure is lowered and cardiopulmonary function is improved. Inhaled NO improves cardiopulmonary function in adult patients as well with severe PAH, but this can only be a short-term measure.

VASOACTIVE PEPTIDES

Over the past 50 years it has been realized that a large number of peptides are utilized by the body as signal molecules in the nervous system as well as for other cell-to-cell communications. Some of these have pronounced direct effects on the vasculature, and may be termed 'Vasoactive peptides'. These may be grouped into those

which constrict and those which dilate blood vessels.

Vasoconstrictor peptides	Vasodilator peptides
1. Angiotensin II (Ang II)	1. Bradykinin and Kallidin (Kinins)
2. Vasopressin (AVP, ADH)	2. Natriuretic peptides (ANP, BNP)
3. Endothelins (ET)	3. Vasoactive intestinal peptide (VIP)
4. Neuropeptide Y (NPY)	4. Substance P
5. Urotensin	5. Calcitonin gene-related peptide (cGRP)
	6. Adrenomedullin

Angiotensin II (Ch. 36) and Vasopressin (Ch. 43) are considered in other chapters.

VASOCONSTRICTOR PEPTIDES

Endothelins (ETs)

Endothelins are three (ET-1, ET-2, ET-3) closely related 21 amino acid peptides having potent vasoconstrictor action. Vascular endothelium primarily generates ET-1 which is functionally the most relevant ET. It is also produced by nervous tissue, lungs, kidney, breast, endometrium and some other cells, while ET-2 and ET-3 have been located in kidney, brain and intestines. ET-1 is synthesized in the endothelial (and other) cells as a larger peptide called *prepro- endothelin-1* (*prepro ET-1*), which is broken down first to a 38 amino acid peptide named 'Big ET-1' and then by an *endothelin converting enzyme* (ECE) to ET-1 (*see* Fig. 37.3). Control of ET-1 synthesis is exercised at the level of expression of prepro ET-1 gene; synthesis is enhanced by transforming growth factor β (TGF β), cytokines (mainly IL-1) and vasoconstrictors like angiotensin II, vasopressin (AVP). On the other hand, vasodilators like NO, prostacyclin (PGI₂) and atrial natriuretic peptide (ANP) suppress production of prepro ET-1. Synthesis of prepro ET-1 is immediately translated into ET-1 production and release from the cell.

Though ET-1 is released into the circulation, it primarily acts locally to serve as *paracrine*

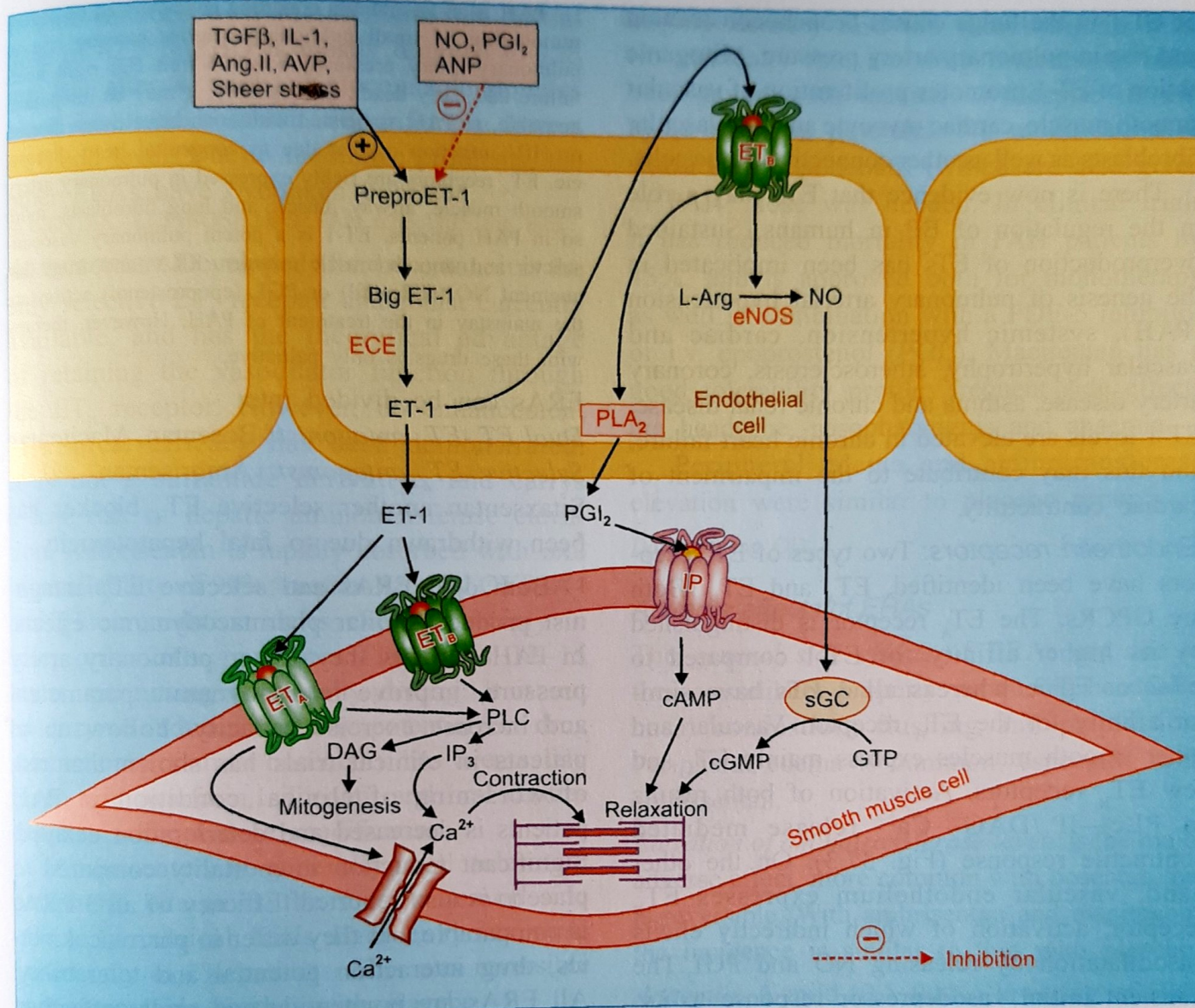


Fig. 37.3: Schematic depiction of endothelin-1 (ET-1) production in the endothelium, and its direct contractile (through ET $_A$ + ET $_S$ receptors), and indirect relaxant (through ET $_B$ receptors) effects on vascular smooth muscle. ECE—Endothelin converting enzyme; Ang II—Angiotensin II; TGF β —Transforming growth factor β ; AVP—Arginine vasopressin; PGI $_2$ —Prostacyclin; IP—prostacyclin receptor; ANP—Atrial natriuretic peptide; L-Arg—L-arginine; NO—Nitric oxide; sGC—soluble guanylyl cyclase; cGMP—cyclic GMP; cAMP—cyclic AMP; IL-1—Interleukin; PLA $_2$ —Phospholipase A $_2$; eNOS—Endothelial nitric oxide synthase; IP $_3$ —Inositol trisphosphate; DAG—Diacylglycerol.

or even *autocrine* signal molecule. ETs are rapidly cleared from blood ($t_{1/2}$ 4–7 minutes) by peptidases in lungs and kidneys as well as by receptor binding.

Actions and roles of endothelins

The most prominent action of ET-1 is pronounced and relatively long lasting vasoconstriction in most vascular beds, including pulmonary, renal and coronary arteries. However, i.v. injection of

ET-1 initially causes brief fall in BP (due to release of NO and PGI $_2$ from the endothelium) followed by more marked and sustained rise in BP due to direct action on arterial smooth muscle. ET-1 also increases heart rate and cardiac contractility which contributes to the rise in BP. Constriction of renal vessels reduces GFR and urine output. Acting on adrenals, ET-1 promotes aldosterone release which contributes to the salt and water retaining action. Action

of ET-1 in the lungs causes bronchoconstriction and rise in pulmonary artery pressure. Mitogenic action of ET-1 promotes proliferation of vascular smooth muscle, cardiac myocyte and perivascular fibroblasts as well as other connective tissue cells.

There is now evidence that ETs play a role in the regulation of BP in humans. Sustained overproduction of ETs has been implicated in the genesis of pulmonary arterial hypertension (PAH), systemic hypertension, cardiac and vascular hypertrophy, atherosclerosis, coronary artery disease, asthma and chronic renal disease. ET-1 levels are elevated in chronic heart failure, and this may contribute to the impairment of cardiac contractility.

Endothelin receptors: Two types of ET receptors have been identified, ET_A and ET_B . Both are GPCRs. The ET_A receptor is distinguished by its higher affinity for ET-1 compared to ET-2 or ET-3, whereas all 3 ETs have similar affinity for the ET_B receptor. Vascular and other smooth muscles express mainly ET_A and few ET_B receptors. Activation of both results in $PLC-IP_3/DAG-Ca^{2+}$ release mediated contractile response (Fig. 37.3). On the other hand, vascular endothelium expresses ET_B receptor, activation of which indirectly elicits vasodilatation by releasing NO and PGI_2 . The transient initial vasodepressor response to i.v. ET-1 is due to this action. Sustained activation of both ET_A and ET_B receptors is mitogenic and results in vascular/cardiac myocyte, perivascular fibroblast and other connective tissue cell hyperplasia. Several organs, viz adrenals, kidney, intestines and brain express more ET_B than ET_A receptors.

Endothelin receptor antagonists (ERAs): Characterization of ET_A and ET_B receptors and appreciation of the etiological role of ETs in several cardiovascular and pulmonary disorders prompted efforts to develop ERAs, and have resulted in the approval of 3 ERAs for clinical use over the past 15 years. Though these ERAs are being tried in several conditions, the only approved indication so far is pulmonary arterial hypertension (PAH).

PAH is an uncommon condition characterized by severe remodeling of small pulmonary arteries causing rise in pulmonary artery pressure above 25 mm Hg, right heart failure and early death. Etiologically, it may be idiopathic, heritable, or PAH associated with connective tissue disease or HIV infection or that due to congenital heart disease, etc. ET_A receptors are richly expressed in pulmonary artery smooth muscle, airway muscle and lung fibroblasts, more so in PAH patients. ET-1 is a potent pulmonary vasoconstrictor and smooth muscle mitogen. ERAs and drugs that augment NO (sildenafil) or PGI_2 (epoprostenol) action are the mainstay in the treatment of PAH. However, therapy with these drugs is only palliative.

ERAs can be divided into:

Dual ET_A-ET_B antagonist: Bosentan, Macitentan

Selective ET_A antagonist: Ambrisentan

Sitaxsentan another selective ET_A blocker has been withdrawn due to fatal hepatotoxicity.

Both dual ERAs and selective ET_A antagonist produce similar pharmacodynamic effects. In PAH patients they lower pulmonary artery pressure, improve haemodynamic parameters and increase exercise capacity. Follow up of patients in clinical trials has shown that risk of worsening of clinical condition in PAH patients is decreased and deterioration delayed. Significant reduction in mortality compared to placebo is also reported. Efficacy of all 3 ERAs is comparable, but they differ in pharmacokinetics, drug interaction potential and tolerability. All ERAs are nonpeptide and orally effective.

Bosentan: It is the first ERA to be approved for clinical use. Chemically it is a sulfamide and dual ET_A-ET_B antagonist. Both initial depressor and subsequent pressor response to i.v. ET-1 is blocked. Oral bioavailability of bosentan is ~50%. It is metabolized in liver by CYP2C9 and CYP3A4, and excreted in bile; plasma $t_{1/2}$ is 6 hours. One of the metabolites is active. No dose adjustment is needed in renal disease, but it is contraindicated in liver disease, because it causes dose-dependent elevation of hepatic aminotransferases and carries risk of hepatic injury. Monthly monitoring of liver function test is mandatory. Bosentan induces microsomal CYP2C9 and CYP3A4 isoenzymes, and can interact with many drugs, including warfarin, oral contraceptives, glibenclamide and simvastatin.

It auto-induces own metabolism; the initial dose of 62.5 mg BD is mostly doubled to 125 mg BD after 4 weeks. Ketoconazole doubles blood levels of bosentan, and rifampin lowers its blood level. Due to the above problems, the other ERAs have overtaken bosentan.

BOSENTAS, LUPIBOSE, BOZTAN 62.5, 125 mg tabs.

Ambrisentan: Developed after bosentan, it is the only selective ET_A receptor antagonist currently available, and has the theoretical advantage of retaining the vasodilator function through the ET_B receptor. However, no enhancement of clinical efficacy has been demonstrated. It is not a sulfamide derivative, and carries a low risk of hepatic aminotransferase elevation. Ambrisentan is rapidly absorbed with oral bioavailability of 90%; metabolized by CYP3A4 and glucuronidation. It is excreted mainly in bile; only 20% in urine. The $t_{1/2}$ is 12–15 hours, permitting once daily dosing. It is not an enzyme inducer, has low potential for drug interactions (contrast bosentan); has been safely given with warfarin, sildenafil and oral contraceptives without dose modification.

Ambrisentan is generally well tolerated; peripheral edema is the most common side effect. In clinical trials liver enzymes were raised only in 2% patients. This was reversible and comparable to that with placebo.

Ambrisentan has been combined with tadalafil (a PDE-5 inhibitor), and this has improved efficacy as well as reduced the risk of clinical failure (AMBITION trial) in PAH patients.

Dose: 5–10 mg OD.

PULMONEXT, AMBRICAN, ENDOBLOC, ZAMBRI 5 mg, 10 mg tabs.

Macitentan: It is a new dual ERA with higher affinity for ET_A than for ET_B receptors. Macitentan has been found to bind tightly to the ET_A receptor producing unsurmountable blockade, which probably contributes to its enhanced activity. Oral absorption of macitentan is slow, takes 8–10 hours. It is oxidized by CYP3A4 to an active metabolite which has a much longer $t_{1/2}$ than the 17 hours $t_{1/2}$ of macitentan itself. Excretion is

largely urinary and the remaining in bile. No clinically relevant drug interactions have been noted with macitentan despite its enzyme inducing potential. No modification of warfarin and sildenafil (which are also given in PAH) dose was needed. In clinical trials it has reduced mortality in PAH patients by 45%, and is approved both for monotherapy as well in combination with a PDE-5 inhibitor or i.v. epoprostenol (PGI_2). Macitentan has a good tolerability profile; frequent side effects are headache, nasopharyngitis and anaemia.

Peripheral edema and aminotransferase elevation were similar to placebo group.

Dose: 10 mg OD.

Adverse effects of ERAs

The *general side effects* of ERAs are due to their vasodilator property, producing headache, nasal congestion, flushing and palpitation. Peripheral edema is common, especially with ambrisentan.

Elevation of aminotransferase levels is the major adverse effect, more common with bosentan, but is reversible. With ambrisentan and macitentan, the incidence is similar to that with placebo.

Anaemia: A mild (0.5–0.8%) lowering of Hb% and haematocrit occurs with all ERAs. It is probably due to fluid retention and haemodilution.

Teratogenicity: All ERAs are teratogenic in animals. In clinical trials, ERAs have not been given to pregnant women, and there is no human data, but all ERAs are contraindicated in pregnancy.

Orally administered ERAs are now a first line well tolerated option for the treatment of PAH of various etiologies. They compare favourably with oral PDE-5 inhibitors (sildenafil, tadalafil) and can be combined with them for enhanced efficacy. Continuous long term i.v. infusion of epoprostenol (PGI_2 analogue) has been shown to afford relief as well as reduce mortality in PAH cases nonresponsive to oral

medication or those with severe (functional class III or IV) disease.

ERAs are also being evaluated for use in refractory chronic heart failure.

Neuropeptide Y (NPY)

Neuropeptide Y (NPY) first isolated from pig brain in 1982, is one of the most abundant neuropeptides in the brain and is also present in peripheral sympathetic nerves. It is the leading member of a family of three 36 amino acid peptides, the other two are *pancreatic polypeptide (PP)* and *peptide YY (PYY)*. While NPY is a neurotransmitter, PP and PYY are hormones. PP is secreted by pancreatic islets in response to food ingestion and acts in the CNS as well as on gut to suppress appetite, delay gastric emptying and promote fuel utilization. Hormonal cells in the ileum secrete PYY according to the quantity of food, and it serves to regulate appetite.

In the periphery NPY is mainly localized in the sympathetic nerves, especially those innervating blood vessels. It is stored in separate synaptic vesicles than those storing NA (see Fig. II.3B). Nerve impulse releases NPY along with NA, and it appears to serve both as transmitter as well as cotransmitter (see p. 108). NPY exerts postjunctional as well as prejunctional actions. The postjunctional action in the vasculature is a direct slow but longlasting contractile effect, as well as potentiation of NA-induced vasoconstriction. The prejunctional action is inhibition of nerve impulse induced transmitter release. Not only NA release from sympathetic nerve endings, but also ACh release from parasympathetic nerves is attenuated.

NPY causes vasoconstriction in nearly all vascular beds, especially renal and cerebral vessels. It has a direct positive inotropic and chronotropic effect on heart and increases Na⁺ and water excretion by the kidney, despite renal vasoconstriction. In the brain, NPY is present in several cortical and subcortical areas and plays a key role in regulating food intake (NPY

strongly promotes feeding), energy homeostasis, stress response, autonomic out flow, HPA axis activation and several other functions. The presynaptic action of NPY in the brain is to inhibit release of various neurotransmitters, viz. NA, DA, 5-HT, glutamate, GABA, as well as that of hypothalamic GnRH, AVP and oxytocin.

NPY receptors: In humans, four types of Y receptors (Y₁, Y₂, Y₄, Y₅) have been characterized for NPY and related peptides, of which NPY has high affinity for three (Y₁, Y₂, Y₅), while Y₄ has preferential affinity for PP; so that it may be regarded as PP receptor. The original Y₃ receptor is now considered to be CXC chemokine receptor and not a Y receptor. All Y receptors are GPCRs, which transduce their response by raising cytosolic Ca²⁺ or by inhibiting adenyl cyclase. The vasoconstrictor and other peripheral post junctional actions of NPY are mediated mainly by Y₁ receptor, while Y₂ is the prejunctional receptor for NPY in the periphery as well as in the brain. Both Y₁ and Y₂ receptors are expressed in many areas of the brain. The Y₅ receptor is restricted to the brain, in areas which control food intake or hypothalamo-pituitary function and in limbic areas.

Several selective nonpeptide Y₁ receptor antagonists have been produced which block vasoconstrictor action of NPY. Their use has indicated that NPY is probably not involved in the regulation of normal BP or cardiac function, but that it may play a role in the development of hypertension or heart failure. Selective Y₂ and Y₅ antagonists have also become available for investigation. Some of the Y₁ and Y₅ antagonists are orally active and have undergone limited clinical trial as antiobesity agents, but none has been approved for clinical use.

Urotensin II (UII)

Urotensin II (UII) is a 11 amino acid cyclic vasoconstrictor neuropeptide identified in 1980 from the urophysis of teleost fish and later demonstrated to be present in mammals including humans. It has been located in the cholinergic neurones of the brainstem and spinal cord as well as in kidney, heart and lungs. UII strongly contracts arterial smooth muscle with less effect on veins, and depresses the heart. A variety of other actions on the CNS as well as on peripheral organs have been shown, but their physiological relevance is uncertain. Though UII circulates in blood, it does not appear to play significant role in the regulation of BP.

The receptor for UII has been identified as a cell surface GPCR and named *UT receptor*. It is coupled to Gq protein and produces vasoconstriction (as well as other effects) through the IP₃/DAG – intracellular Ca²⁺ release pathway. The UT receptor is widely expressed in the CNS, heart, blood vessels, lung, kidney and adrenal medulla.

Though UII appears to be physiologically unimportant in man, it may play a role in certain cardiovascular

and other diseases. Raised UIL levels have been noted in hypertension, advanced heart failure, diabetic renal failure, etc. Many peptide and nonpeptide UT receptor antagonists have been produced, and some have been clinically tested, particularly in diabetic nephropathy and in asthma, but none has been developed as a drug.

VASODILATOR PEPTIDES

Bradykinin and Kallidin (Plasma kinins)

Plasma kinins are polypeptides split off from a plasma globulin *Kininogen* by the action of specific enzymes *Kallikreins*. The two important plasma kinins, *Kallidin* (decapeptide) and *Bradykinin* (nonapeptide) were discovered around 1950 by two independent lines of investigation into the hypotensive activity of urine and certain snake venoms. These and some other biological fluids were found to act indirectly: they contained enzymes which generated vasodilator substances in the plasma.

Kinins are generated by proteolytic reactions triggered by tissue injury, inflammation, allergic reaction, etc., and play important mediator roles.

Generation and metabolism Kininogens are α_2 globulins present in plasma which also contains inactive kininogenase *prekallikrein*.

Prekallikrein is activated by Hageman factor (factor XII) which itself is activated by tissue injury and contact with surfaces having negative charge, e.g. collagen, basement membrane, bacterial liposaccharides, urate crystals, etc. *Plasmin* facilitates contact activation of Hageman factor

(Fig. 37.4). Kinins are also generated by trypsin, proteolytic enzymes in snake and wasp venoms and by kallikrein present in kidney, pancreas and other tissues. Bradykinin is generated from high molecular weight (HMW) kininogen by the action of plasma kallikrein, because HMW-kininogen does not cross the capillaries. On the other hand, kallidin can be produced from both low molecular weight (LMW) kininogen as well as HMW-kininogen by the action of tissue kallikreins. Bradykinin can also be generated from kallidin on the removal of lysine residue by an aminopeptidase.

Plasma and tissues also contain kininogenase inhibitory factors of which *complement (C1) esterase inhibitor (C1 Inh)* is the most important. Moreover, kallikreins are normally present in their inactive zymogen forms. Thus, physiologically only small amounts of kinins are generated in plasma and tissues.

Kinins are very rapidly degraded, primarily in lungs, but also in other tissues and have a $t_{1/2}$ of < 1 min. The principal degrading enzyme is *Kininase II*, also known as 'angiotensin-II converting enzyme' (ACE) which splits off 2 amino acids from the carboxyterminal of the peptide chain. Another carboxypeptidase *Kininase I* removes only one amino acid (arginine) producing selective B_1 receptor agonistic metabolites (desArg bradykinin and desArg kallidin) which are further degraded by other peptidases.

Actions

Bradykinin and kallidin have similar actions.

1. **Blood vessels** Kinins are more potent vasodilators than ACh and histamine. The dilatation is mediated through endothelial NO and PGI₂ generation, and involves mainly the arterioles. In contrast, large arteries, most veins and

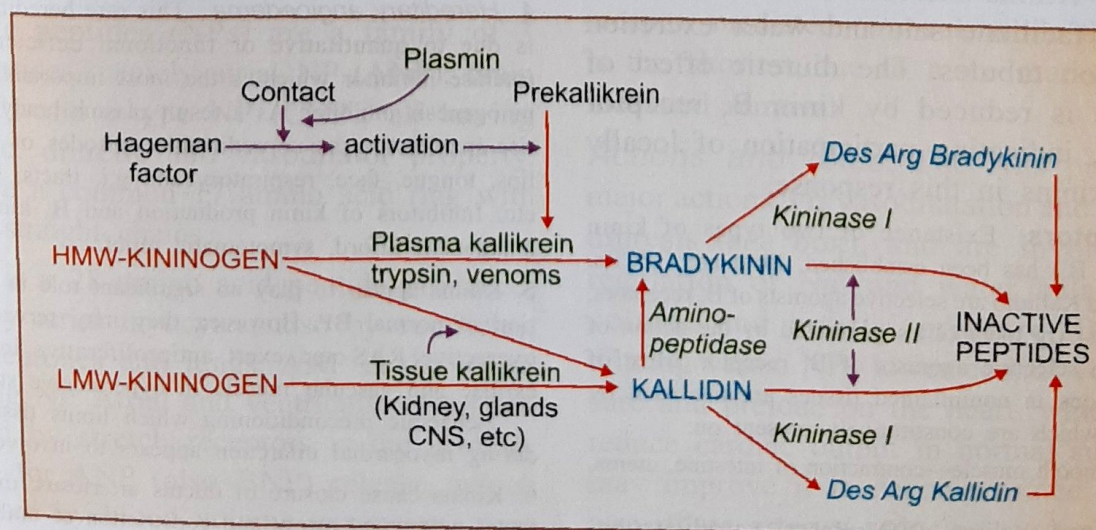


Fig. 37.4: Generation and degradation of bradykinin and kallidin
HMW—high molecular weight; LMW—low molecular weight.

vessels with damaged endothelium are constricted by direct action of kinins on the smooth muscle. In addition, they can release histamine and other mediators from mast cells. Injected i.v. kinins cause flushing, throbbing headache and fall in BP. They markedly increase capillary permeability due to separation of endothelial cells → exudation and inflammation occurs if they are injected in a tissue. Intradermal injection produces wheal and flare (similar to histamine).

Kinins have no direct action on heart; reflex stimulation occurs due to fall in BP.

2. Smooth muscle Kinins induce contraction of intestine which is characteristically slow (*bradys*—slow, *kinein*—to move). They cause marked bronchoconstriction in guineapigs and in asthmatic patients. Action on other smooth muscles is not prominent. Some smooth muscles may be relaxed.

3. Neurones Kinins strongly stimulate nociceptive afferents and produce a burning sensation. Applied to blister base or injected intraperitoneally or in the brachial artery, bradykinin produces intense, transient pain and has been used in analgesic testing.

Kinins release CAs from adrenal medulla. Injected directly in brain they produce a variety of effects including enhanced sympathetic discharge. They increase permeability of the blood-brain barrier.

4. Kidney Kinins increase renal blood flow as well as facilitate salt and water excretion by action on tubules. The diuretic effect of furosemide is reduced by kinin B₂ receptor antagonists, indicating participation of locally generated kinins in this response.

Kinin receptors Existence of two types of kinin receptors (B₁, B₂) has been established. Both are GPCRs. Bradykinin and Kallidin are selective agonists of B₂ receptors, while their des-Arg metabolites generated by the action of kininase I are selective agonists of B₁ receptor. Most of the kinin actions in noninflamed tissues are mediated by B₂ receptors which are constitutively present on:

- Visceral smooth muscle—contraction of intestine, uterus, airway.
- Vascular endothelium—NO release, vasodilatation, increased permeability.
- Sensory nerves—acute pain.

The B₂ receptor is coupled to Gq and Gi proteins which utilizes the phospholipase C—IP₃/DAG—intracellular Ca²⁺ mobilization transducer mechanism. Endothelial NO synthase (eNOS) is activated causing vasodilatation. Certain responses to kinins, e.g. bronchoconstriction, renal vasodilatation and activation of pain nerve endings appear to be mediated by stimulation of phospholipase A and generation of PGs at the local site. The activated Gq protein also mediates production of NFκB which triggers MAP kinase pathway leading to generation of proinflammatory mediators.

The B₁ kinin receptors are minimally expressed in normal tissues, but are induced by inflammation and colocalize with kininase I enzyme, so that the B₁ agonistic des-Arg kinins are produced at the same site. Activated B₁ receptor also transduces through Gq and Gi proteins and cause contraction of veins, large vessels, enhanced PG synthesis, leukocyte recruitment, generation of inducible NOS (iNOS) and chronic pain in inflamed tissues.

Pathophysiological roles

1. Mediation of inflammation Kinins produce all the signs of inflammation—redness, exudation, pain and leukocyte mobilization. Tissue injury can cause local kinin production which then sets in motion the above defensive and reparative processes. Activation of B₁ receptors on macrophages induces production of IL-1, TNF-α and other inflammatory mediators.

2. Mediation of pain By directly stimulating pain nerve endings and by increasing PG production, kinins appear to serve as mediators of pain. The B₂ antagonists block acute pain produced by bradykinin, but induced B₁ receptors appear to mediate pain of chronic inflammation.

3. Functional hyperemia (in glands during secretion) and *regulation of microcirculation*—especially in kidney may be occurring through local kinin production.

4. Hereditary angioedema This rare hereditary disorder is due to quantitative or functional deficiency of C-1 esterase inhibitor which is the most important natural kininogenesis inhibitor. As a result plasma bradykinin levels rise manifesting as unpredictable episodes of swelling of lips, tongue, face, respiratory and g.i. tracts, hands, feet, etc. Inhibitors of kinin production and B₂ kinin receptor antagonists afford, symptomatic relief.

5. Kinins appear to play no significant role in the regulation of normal BP. However, they may serve to oppose overactive RAS and exert antiproliferative influence on cardiac and vascular muscle in hypertensive states.

Ischaemic preconditioning which limits tissue damage during myocardial infarction appears to involve kinins.

6. Kinins cause closure of ductus arteriosus, dilatation of foetal pulmonary artery and constriction of umbilical vessels. Thus, they may be involved in adjusting from foetal to neonatal circulation.

7. Kinins play a major role in the development of angioedema. They also appear to be involved in *shock, rhinitis, asthma, ACE inhibitor induced cough, carcinoid, postgastrectomy dumping syndrome*, fluid secretion in *diarrhoea, acute pancreatitis* and certain *immunological* reactions.

Because of evanescent and unpleasant actions, kinins have no clinical use.

Bradykinin antagonists

After characterization of B_1 and B_2 kinin receptors, and in view of prominent pathophysiological roles of kinins, several peptide and nonpeptide kinin antagonists have been produced and tested for clinical use. However, only one (Icatibant) has been marked so far in UK and Europe.

Icatibant: It is a synthetic decapeptide bradykinin B_2 receptor antagonist which is resistant to kinin degrading enzymes, and has a duration of action upto 6 hours after s.c. injection. It effectively reverses the swelling and other symptoms in hereditary angioedema, and is currently the best treatment option for this condition. Its utility in treating drug induced angioedema, burns, pancreatitis and other conditions involving kinins, is being explored.

A recent controlled trial has reported icatibant injected s.c. to be more effective in resolving ACE inhibitor induced angioedema than the standard therapy with i.v. prednisolone and an antihistaminic.

(Icatibant is marketed in UK, Europe as **FIRAZYR 30 mg/3 ml prefilled syringe for s.c. injection in abdominal area**). Compound 173657, comp. SSR 240612 and few other orally active nonpeptide B_2 antagonists have undergone trials and are being developed as analgesics, antiinflammatory drugs, and for treating pancreatitis, head injury, etc.

Natriuretic peptides (ANP, BNP, CNP)

Natriuretic peptides (NPs) are a family of 3 cyclic peptides, namely atrial NP (ANP), brain NP (BNP) and c-type NP (CNP), all having natriuretic, diuretic and vasodilator property. They have a common 17 amino acid ring with differing straight chains.

ANP: It is a 28 amino acid peptide produced, mainly by atrial myocytes; also by ventricles, certain neurones and lungs. ANP is the major circulating NP. Atrial and ventricular distention perceived by stretch receptors is the primary stimulus for ANP (also BNP) release, which is triggered by blood volume expansion, sympathetic stimulation (α_1 receptor mediated),

AngII, endothelin, vasopressin and aldosterone as well. Plasma ANP level is raised in CHF, renal failure, primary aldosteronism and in inappropriate ADH secretion syndrome. ANP is synthesized in cardiac myocytes as a large peptide *prepro-ANP*, which is then split to *pro ANP* and finally to ANP which is released.

BNP: Produced mainly by ventricles, also by brain and some other organs, BNP exists in 2 forms—either 32 or 26 amino acids. It is also first synthesized as *prepro BNP*, and then broken down to BNP in 2 steps. Releasing stimuli for BNP are the same as for ANP, and the actions of the two peptides are identical. However, circulating levels of BNP are lower than those of ANP.

CNP: This 22 amino acid NP is produced mainly by vascular endothelium and neurones in the CNS; also by kidneys and intestines. Circulating levels of CNP are very low. The primary action of CNP is vasodilatation, while natriuresis and diuresis is mild. The physiological role of CNP is not clear.

Clearance of NPs: Occurs both by enzymatic degradation as well as by receptor mediated internalization followed by intracellular breakdown (see below).

A neutral endopeptidase (NEP; also called *Neprilysin*) present in kidney, lungs and liver rapidly degrades NPs. The plasma $t_{1/2}$ of ANP is short (2–5 min in humans), BNP has a longer $t_{1/2}$, (4–20 min) probably due to less efficient receptor mediated removal, while that of CNP is the shortest due to more marked receptor mediated removal.

Actions and roles: The NPs produce two major actions, viz vasodilatation and natriuresis-diuresis (see box), and are involved in the regulation of Na^+ and water balance, blood volume and arterial pressure. By directly dilating veins, NPs lower central venous pressure and preload on the heart, which tends to reduce cardiac output in normal subjects, but may improve it in decompensated heart. NPs also dilate arteries reducing systemic vascular resistance, and BP.

Vascular and renal actions of ANP/BNP

- Systemic arterial and venous dilatation
- ↓ Sympathetic flow
 - ↓ Venous pressure and preload on heart
 - ↓ Systemic arterial pressure
- Natriuresis and diuresis
- ↑ Renal blood flow
- ↑ Glomerular filtration rate and filtration fraction
- ↓ Proximal tubular Na^+ reabsorption
- ↓ Renin release from kidney
 - ↓ Plasma angiotensin II
 - ↓ Plasma aldosterone

In addition to direct vasodilator action, NPs decrease sympathetic flow by acting in the CNS as well as by reducing NA release from sympathetic nerve terminals.

Acting on the kidneys, NPs increase renal blood flow, GFR and filtration fraction. They also decrease proximal tubular Na^+ reabsorption resulting in natriuresis and diuresis. Moreover, they inhibit renin release from the kidney which

translates into lowered plasma AngII reducing aldosterone production. This also contributes to the natriuresis (Fig. 37.5). In contrast to furosemide and thiazide induced natriuresis, the ANP induced natriuresis is not accompanied by K^+ loss, because it also suppresses the renin-AngII-aldosterone mechanism. Lowered circulating AngII contributes to the hypotensive action as well.

Overall, the NPs seem to act as a counter regulatory system to the renin-AngII-aldosterone system to maintain blood volume and blood pressure homeostasis, as well as Na^+ - K^+ balance.

NP receptors: The NPs act by combining with specific cell surface receptors, three subtypes of which have been identified, namely ANP_A (or NPR_1), ANP_B (or NPR_2) and ANP_C (or NPR_3).

ANP_A and ANP_B receptors: Both these receptors are transmembrane enzyme-linked receptors (see p. 58–59) and Fig. 4.8) which have guanylyl cyclase activity at the cytosolic domain and are

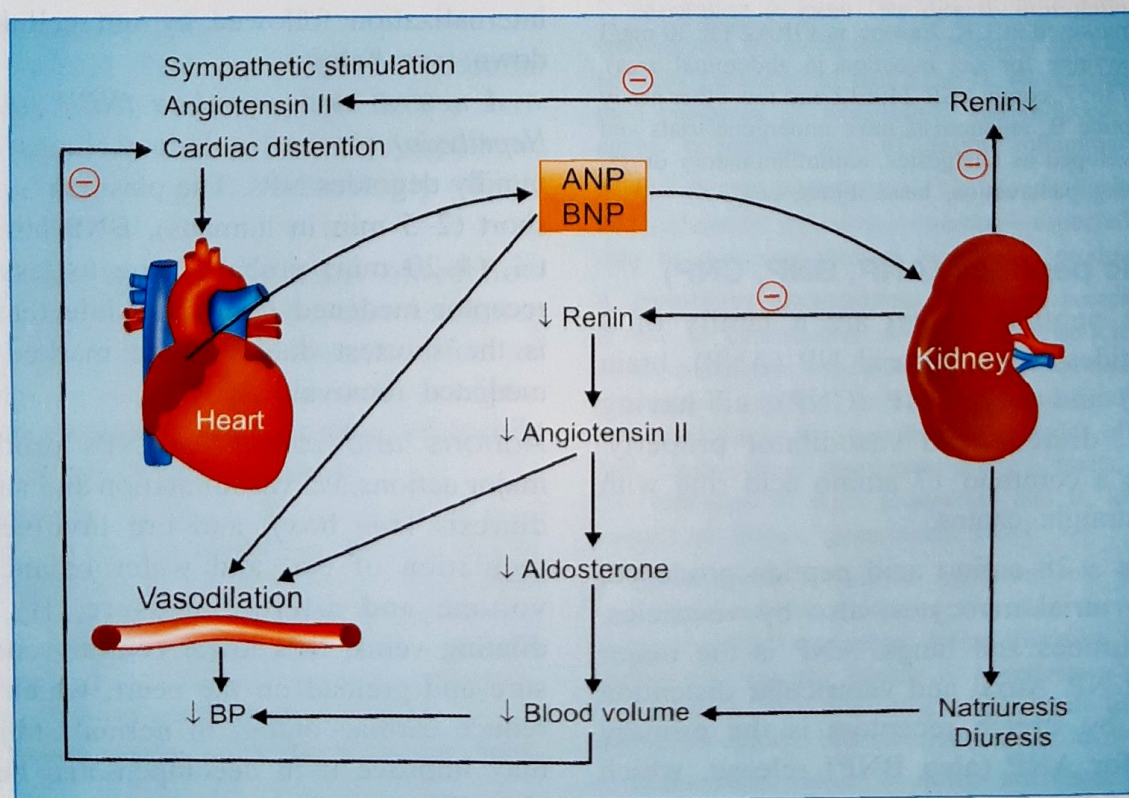


Fig. 37.5: Counter-regulatory operation of natriuretic peptides against the renin-angiotensin II-aldosterone system to maintain blood volume, blood pressure, salt and water homeostasis
ANP—atrial natriuretic peptide; BNP—brain natriuretic peptide.

located on the surface of target cells. Binding of ANP or BNP at the extracellular domain promotes receptor dimerization and activation of the guanylyl cyclase. The raised cytosolic cGMP activates PK_G which brings about vascular smooth muscle relaxation, natriuresis and other responses by lowering intracellular Ca²⁺ and dephosphorylating MLCK (see Fig. 40.3). While ANP_A receptor has equal affinity for ANP and BNP, the ANP_B subtype has high affinity for CNP and is involved mainly in causing vasodilatation.

ANP_C receptor: Located mostly on vascular endothelium, it primarily serves as a clearance receptor, and has equal affinity for ANP, BNP and CNP.

On binding the NP, the receptor dimerizes and is internalized. The peptide is broken down intracellularly and the receptor is returned to the surface. The ANP_C receptor is also linked to adenylyl cyclase inhibition and PL_C activation, but the physiological consequences of these are not well understood.

Clinical application: A recombinant form of BNP called *Nesiritide* is used clinically to a limited extent because of need for i.v. administration and brief life-span in the body. Continuous i.v. infusion of nesiritide is occasionally employed to treat severely decompensated patients of CHF. It benefits by causing natriuresis-diuresis, vasodilatation—reduction in cardiac pre- and after-load and lowering of renin-AngII-aldosterone levels. However, hypotension and renal injury are the risks, and cost is prohibitive.

Serial measurements of plasma BNP levels have been used to monitor progression of heart failure, and to assess response to therapy: levels fall with improvement.

Neutral endopeptidase (NEP; neprilysin) inhibitors: This is a new class of drugs which inhibit NEP enzymes that degrade NPs, bradykinin, substance P and adrenomedullin. By raising levels of natriuretic and vasodilator peptides they have potential utility in CHF. A nonpeptide NEP inhibitor *Sacubitril* has been approved (in 2015) for the treatment of class II to IV CHF when combined with the ARB valsartan.

Sacubitril: It is an orally active NEP inhibitor which increases the blood levels of NPs and other vasodilator peptides resulting in natriuresis, diuresis, vascular relaxation, lowering of AngII

as well as aldosterone levels. Combined with valsartan, it affords symptomatic improvement in CHF patients. Sacubitril is a prodrug that is absorbed after oral ingestion and rapidly activated by esterases to *sacubitrilat*. The absolute oral bioavailability is 60%, and food does not alter absorption. Passage of sacubitrilat across blood-brain barrier is limited and it is not significantly metabolized further. Excreted in both urine and faeces, the plasma t_{1/2} of sacubitrilat is ~12 hours. No significant drug interactions with drugs generally used in CHF have been observed.

In a large multicentric randomized clinical trial (PARADIGM-HF, 2014), a 1:1 combination of sacubitril + valsartan (200 mg BD) was compared with enalapril (10 mg BD) in 8442 patients of class II to class IV CHF already receiving CHF therapy with one or more of diuretics, β blockers and aldosterone antagonist. The sacubitril-valsartan combination caused 20% greater reduction in death or hospitalization for heart failure than enalapril. The most common side effects were hypotension, hyperkalemia and renal impairment. Angioedema was infrequent, and its tolerability profile was similar to enalapril.

Thus, sacubitril-valsartan appears to be a more effective and well tolerated alternative to ACE inhibitors for reducing morbidity and mortality in patients with advanced heart failure. However, it is expensive.

ENTRESTO, VYMADA 50, 100, 200 mg tabs. (containing 24 + 26 mg or 49 + 51 mg or 97 + 103 mg sacubitril + valsartan.

Vasoactive intestinal peptide (VIP)

First isolated from intestines this 28 amino acid neuropeptide is broadly expressed in the CNS, peripheral cholinergic and peptidergic neurones in the ANS, especially those innervating blood vessels, heart, intestines, pancreas, urogenital tract and in some immunological cells. VIP is also a cotransmitter with ACh in many parasympathetic nerves (see Fig. 11.3, p. 108). It produces a slow, but long lasting response. Though present in circulation, the t_{1/2} of VIP in blood is very short (~ 2 min), and it appears to function mainly as a paracrine transmitter rather than as a hormone.

VIP is a potent vasodilator; coronaries and most vessels are dilated; BP falls on i.v. injection of VIP. Cardiac contractility and heart rate are increased. Intestinal, biliary, bronchial and genitourinary smooth muscle is relaxed. Secretion of salt and water across intestinal mucosa is increased; overactivation may cause watery diarrhoea. VIP promotes glycogenolysis in liver and appears to regulate certain metabolic processes. A role in modulating immune function has also been ascribed to VIP.

Two subtypes, $VPAC_1$ and $VPAC_2$ of VIP receptors have been designated. Both are GPCRs and are richly expressed in the CNS, heart, blood vessels, intestines, pancreas and immune cells. These GPCRs are linked to the Gs protein; vasodilatation, cardiac stimulation, smooth muscle relaxation and many other responses are mediated by adenylyl cyclase activation and generation of cAMP. Some other responses are produced via generation of IP_3 and mobilization of cytosolic Ca^{2+} .

Because of very short lifespan, VIP as such is not suitable for clinical use. Some stable analogues have been produced and their clinical application in cardiovascular, neurological, gastrointestinal and inflammatory diseases, as well as in COPD and diabetes is being explored, but none has been found satisfactory yet. VIP antagonists are also being investigated.

Substance P (SP)

A substance that contracted intestinal smooth muscle was detected in certain tissue extracts by Von Euler and Gaddum in 1931 and named substance P. Later it was found to be a 11 amino acid neuropeptide distributed widely in the brain, spinal cord, peripheral sensory nerves, enteric nervous system, blood vessels and skin. Two other related decapeptides *Neurokinin A (NKA)* and *Neurokinin B (NKB)* have also been found, and together they constitute the *Tachykinin* (contrast *bradykinin* which produces slow contraction) family. All three have similar actions, but SP is the dominant member. SP, NKA and NKB are produced by the cleavage of a large polypeptide *preprotachykinin* and all three serve as neurotransmitter or neuromodulator in the CNS as well as in the periphery, where SP is primarily associated with pain and inflammation.

Tachykinin receptors: SP and others act by binding to three subtypes of tachykinin receptors NK_1 , NK_2 and NK_3 ; all of which are GPCRs coupled to Gq protein. The responses are transduced by generation of IP_3 and mobilization of intracellular Ca^{2+} triggering smooth muscle contraction, glandular secretion, release of NO and other messengers, cell proliferation, production of afferent impulse, etc. The NK_1 receptor (NK_1R) has the highest affinity for SP and serves as its major receptor. Binding of SP to NK_1R also leads to receptor internalization

into endosomes where SP is degraded and the NK_1R is returned to the surface.

Actions and role of substance P: SP is a potent arteriolar dilator; acts on endothelial NK_1 receptors to release NO which then relaxes the underlying smooth muscle. Rapid fall in BP occurs when SP is injected i.v. Subcutaneous injection produces red spot, wheal and flare by local vasodilatation, exudation and axon reflex. However, veins are often contracted due to direct action on their smooth muscle. Intestinal, bronchial and other smooth muscles are rapidly and strongly contracted. Secretion from salivary glands and intestinal mucosa is increased. Acting on the kidney, SP promotes natriuresis and diuresis.

In the brain, SP and NK_1R are particularly localized in the hypothalamus, amygdala, periaqueductal gray, nucleus tractus solitarius (NTS), area postrema, and appear to be involved in mood, behaviour, anxiety, stress response, depression, reinforcement, nociception, etc. The chemoreceptor trigger zone (CTZ) and NTS express NK_1R along with 5-HT₃ and other receptors which respond to noxious stimuli and coordinate the act of vomiting. It has been found that SP is released along with other mediators in response to tissue injury, especially that due to cancer chemotherapy and radiotherapy. The SP reaches CTZ and NTS which are not protected by blood-brain barrier and participates in triggering nausea and vomiting. Accordingly, the NK_1R antagonists have antiemetic property.

SP is involved in pain perception at various levels. It is elaborated by pain nerve endings in response to injury, and can trigger neurogenic inflammation. SP is released from primary pain afferents in the dorsal horn of spinal cord, and together with glutamate serves to transmit pain impulses. SP is one of the first responders to noxious stimuli. It initiates expression of cytokines which in turn induce SP and NK_1R , thus sustaining the reaction and inducing cellular proliferation. As such, SP has a role in inflammatory response. Recently, it has been found that SP and NK_1R , are richly

expressed on certain tumour cells, and that SP along with cytokines induced by it stimulate growth, proliferation and angiogenesis in these tumours. Thus, NK₁R antagonists may have potential utility in certain cancers.

SP (NK₁R) antagonists: Realizing the pathophysiological importance of SP, several non-peptide orally active NK₁R antagonists have been produced and are being tested as potential drugs for anxiety, mood disorders, depression, inflammatory bowel disease, arthritis, eczema and cancer. However, the only drug that has succeeded clinically so far is *Aprepitant* (and its parenteral prodrug *Fosaprepitant*) which is being used as an adjuvant antiemetic for cancer chemotherapy induced vomiting and for post-operative nausea and vomiting (see Ch. 48).

Calcitonin gene-related peptide (CGRP)

Discovered ~30 years ago in medullary thyroid carcinoma, it is a 37 amino acid neuropeptide produced by alternative processing of the calcitonin gene. CGRP is co-located with calcitonin in the C cells of thyroid, but is primarily produced by neurones in the central and peripheral nervous system; also in enteric neurones, around blood vessels and in lungs. There are two (α and β) forms of CGRP which have similar biological activity, but are products of separate genes.

CGRP acts on an unusual type of receptor which has two parts, a *calcitonin receptor-like receptor (CLR)* and a *receptor activity modifying protein (RAMP)* needed for the receptor to function. CGRP is a very potent vasodilator which acts *via* the adenylyl cyclase—cAMP mechanism in the vascular smooth muscle, and not through endothelial NO release. Injected i.v. CGRP causes fall in BP and tachycardia by dilating arteries, especially coronaries. CGRP is released mainly from the sensory C and A δ fibres (where it is mostly collocated with SP). The periarterial nerves are rich in CGRP, and it appears to serve both afferent (nociceptive) as well as efferent (neurogenic inflammation) function. CGRP is also found in the dorsal horn of spinal cord and may be an important link in pain transmission. Injected in the brain CGRP produces a variety of effects.

Several lines of evidences have pointed to a role of CGRP in migraine. It appears that sensory neurones of the trigeminal ganglion release CGRP around meningeal vessels—cause dilatation, plasma extravasation and mast cell degranulation→neurogenic inflammation is triggered and a migraine attack is

precipitated. Cranial CGRP level is increased during migraine, and sumatriptan treatment normalizes it. CGRP injected i.v. has been found to produce headache in individuals susceptible to migraine.

Some peptide as well as nonpeptide CGRP antagonists have been produced, and two agents *olcegepant* and *telcagepant* have been tested clinically in migraine, and found to be effective. However, olcegepant has very low bioavailability, and telcagepant was withdrawn after phase III trial due to hepatotoxicity.

Erenumab It is a fully human monoclonal antibody which blocks CGRP receptor, and has been approved by US-FDA (May 2018) for prophylaxis of migraine. Administered as 70 mg once a month s.c. injection through a single-use prefilled autoinjector, erenumab (marketed as AIMOVIG inj.) significantly reduced the number of migraine days per month in those suffering 4-14 migraine days per month. In some patients the reduction in migraine days was > 50%, and few patients required a double dose (140 mg/month). Erenumab has been well tolerated; injection site reaction was the most common adverse effect.

A protective role of CGRP against development of hypertension has been proposed. Due to presence in enteric nervous system, CGRP has been implicated in visceral painful disorders like irritable bowel syndrome.

Adrenomedullin (AM)

Adrenomedullin (AM) is a 52-amino acid peptide belonging to the calcitonin family that was first obtained from human pheochromocytoma. It is now known to be present in normal adrenal medulla, hypothalamus, anterior pituitary, heart, lungs, blood vessels, kidneys and intestines, as well as circulates in blood. AM produces long-lasting vasodilatation in majority of vascular beds resulting in hypotension and reflex tachycardia. Direct cardiac stimulation and centrally mediated increased sympathetic outflow also occurs. Other actions are natriuresis and renin release.

The AM receptor is structurally and functionally very similar to the CGRP receptor, i.e. it is a GPCR consisting of a CLR part coupled to a subtype of RAMP. On activation by AM, the receptor interacts with Gs protein to increase cAMP production in target cells, including vascular smooth muscle, and releases NO from endothelium. Though the physiological significance of AM is still not defined, it has been hypothesized to be playing a counterbalancing role to the RAS and endothelin system. Raised AM levels have been found in systemic and pulmonary hypertension, CHF, kidney failure and in acute myocardial infarction, where AM may be serving as a protective mechanism. No AM based clinically useful drug has been developed so far.