Chapter-2

Review of Literatures
Chapter-2.1

Calcimimetic Drug-Cinacalcet
2.1.1 Chronic Kidney Disease

According to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) Workgroup on the Diagnosis and Treatment of Chronic Kidney Disease (CKD), CKD is defined as functional abnormalities of the kidney lasting longer than 3 months, with or without reduced glomerular filtration rate (GFR).\textsuperscript{32} CKD can also be defined by the presence of urinary albumin with an excretion rate higher than 300 mg per 24 hours or in a ratio of more than 200 mg of albumin to 1 g of creatinine.\textsuperscript{32}

The National Health and Nutrition Examination Survey (conducted 1999-2000) found that 80,000 people are diagnosed annually with CKD.\textsuperscript{33}

CKD staging is classified by kidney damage and reductions in GFR, as shown in following Table 2.1.1.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GFR (ml/min/1.73m(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage and normal/elevated GFR</td>
<td>≥ 90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage and mild reduction in GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>3</td>
<td>Moderate reduction in GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>4</td>
<td>Severe reduction in GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>15 (or dialysis)</td>
</tr>
</tbody>
</table>

GFR-Glomerular Filtration Rate

Between 1990 and 2002, diabetes mellitus and hypertension was responsible for the majority of new kidney failure cases (44.6% due to diabetes mellitus and 26.9% to hypertension).\textsuperscript{34} Glomerulonephritis, ischemia, infection, polycystic kidney disease, obstruction, toxins, and autoimmune and infiltrative diseases are some of the disorders which can cause CKD.\textsuperscript{33}

Adding to the burden of CKD is the development of secondary hyperparathyroidism (SHPT), a common complication to display in elevated parathyroid hormone (PTH) levels as a result of decreased renal function, vitamin-D deficiency, and impaired mineral metabolism.\textsuperscript{35} SHPT occurs in most patients during the progression of CKD. It is associated with several comorbidities, including renal osteodystrophy (ROD), extraskeletal calcification, and cardiovascular disease (CVD), which can result in increase in the mortality.\textsuperscript{35}
Several treatment options are available to slow the progression of CKD. The management of SHPT is a component of CKD care which is mostly overlooked and undertreated by both the primary care physician and the nephrologists. This when untreated can lead to CVD and ROD. In many patients, kidney disease is often diagnosed comparatively late; therefore the staging at diagnosis is often stage 3 or 4. The extra renal complications begin to appear during the same stages i.e 3 or 4.\(^36-39\) Thus, many patients who go through the CKD diagnosis often have chronic exposure to reductions in vitamin-D and serum calcium, and elevations in PTH and phosphorus, and may produce significant skeletal and/or cardiovascular sequelae.

The most common stage of CKD is stage-III, with a rate reaching 30% in patients older than 70 years of age.\(^33\) This high number of CKD patients are challenge for both nephrologists and primary care physicians, especially when dealing with blood pressure, anaemia, volume status, and, importantly, the combination of secondary hyperparathyroidism and mineral bone disease.\(^40\)

(A) Calcium and Phosphorus Homeostasis\(^40\)

The homeostasis of calcium and phosphorus can be result of complex relations between calcemia, phosphatemia, and different hormones and factors working synergistically to keep a normal balance of these minerals (Figure 2.1.1).

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![Figure 2.1.1 Normal calcium and phosphorus homeostasis. PTH- parathyroid hormone; FGF-23-fibroblasts growth factor-23](image-url)

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2.1.2 Secondary Hyperparathyroidism (SHPT)

One of the major complications of CKD is SHPT, caused due to the disturbances in the regulation of PTH, calcium, phosphorus, and vitamin-D. Hyperphosphatemia appears to be important in the development of SHPT but the complication often occurs early i.e. before the development of hyperphosphatemia in stage-3 of kidney failure.\(^{41,42}\)

A common complication manifested in elevated parathyroid hormone (PTH) levels as a direct result of decreased renal function, vitamin-D deficiency, and impaired mineral metabolism is the development of secondary hyperparathyroidism (SHPT), which adds to the burden of CKD. In most patients, SHPT occurs during the progression of CKD. It is associated with several comorbidities, including renal osteodystrophy (ROD), extraskeletal calcification, and cardiovascular disease (CVD), which results in increased mortality.\(^35\)

SHPT secondary to CKD is an excessive production of PTH due to several changes that occur in bone and mineral metabolism as a result of decrease in kidney function. The deficiency of activated vitamin-D and an increase in phosphorus excretion by the remaining functional nephrons, are the first changes that usually occur with declining kidney function. Both of these changes stimulate an increase in PTH synthesis and secretion.\(^43\)

A majority, if not all, of untreated CKD patients will suffer from SHPT as a result of inability of kidneys to maintain mineral homeostasis.

(A) Pathophysiology of Secondary Hyperparathyroidism (SHPT)

Parathyroid Hormone

The four pea sized glands located on the thyroid gland in the neck are called as the parathyroid glands. Although their names are similar, the thyroid and parathyroid glands are entirely different glands, each producing different hormones with specific functions. The parathyroid glands secrete parathyroid hormone (PTH), which help in maintaining the correct balance of calcium and phosphorus in the body. PTH can involved in the homeostasis of bone metabolism by regulating the level of calcium in the blood, release of calcium from bone, absorption of calcium from the intestine, and excretion of calcium in the urine. So, the secretion of PTH by the parathyroid gland is affected by the levels of calcium and other minerals involved in bone metabolism, such as phosphorus and vitamin-D.\(^43\)
The most important regulator of calcium metabolism is parathyroid hormone (PTH). It is a polypeptide containing 84 amino acids and is secreted by the leader cells of the parathyroid glands in response to Hypocalcemia and hyperphosphatemia. It has a short half-life (2 to 4 minutes) before being degraded to various inactive fragments. PTH acts mainly on 2 organs: the bone and the kidney.

1. It stimulates the osteoclasts and causes bone resorption, resulting in an increase in the serum concentration of calcium and phosphorus.

2. PTH stimulates the 1-hydroxylase activity in the kidney which leads to an increase in 1,25 dihydroxyvitamin-D production. It also increases the reabsorption of calcium in the distal renal tubules, decreasing calcium clearance. Opposite effect occurs phosphorus clearance. PTH can decrease the reabsorption of phosphorus in the proximal renal tubules from 85% in healthy individuals to less than 15% in dialysis patients.

3. PTH has no direct established activity on the intestine. But it can indirectly increase intestinal calcium and phosphorus absorption via stimulation of 1,25 dihydroxyvitamin-D production. The results of high PTH are hypercalcemia, hypophosphatemia, and high urinary calcium and phosphorus.

4. Calcium has a negative feedback effect on the parathyroid glands through the calcium sensing receptor. Recently, phosphorus has been shown to have a direct stimulatory effect on the parathyroid glands.

**Vitamin-D**

The term “vitamin-D” is used generically to refer to many substances or forms of vitamin-D. In the body, vitamin D3 is the active form of vitamin-D. Precursors to the hormone vitamin D3 are obtained from food sources and exposure to ultraviolet light. These precursors then undergo two important enzymatic reactions. The resulting calcitriol or active vitamin D3 [1,25-(OH)2D3] molecule is the active form that binds to the vitamin-D receptor (VDR). Under normal circumstances, vitamin D3 plays a vital role in regulating PTH synthesis and release. By stimulating the parathyroid VDR, it down regulates the production of PTH. Vitamin D3 also decreases PTH indirectly by stimulating VDRs in the gut, thereby increasing calcium absorption and serum calcium. As kidney function declines, there is a decrease of renal 1α-hydroxylase activity that is responsible for the final hydroxylation reaction in calcitriol synthesis. In worsening CKD, the kidney becomes less able to perform 1α-
Chapter 2.1 Review of Literature-Cinacalcet

hydroxylation and, consequently, active vitamin D3 levels become deficient and increase PTH concentrations.43

Vitamin-D is an essential factor in the regulation of calcium and phosphorus balance. It is synthesized in the skin but is also present in the diet. 1,25 dihydroxyvitamin-D is its active form. Its main action is to enhance the availability of calcium and phosphorus for formation of new bone. Important actions of vitamin-D in many other tissues have been shown in some recent studies. Vitamin-D enhances the intestinal absorption of calcium and phosphorus, causing increase their serum levels.
1. Vitamin-D is a required factor in the bone resorption process, along with PTH.
2. It also increases the reabsorption of urinary calcium and phosphorus in the renal tubules.
3. It has a direct effect on the parathyroid glands to suppress PTH secretion through the vitamin-D receptors.40

Fibroblasts Growth Factor-2340

Until recently, it was thought that the phosphorus homeostasis was mainly achieved by PTH and vitamin-D. Recent studies identified fibroblasts growth factor (FGF)-23 as a new protein with phosphaturic activity. It is mainly secreted by osteocytes and is now considered to be the most important factor for regulation of phosphorus homeostasis.
1. It acts mainly on the kidney to increase phosphorus clearance through the Klotho receptor.
2. FGF-23 also inhibits the 1-hydroxylase activity which causes a low 1,25 dihydroxyvitamin-D level.
3. The principal stimulator for FGF-23 is hyperphosphatemia.
4. It is not yet proven if there is any direct relation between PTH and FGF-23.

(B) Calcium and Phosphorus Metabolism in Renal Failure40

When GFR falls, the phosphorus clearance decreases significantly, leading to phosphorus retention.
The principal cause of secondary hyperparathyroidism is thought to be hyperphosphatemia, subclinical when estimated GFR is >30 mL/min (Figure 2.1.2). Phosphorus induces PTH secretion by 3 mechanisms:
1. Direct stimulatory effect on the parathyroid glands.
2. Induction of mild hypocalcemia by precipitating with calcium as CaHPO4. Decreased calcium release from bone pools can also cause hypocalcemia.
3. Stimulation of FGF-23, which leads to severe inhibition of 1\_ hydroxylase and depressed level of 1,25 dihydroxyvitamin-D. The downregulation of the vitamin-D receptors on the parathyroid glands leads to vitamin-D resistance. A high PTH level is caused by the loss of negative feedback on the parathyroid glands.

![Figure 2.1.2 Calcium and phosphorus metabolism in renal failure. PTH-parathyroid hormone; FGF-23-fibroblasts growth factor-23.](image)

(C) Phosphorus Metabolism

As the glomerular filtration rate (GFR) declines to <60 ml/min/1.73 m\(^2\), phosphorus excretion becomes altered in the nephron. Although half of the nephrons are not working to excrete phosphorus, the remaining nephrons compensate by hyper-excreting the daily phosphorus load to maintain normal serum phosphorus concentrations. Compensation can generally continue until the GFR declines to <25–40 ml/min/1.73 m\(^2\). With progressive CKD, when the remaining nephrons can no longer sufficiently excrete the phosphorus load, hyperphosphatemia is detected. Calcium, a divalent cation, and phosphorus, a monovalent anion, have a high binding affinity for each another. In the serum, as the concentration of one or both ions increases, there is an increased risk for an ionic bond to form, creating an insoluble complex. This process may lead to extraskeletal calcification and potentially calciphylaxis or cardiac disease.\(^4\) Additionally, the precipitation may decrease serum calcium
concentrations, further stimulating PTH secretion. In fact, PTH production and secretion may be stimulated by hypocalcemia, hyperphosphatemia, and vitamin-D deficiency.\textsuperscript{45,46} Because PTH is chiefly responsible for preventing hypocalcemia, it stimulates osteoclasts to lyse bone, releasing calcium into the serum. Under normal conditions, there is homeostasis involving osteoclast activity and osteoblast synthetic activity. SHPT produces an imbalance of these activities leading to enhanced bone breakdown that eventuates in renal osteodystrophy.\textsuperscript{47,48}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.1.3.png}
\caption{Pathophysiology of secondary hyperparathyroidism}
\end{figure}
(D) Impact and Consequences of SHPT

1. Bone Disease
Renal osteodystrophy refers to several bone disorders that accumulate from the pathophysiology of bone and mineral metabolism in CKD which are osteitis fibrosa cystica, osteomalacia, and adynamic bone disease. Osteitis fibrosa cystic is referred to as high-turnover bone disease and is associated with elevated PTH concentrations that stimulate osteoclast activity, bone breakdown, and resorption. Osteomalacia (“soft bone”) is characterized by a low turnover of bone and abnormal mineralization and has historically been associated with aluminum toxicity.\(^{39,41}\)

Adynamic bone disease is referred to as low-turnover disease with normal mineralization and may result from low PTH levels.\(^{42}\) The prevalence of adynamic bone disease is increasing and may be the consequence of PTH over-suppression from the use of vitamin D agents, calcimimetics, and phosphate binders, singly or in combination.

2. Impact of Alterations: Extraskeletal Calcification
Alterations in calcium, phosphorus, vitamin-D, and PTH cause other deleterious consequences in patients with CKD in addition to bone mineral defects and disease. Extraskeletal calcification (primarily cardiovascular calcification) has been documented in patients with CKD\(^ {43}\) and is directly correlated to an increase in cardiovascular morbidity and mortality.\(^ {44}\) Patients with CKD, especially end-stage renal disease (ESRD), have an increased risk of cardiovascular morbidity and mortality. In fact, research has shown that the primary cause of death in patients with ESRD is cardiovascular disease.\(^ {45}\) A study of patients on hemodialysis found that even when stratified for variables such as sex, race, and presence of diabetes, dialysis patients still had a cardiovascular mortality rate nearly 30 times greater than the general population.\(^ {46}\)

Certainly comorbid disorders, such as diabetes, hypertension, hyperlipidemia, and anemia, play a role in these findings. However, recent research has also identified cardiovascular calcification as a contributing factor. Correlations have been made between cardiovascular calcification and factors such as hyperphosphatemia, increased calciumphosphorus product (Ca × P), hypercalcemia, vitamin-D therapy, and increased doses of calcium-containing phosphate binders and calcium supplements. The balance of calcium, phosphorus, vitamin D, and iPTH is complex and interrelated. Patients must adhere to dietary restrictions, dialysis therapies, and complicated medication regimens. These factors create barriers to achieving and maintaining control of SHPT. In fact, one study of nearly 200 chronic hemodialysis
outpatients revealed that < 10% of patients could be simultaneously maintained within the target ranges of the above parameters.\textsuperscript{47}

(E) Treatment of secondary hyperparathyroidism

1. Goals of SHPT Treatment\textsuperscript{43}

The ultimate goals of treating SHPT are to normalize mineral metabolism, prevent bone disease, and prevent extraskeletal manifestations of the altered biochemical processes. The markers of calcium, phosphorus, vitamin-D, and iPTH are used as surrogate measures of disease progression.

It is important to identify SHPT early. Abnormalities can occur subtly, usually without any symptoms, and may progress to cause more complications if not detected early.

Until recently, it was thought that hyperphosphatemia was the earliest sign of SHPT and bone metabolism disorders. However, when patients reach Stage-3 CKD, it is highly probable that none of the biochemical parameters routinely assessed will be abnormal. In fact, the iPTH level is often increased before clinical hyperphosphatemia occurs. For this reason, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KQODI) guidelines recommend that all patients with a GFR < 60 ml/min/1.73 m\textsuperscript{2} undergo evaluation of serum calcium, phosphorus, and iPTH levels.

(F) Current Treatment Options for Secondary Hyperparathyroidism

1. Dietary phosphate restriction\textsuperscript{43}

Generally as the GFR declines to < 0 ml/min/1.73 m\textsuperscript{2}, hyperphosphatemia becomes prevalent. One of the first interventions recommended to lower serum phosphate concentrations is dietary phosphate restriction. Foods that are high in phosphate content include dairy products, meats, beans, dark sodas, beer, and nuts. Many foods that are high in phosphorus are also primary sources of protein, particularly meats. Generally, patients are instructed to reduce their intake of or avoid foods that are high in phosphorus but not high in protein. Examples of foods to avoid include cheese, milk, ice cream, beer, and dark sodas. Protein sources are not withheld because poor nutrition can lead to hypoalbuminemia, which has been associated with increased morbidity and mortality in CKD. Dietary phosphate restriction alone is often insufficient to maintain serum phosphorus concentrations in the target range. In this case, phosphate binders may be used to prevent hyperphosphatemia.
2. Phosphate binding agents

Phosphate binding agents bind to dietary phosphate in the gut, forming an insoluble complex that is excreted in the feces causing decrease in serum phosphate concentrations. Optimally, these agents can administer with food and are generally taken 3 times daily with meals. More frequent administration may be needed in patients requiring enteral feedings. Patient acceptance and adherence is the greatest challenge for successful use of phosphate binders. Patient education is mandatory as these medicines must be taken several times a day and may significantly increase patients’ medication burden. In order to achieve target concentrations of phosphorus and calcium phosphate binders from different classes can be combined. Actually, the combined use of a calcium-containing phosphate binder and a non–calcium-containing phosphate binder may reduce the serum phosphorus level while maintaining the calcium concentration.

Similarly, patients with hyperphosphatemia with concurrent hypercalcemia may need the use of one or more non–calcium-containing phosphate binders (e.g., sevelamer hydrochloride, lanthanum carbonate, and aluminum). Often, CKD patients can require therapy to lower iPTH and serum phosphorus concentrations. Phosphate binders can typically used concurrently with vitamin-D therapy or a calcimimetic agent to control all of the biochemical parameters involved (i.e., calcium, phosphorus, Ca × P, and iPTH).

3. Vitamin D and Its Derivatives

One of the oldest treatments for secondary hyperparathyroidism is Vitamin-D. It is known that calcitriol deficiency and resistance are major contributors to the pathophysiology of the disease and that calcitriol supplementation is effective in suppressing high levels of PTH. On the other hand, calcitriol enhances the intestinal absorption of calcium and phosphorus, increasing their blood levels and possibly increasing their product. Several observational studies have shown improved survival in patients treated with intravenous vitamin-D, but randomized controlled studies to confirm survival benefits are still lacking. In fact, a meta-analysis done in 2007 showed no difference in mortality, bone pain, vascular calcifications, or rate of parathyroidectomies between patients treated with vitamin-D compounds and those who received placebo. Calcitriol (1,25 dihydroxy vitamin-D3) is the natural form of vitamin-D produced by the human body. Studies have shown that intermittent, intravenous administration of calcitriol is more effective than daily oral calcitriol in lowering serum PTH levels. Several forms of vitamin-D and its derivatives are available on the market.
4. Ergocalciferol

Ergocalciferol is vitamin-D2 or the nutritional vitamin-D. In order to get activated, ergocalciferol needs to be metabolized in the liver and the kidneys. This requires at least some activity of the 1-hydroxylase. Recently, it has been known that low levels of 25-hydroxyvitamin-D and not only of the vitamin D active form (1,25 dihydroxy vitamin-D)—can contribute to the development of secondary hyperparathyroidism. Ergocalciferol is indicated only in patients with CKD stages III and IV when the 25-hydroxy vitamin-D level is 30 ng/mL. There is currently insufficient evidence to support the benefits of ergocalciferol use in dialysis patients (CKD stage V).

5. Selective Vitamin-D Analogue

The development of selective agents having more affinity to the kidney rather than intestinal receptors was the result of the problems occurring with the increased intestinal absorption of calcium and phosphorus after administration of cacitriol. These second-generation agents cause less hypercalcemia and hyperphosphatemia as compared to traditional calcitriol. The two agents which are available and widely used in the United States are paricalcitol and doxercalciferol. In a large study conducted on 69,492 patients undergoing dialysis in Fresenius facilities, the patients treated with paricalcitol had a 16% lower mortality rate than those who received calcitriol. They also showed lower levels of calcemia and phosphatemia and a better PTH control.

6. Calcimimetic agents

The sensitivity of the calcium-sensing receptor (CaR) in the parathyroid gland is increased by the novel agents known as Calcimimetics. Calcimimetic agents induce a conformation change in the CaR that lowers the activation threshold for serum calcium by binding to the transmembrane domain of the CaR. Thus, these drugs decrease PTH secretion at calcium levels below the normal physiological concentration. Cinacalcet HCl is the only calcimimetic agent which has been approved by USFDA. Cinacalcet is available in the market as a once daily oral formulation for the treatment of SHPT in adult CKD stage-5 patients on dialysis and also for the treatment of hypercalcemia in patients with parathyroid carcinoma.

It has Proven safe and effective in clinical trials, cinacalcet has been shown to substantially decrease PTH levels without concomitant elevations in calcium, phosphorus, or Ca x P.34
Cinacalcet is effective in decreasing iPTH concentrations and to maintain calcium and phosphorus concentrations.\(^{49,50}\) When used alone, with phosphate binders, or in combination with phosphate binders and vitamin-D therapy, a new treatment strategy is offered by Cinacalcet.\(^{43}\)

### 2.1.3 The calcium-sensing receptor (CaR)

The sigmoidal, inverse relationship between serum levels of PTH and calcium illustrates an exquisitely sensitive control mechanism in which only minute changes in serum calcium are required to induce large changes in PTH. Although the calcium-sensing receptor can be found in many tissues, its ability to sensitively control serum calcium levels is largely dependent upon its presence on the chief cells of the parathyroid gland (CaRs on the luminal side of the medullary collecting duct epithelium of the kidneys also participate in calcium regulation).

The responsiveness of the parathyroid glands to changes in serum calcium caused researchers to speculate about the presence of a cell-surface calcium receptor for many years. Indirect evidence of such a receptor was provided by Akerstrom and colleagues in the late 1980s, but it was not until 1993 that Brown \textit{et al.} cloned the bovine calcium-sensing receptor. The calcium-sensing receptor is part of a superfamily of G-protein-coupled transmembrane receptors.

It has three major domains, an extracellular 612-amino-acid ligand-binding portion, a hydrophobic 250-amino-acid membrane-spanning section and a cytosolic C-terminal tail of approximately 250 amino acids.\(^{51}\)

![Parathyroid and renal actions of the calcium-sensing receptor](image)

\textit{Figure 2.1.4 Parathyroid and renal actions of the calcium-sensing receptor}
The calcium-sensing receptor is a member of the G-protein-coupled superfamily of receptors. It differs from most of G-protein-coupled receptors in that it possesses a large extracellular amino-terminal domain and a smaller intracellular carboxy-terminal domain. The calcium-sensing receptor is not specific for Ca\textsuperscript{2+} as it also responds to a variety of divalent and trivalent cations, including magnesium, aluminum and gadolinium, as well as polycations, all of which have in common a net positive charge at physiological pH. A schematic diagram of the CaR on the parathyroid cell membrane is shown in Figure 2.1.4. As stated, the calcium-sensing receptor is not specific for Ca\textsuperscript{2+}, but has the highest affinity for the calcium-sensing receptor compared with other cations.

Activation of calcium-sensing receptor on the parathyroid gland cells by Ca\textsuperscript{2+} leads to the activation of a number of secondary messengers in a cascade of events eventually resulting in the inhibition of synthesis and secretion of PTH (Figure 2.1.5). The CaR, following activation by elevated serum levels of Ca\textsuperscript{2+}, couples to phospholipase C (PLC) via a G-protein (probably G\textsubscript{q} or G11) and then indirectly to phospholipase A2 (PLA2). PLA2 then acts on membrane phospholipids to release arachidonic acid, which in turn is converted to leukotrine metabolites that inhibit PTH secretion.

Activation of the calcium-sensing receptor results in G\textsubscript{q}/G11 mediated activation of phosphatidylinositol (PI)-PLC, which in turn mobilizes intracellular calcium leading to the inhibition of PTH secretion. PI-PLC also activates protein kinase C (PKC), leading to PKC-mediated stimulation of the MAPK cascade. Calcium-sensing receptor activates MAPK via a pertussis toxin-sensitive G-Protein, thought to be Gi, leading to downstream activation of a tyrosine kinase-dependent process involving ras and raf-dependent pathways.

Activated MAPK phosphorylates and activates PLA2, releasing free arachidonic acid. Arachidonic acid is metabolized to active mediators, such as hydroxyperoxyeicosatetraenoic acid or hydroxyeicosatetraenoic acid (20-HETE), which can then decrease PTH secretion.
Figure 2.1.5 Schematic diagram for mechanisms for Ca\textsuperscript{2+}-sensing receptor (CaR)-induced activation of extracellular signal-regulated kinase (ERK)1/2 and cytosolic (c)PLA2 in the parathyroid cell.

The way in which extracellular Ca\textsuperscript{+} acts on the calcium-sensing receptor varies between cell types, and this can be illustrated in the kidney, where hypercalcaemia can alter many aspects of renal function, including a reduction in glomerular filtration rate and an increase in renal vasoconstriction. In the thick ascending limb of Henle, hypercalcaemia reduces cAMP generation induced by various hormones at this site.

The calcium-sensing receptor is expressed on the basolateral membrane. When Ca\textsuperscript{2+} activates the receptor, there is a resultant enhancement in the formation of arachidonic acid-derived 20-HETE, which can reversibly inhibit apical K\textsuperscript{+} channels, as well as directly inhibiting Na\textsuperscript{+},K\textsuperscript{+}
and Cl- activity. The inhibition of cAMP formation by the action of Ca+ on the CaR leads, through a number of intracellular pathways, to a decrease in Na+, K+ and Cl- reabsorption via their common transporter in the thick ascending limb and a decrease in K+ transfer across a specific potassium channel. In addition, CaR-mediated inhibition of cAMP reduces the stimulatory action of vasopressin and PTH on the tubule epithelium, in this area of the nephron and further downstream, respectively.

The Na+,K+ , Cl- co-transporter can transport NH4+ instead of K+ and can therefore also regulate net urinary acid excretion, suggesting that CaR-mediated process may also be responsible for maintaining a correct acid-base balance. Activation of calcium-sensing receptors on the renal tubules produces several different effects. CaR activation decreases Cl- resorption and K+ secretion through the apical channel in the thick ascending limb. This decreases the lumen-positive voltage in the tubular lumen leading to decreased Ca2+ and Mg 2+ reabsorption and, consequently, their increased distal delivery.

In the distal tubule, the calcium-sensing receptor is expressed on the basolateral side of the epithelium. Stimulation of the CaR by calcium in the distal tubules leads to inhibition of calcium transport. CaR are also expressed on the luminal side of the medullary collecting duct epithelium of the kidney. Calcium-sensing receptor at this site use the same vesicles as the vasopressin-activated aquaporin-2 water channels, whose reduced insertion in the apical cell membrane in response to a rise in luminal Ca2+ concentration above a certain level leads to a decrease in water reabsorption. This, in turn, could be considered to be an efficient mechanism against excessive calcium concentration and calcium crystal formation in the terminal urine.

Thus, the calcium-sensing receptor is not only involved in PTH secretion, but also involved in many different processes in the kidney, participating directly and indirectly in the maintenance of calcium homoeostasis and several other homoeostatic regulations.52

### 2.1.4 Effect of calcimimetics on PTH secretion and parathyroid gland cell proliferation

The awareness of the role of the calcium-sensing receptor and the effect of calcium on the parathyroid gland had provided an ideal target for the development of new drugs that may be able to control levels of PTH. Calcimimetics modulate the CaR, which makes it more
sensitive to the effects of extracellular calcium leading to the inhibition of PTH secretion. So, these compounds could be potentially used in the treatment of SHPT.

The onset of SHPT occurs long before the start of dialysis in patients with chronic renal failure. Evidence from animal models of hyperparathyroidism and from human parathyroid gland cell cultures, published data shows that treatment with calcimimetic agent (Cinacalcet) produced a benefit in the control of serum PTH levels in pre-dialysis patients.53

Figure 2.1.6 Calcimimetics act on the calcium-sensing receptor (CaR) by mimicking and/or potentiating the effects of calcium. Increased serum calcium (Ca^{2+}) levels activate the CaR of the parathyroid cell, which leads—via intracellular signalling mechanisms—to decreased parathyroid hormone (PTH) synthesis and secretion. Calcimimetics potentiate the effect of serum calcium and thus can lower serum calcium and serum phosphate (PO_4) levels in dialysis patients with secondary hyperparathyroidism.
2.1.5 Cinacalcet Hydrochloride\textsuperscript{54}

(A) Chemical Name

The hydrochloride salt of cinacalcet is described chemically as N-\[1-(\text{R})-(\cdot)-(1-naphthyl)ethyl]-3-[3(trifluoromethyl)phenyl]-1-aminopropane hydrochloride.

(B) Structural formula

![Structural formula of Cinacalcet Hydrochloride]

(C) Empirical formula: $\text{C}_{22}\text{H}_{22}\text{F}_{3}\text{N}\cdot\text{HCl}$

(D) Molecular weight

Cinacalcet Hydrochloride: 393.9 g/mol
Cinacalcet: 357.4 g/mol.

(E) Indications

**Secondary Hyperparathyroidism**
Cinacalcet is indicated for the treatment of secondary hyperparathyroidism (HPT) in patients with chronic kidney disease (CKD) on dialysis.

**Parathyroid Carcinoma**
Cinacalcet is indicated for the treatment of hypercalcemia in patients with parathyroid carcinoma.

**Primary Hyperparathyroidism**
Cinacalcet is indicated for the treatment of severe hypercalcemia in patients with primary HPT who are unable to undergo parathyroidectomy.
(F) Dosage and Administration

Secondary Hyperparathyroidism in Patients with Chronic Kidney Disease on Dialysis
The recommended starting oral dose of cinacalcet is 30 mg once daily. Serum calcium and serum phosphorus should be measured within 1 week and intact parathyroid hormone (iPTH) should be measured 1 to 4 weeks after initiation or dose adjustment of cinacalcet. Cinacalcet should be titrated no more frequently than every 2 to 4 weeks through sequential doses of 30, 60, 90, 120, and 180 mg once daily to target iPTH levels of 150 to 300 pg/mL. Serum iPTH levels should be assessed no earlier than 12 hours after dosing with cinacalcet. Cinacalcet can be used alone or in combination with vitamin D sterols and/or phosphate binders.

During dose titration, serum calcium levels should be monitored frequently and if levels decrease below the normal range, appropriate steps should be taken to increase serum calcium levels, such as by providing supplemental calcium, initiating or increasing the dose of calcium-based phosphate binder, initiating or increasing the dose of vitamin D sterols, or temporarily withholding treatment with cinacalcet.

Parathyroid Carcinoma and Primary Hyperparathyroidism
The recommended starting oral dose of cinacalcet is 30 mg twice daily. The dose of cinacalcet should be titrated every 2 to 4 weeks through sequential doses of 30 mg twice daily, 60 mg twice daily, and 90 mg twice daily, and 90 mg 3 or 4 times daily as necessary to normalize serum calcium levels.

(G) Mechanism of Action
Secondary HPT in patients with CKD is a progressive disease, associated with increases in PTH levels and derangements in calcium and phosphorus metabolism. Increased PTH stimulates osteoclastic activity resulting in cortical bone resorption and marrow fibrosis. The goals of treatment of secondary HPT are to lower the levels of PTH, calcium, and phosphorus in the blood in order to prevent progressive bone disease and the systemic consequences of disordered mineral metabolism. Reductions in PTH are associated with a decrease in bone turnover and bone fibrosis in patients with CKD on dialysis and uncontrolled secondary HPT.

The calcium-sensing receptor on the surface of the chief cell of the parathyroid gland is the principal regulator of PTH synthesis and secretion. Sensipar (cinacalcet) directly lowers PTH levels by increasing the sensitivity of the calcium-sensing receptor to extracellular calcium. The reduction in PTH is associated with a concomitant decrease in serum calcium levels.
Measurements of PTH during the Sensipar (cinacalcet) studies were obtained using the Nichols iPTH immunoradiometric assay.

(H) Pharmacodynamics
Reduction in iPTH levels correlated with the plasma cinacalcet concentrations in patients with CKD. The nadir in iPTH level occurs approximately 2 to 6 hours postdose, corresponding with the maximum plasma concentration (Cmax) of cinacalcet. After steady-state cinacalcet concentrations are reached (which occurs within 7 days of dose change), serum calcium concentrations remain constant over the dosing interval in patients with CKD.

(I) Pharmacokinetics
1. Absorption and Distribution
After oral administration of cinacalcet, Cmax is achieved in approximately 2 to 6 hours. Cinacalcet Cmax and AUC(0-inf) were increased by 82% and 68%, respectively, following administration with a high-fat meal compared with fasting in healthy volunteers. The Cmax and AUC(0-inf) of cinacalcet were increased by 65% and 50%, respectively, when cinacalcet was administered with a low-fat meal compared with fasting.
After absorption, cinacalcet concentrations decline in a biphasic fashion with a terminal half-life of 30 to 40 hours. Steady-state drug levels are achieved within 7 days, and the mean accumulation ratio is approximately 2 with once-daily oral administration. The median accumulation ratio is approximately 2 to 5 with twice-daily oral administration. The AUC and Cmax of cinacalcet increase proportionally over the dose range of 30 to 180 mg once daily. The pharmacokinetic profile of cinacalcet does not change over time with once-daily dosing of 30 to 180 mg. The volume of distribution is approximately 1000 L, indicating extensive distribution. Cinacalcet is approximately 93% to 97% bound to plasma protein(s). The ratio of blood cinacalcet concentration to plasma cinacalcet concentration is 0.80 at a blood cinacalcet concentration of 10 ng/mL.

2. Metabolism and Excretion
Cinacalcet is metabolized by multiple enzymes, primarily CYP3A4, CYP2D6, and CYP1A2. After administration of a 75mg radiolabeled dose to healthy volunteers, cinacalcet was metabolized via: 1) oxidative N-dealkylation to hydrocinnamic acid and hydroxy-hydrocinnamic acid, which are further metabolized via βoxidation and glycineconjugation; the oxidative N-dealkylation process also generates metabolites that contain the naphthalene ring;
and 2) oxidation of the naphthalene ring on the parent drug forming dihydrodiols, which are further conjugated with glucuronic acid. The plasma concentrations of the major circulating metabolites, including the cinnamic acid derivatives and glucuronidated dihydrodiols, markedly exceed the parent drug concentrations. The hydrocinnamic acid metabolite and glucuronide conjugates have minimal or no calcimimetic activity. Renal excretion of metabolites was the primary route of elimination of radioactivity. Approximately 80% of the dose was recovered in the urine and 15% in the feces.

3. Hepatic Impairment
The disposition of a 50 mg cinacalcet single dose was compared between patients with hepatic impairment and patients with normal hepatic function. Cinacalcet exposure (AUC(0-inf)) was comparable between healthy volunteers and patients with mild hepatic impairment. However, in patients with moderate and severe hepatic impairment (as indicated by the Child-Pugh method), cinacalcet exposures (AUC(0-inf)) were 2.4 and 4.2 fold higher, respectively, than that in healthy volunteers. The mean half-life of cinacalcet increased from 49 hours in healthy volunteers to 65 hours and 84 hours in patients with moderate and severe hepatic impairment, respectively. Protein binding of cinacalcet is not affected by impaired hepatic function.

4. Renal Impairment
The pharmacokinetic profile of a 75 mg cinacalcet single dose in patients with mild, moderate, and severe renal impairment, and those on hemodialysis or peritoneal dialysis is comparable with that in healthy volunteers.

5. Geriatric Patients
The pharmacokinetic profile of cinacalcet in geriatric patients (age ≥ 65 years, n = 12) is similar to that for patients who are < 65 years of age (n = 268).

6. Pediatric Patients
The pharmacokinetics of cinacalcet has not been studied in patients < 18 years of age.
Chapter-2.2

Antianginal Drug-Ivabradine
2.2.1 Ischemic heart disease and Chronic stable Angina

Ischemic heart disease (IHD), also known as coronary heart disease or coronary artery disease, is defined as an imbalance between myocardial oxygen demand and supply. The majority of patients with IHD (nearly 60%) suffer from angina pectoris, or simply angina.\textsuperscript{55} The most common symptom of ischemia is angina which is defined as discomfort in the chest or adjacent areas due to compromised blood supply to the myocardium.\textsuperscript{56} The predictable occurrence of ischemic symptoms with physical activity or other conditions that increase oxygen demand is Chronic stable angina. Another sign of IHD is acute coronary syndrome (ACS), which includes unstable angina and myocardial infarction (MI).

(A) Pathophysiology\textsuperscript{56}

Ischemic heart disease is caused by the narrowing of one or more of the major coronary arteries. The characteristic feature of chronic stable angina is an established atherosclerotic plaque which obstructs coronary blood flow. The Pathophysiology of atherosclerosis is shown in Figure 2.2.1.

Atherosclerotic plaques develop as lipids [e.g., low density lipoprotein (LDL) cholesterol] from the bloodstream and penetrate and deposit in the intimal layer of the coronary arteries. In response to lipid deposition, inflammatory mediators are released. This causes endothelial dysfunction, promotes migration and proliferation of smooth muscle cells in order to “stabilize” the growing plaque by forming a thick fibrous cap, and producing a prothrombotic environment.\textsuperscript{57} Atherosclerotic plaques, have a tendency to rupture, particularly those “vulnerable” plaques which are characterized by a large lipid core and thin fibrous cap. This triggers platelet activation and aggregation, stimulation of the clotting cascade, and causes the release of more inflammatory mediators. A thrombus may form at the site of injury, in response to plaque rupture which acutely impedes blood flow and precipitates acute ischemia, and perhaps infarction. The inflammatory response to plaque rupture may “heal” the plaque by promoting progressive stenosis progresses. The lumen of the coronary artery begins to narrow as the atheroma grows in size causing decrease in the coronary blood flow. Several risk factors which affect the development of IHD are given in Table 2.2.1.
Table 2.2.1 Major risk factors for ischemic heart disease

<table>
<thead>
<tr>
<th>Modifiable</th>
<th>Non-Modifiable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>• Men: &gt;45 years of age</td>
</tr>
<tr>
<td></td>
<td>• Women: &gt;55 years of age</td>
</tr>
<tr>
<td>Dyslipidemia:</td>
<td>Gender</td>
</tr>
<tr>
<td>• Elevated LDL</td>
<td>• Men</td>
</tr>
<tr>
<td>cholesterol</td>
<td>• Post-menopausal women</td>
</tr>
<tr>
<td>• Elevated non-HDL</td>
<td></td>
</tr>
<tr>
<td>cholesterol</td>
<td></td>
</tr>
<tr>
<td>• Elevated triglycerides</td>
<td></td>
</tr>
<tr>
<td>• Low HDL cholesterol</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Family history of premature cardiovascular disease in first-degree relative</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>(e.g., parent or sibling) younger than:</td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>• 55 years of age (male family member)</td>
</tr>
<tr>
<td>Obesity</td>
<td>• 65 years of age (female family member)</td>
</tr>
</tbody>
</table>

LDL = low density lipoprotein; HDL = high density lipoprotein

The plaque characteristic of chronic stable angina is a small lipid-laden core surrounded by a thick fibrous cap. When the plaque occludes 70% or more of the coronary artery, myocardial oxygen supply cannot meet increases in the oxygen demand. In such case ischemia may develop, and the patient may experience angina during periods of exertion or emotional distress.

Vasospasm may occur at the site of an atherosclerotic plaque or at times in the absence of significant atherosclerosis (variant or Prinzmetal’s angina) which impairs coronary blood flow, induces ischemia, and precipitates angina. When ischemia lasts for sufficient duration, it can cause MI. Other complications occurring along with IHD include heart failure, arrhythmias, and death.

When myocardial perfusion is insufficient to meet myocardial oxygen demand it results in Angina, which is a symptom of atherosclerotic coronary artery obstruction. The results of
Chapter 2.2 Review of Literature-Ivabradine

The Framingham Heart Study states that the lifetime risk of coronary heart disease for patients aged 40 years is 31% in females and 48% in males.\textsuperscript{57} Also, many large scale epidemiological studies such as the Chicago epidemiological studies and the National Health and Nutrition Examination Survey (NHANES) support elevated resting HR as a risk marker for both cardiovascular and all-cause mortalities.\textsuperscript{60,61}

Figure 2.2.1 Pathophysiology of coronary atherosclerosis
(B) Heart rate Reduction in the treatment of Angina

The most important determining factor of myocardial oxygen use is Heart Rate. Thus, an increase in HR is responsible for the majority of cardiovascular episodes in CAD patients. An increased HR can cause myocardial ischaemia and subsequent angina due to an increase in myocardial oxygen demand. It can also cause decrease in myocardial perfusion, due to the shorter diastole duration.\(^{62}\)

Heart rate has identified as an independent risk factor for mortality from cardiovascular diseases. The correlation of high resting heart rate with increased all-cause and cardiovascular mortality is shown by Large-scale epidemiological studies, such as the Framingham Heart Study.\(^{63}\) This phenomenon can be explained by several mechanisms. Accelerated heart rate is involved in the progression of atherosclerosis through mechanical and metabolic processes. Tachycardia results in increased wall stress which can induce endothelial injury and easier penetration of lipids into the vessel wall. This mechanism can also explain the higher incidence of atherosclerotic plaque disruption and new acute coronary events in patients with high resting heart rate.\(^{64, 65}\)

Elevated heart rate usually express activation of the sympathetic nervous system with its attendant injurious metabolic effects resulting in accelerated atherogenesis.

In the management of myocardial ischemia and chronic stable angina, reduction of Heart rate plays an important role. Literature states that myocardial ischemia is a result of an imbalance between myocardial oxygen demand and supply. Heart rate is an important determining factor of oxygen consumption and metabolic demand. Also, higher heart rate may induce or exacerbate ischemia and symptoms of angina, as it shortens the duration of diastole and followed by decreases in the perfusion of epicardial coronary arteries. Above observations elucidate, why rate-slowing drugs, such as beta-blockers, followed by calcium channel antagonists, are considered to be the important in antianginal therapy. The broad use of beta-blockers is limited by their adverse reactions such as fatigue, lethargy, insomnia, negative inotropic and lusitropic effect, depression and erectile dysfunction in men. Also, beta-blockers are contraindicated in decompensated heart failure, chronic obstructive lung disease, peripheral vascular disease, severe atrioventricular conduction defects and finally, Prinzmetal angina related to coronary artery spasm. Beta-blockers might even exacerbate symptoms of angina due to unopposed alpha-receptor activation, in prinzmetal angina related to coronary artery spasm patients, with angiographically normal coronary arteries.\(^{66}\)
Inspite of the use of a combination of anti-anginal agents, angina is most common, which in turn leads to restricting the quality of life of patients suffering from it. The outcomes have improved due to revascularization, but symptoms of angina are still experienced by up to 60% of patients, as seen in the RITA2 trial.67

New agents are needed to improve therapy of angina. Ivabradine is the novel class of agents that exclusively reduce heart rate through inhibition of the sinoatrial If current.

(C) The Role of Cardiac Pacemaker (If) Current

The sinoatrial node is the normal pacemaker of the heart, because action potentials begin earlier in this area than other regions, including the atroioventricular node, the bundle of His and the Purkinje fibers. The pacemaker cells get hyperpolarized at rest. At the next point, a slow diastolic depolarization begins, which turns the membrane voltage to the threshold level for a new action potential. The rate of depolarization actually reflects the heart rate. The If current determines the slope of the depolarization curve towards the threshold level, which is −40 mV in humans. In this manner, time interval between action potentials, which in turn is expressed in the heart rhythm, is regulated by this inward Na+/K+ current known as the If current of the sinoatrial node.66

The myocytes of the SA node are the primary pacemaker cells of the heart and therefore control HR. The pacemaker function involves several ionic currents that influence spontaneous diastolic depolarisation of the SA node. One of the most important of these is the If current. This current has atypical or “funny” properties compared to other current systems, such as mixed Na+-K+ inward movement, activated on hyperpolarisation and intracellular cyclic adenosine monophosphate (cAMP), low single-channel conductance and slow kinetics. The If current is modulated by the autonomic nervous system. A rise in intracellular cAMP under the influence of β-receptor stimulation results in an increase in the If current and the diastolic depolarisation slope, which then leads to a decrease in the duration of diastole, producing an increase in HR. The opposite occurs when the muscarinic receptors are stimulated: diastolic duration increases resulting in a decrease in HR.

The function of the If current is to determine the slope of the diastolic depolarisation curve towards the threshold level which, consequently controls the time interval between successive action potentials, thereby playing a vital role in the pacemaking process. The f channels
responsible for the $I_f$ current are part of the hyperpolarised-activated, cyclic-nucleotide-gated (HCN) channel family of which four distinct isoforms are found. These isoforms vary in terms of their properties and distribution in the different tissues such as the retina, brain and heart. The HCN4 channel is the isoform found in the heart and is active in the SA node. HCN4 channels found in the Purkinje fibers and the atrioventricular node are inactive under normal physiological conditions, but may operate under pathological conditions such as heart failure.\textsuperscript{62}

**Figure 2.2.2 Action potential of the SA node cells\textsuperscript{68}**

\{s = time interval. In phase 4 of the action potential, there is a slow, gradual depolarisation until threshold is reached (-50mV), and marked depolarisation occurs (refer to blue arrow). This spontaneous membrane depolarisation is accounted for by the $I_f$ current. The orange highlighted area of the graph shows when the $I_f$ current is activated.\}

Inhibition of the $I_f$ current is a valuable pharmacological target of reducing HR and providing a new approach to the treatment of ischaemic heart disease.

**2.2.2 Ivabradine, A Spesific And Selective $I_F$ Inhibitor\textsuperscript{62}**

Ivabradine specifically binds to the f channels on the intracellular side of the plasma membrane of the SA node pacemaker cells and slows the Heart rate. Ivabradine, thus selectively inhibits the $I_f$ current. When $I_f$ current is inhibited, the direct electrophysiological result is the decrease in the slope of the diastolic depolarisation in the SA node cells. This ends in an increased time interval between consecutive action potentials and a decrease in HR both at rest and during exercise in animals and humans.
Ivabradine inhibits the f channel in the open phase when channels deactivate upon depolarisation and can relieved during hyperpolarising in the closing phase, by that means acting as an open channel blocker. The inhibition can dose dependent and appears to be current-dependent, it suggest that ivabradine is more active at increased heart rates. β-blockers, unlike ivabradine, reduce If activation by decreasing sympathetic activity and cAMP formation, which results in a lower HR.

However, Spite contributing to the decrease in myocardial oxygen use, the negative inotropic effects of β-blockers also limit the increases in coronary flow associated with HR lowering. Conversely, If inhibition with ivabradine does not alter myocardial inotropy or coronary vasomotor function, thus supporting cardiac output and coronary flow even during exercise. If inhibition may improve the left ventricular function and ventricular remodelling.

Figure 2.2.3 The I_f channel system
2.2.3 Current pharmacologic options for the treatment of chronic stable angina

The main goals in the treatment of chronic stable angina are improving prognosis by preventing myocardial infarction and/or death and improving the functional status and quality of life of patients by relieving the symptoms of angina. General pharmacologic options to reduce symptoms are betablockers, calcium-channel antagonists and organic nitrates.

(A) Organic Nitrates

1. Introduction

These agents are pro-drugs that are sources of nitric oxide (NO). NO activates the soluble isoform of guanylyl cyclase, and thus increase intracellular levels of cyclic GMP. This promotes the dephosphorylation of the myosin light chain and causes reduction of cystolic (Ca2+) and leads to the relaxation of smooth muscle cells in a broad range of tissues. The NO-dependent relaxation of vascular smooth muscle leads to vasodilation; NO-mediated guanylyl cyclase activation inhibits platelet aggregation. It also relaxes smooth muscle in the bronchi and gastrointestinal (GI) tract.


Nitrites, organic nitrates, nitroso compounds, and a variety of other nitrogen oxide-containing substances lead to the formation of the reactive free radical NO. The exact mechanism(s) of denitration of the organic nitrates to liberate NO remains an active area of investigation. NO can activate guanylyl cyclase, increase the cellular level of cyclic GMP, activate PKG (the cyclic GMP-dependent protein kinase), and modulate the activities of cyclic nucleotide phosphodiesterases (PDEs 2, 3, and 5) in a variety of cell types. In smooth muscle, the net result is reduced phosphorylation of myosin light chain, reduced Ca2+ concentration in the cytosol, and relaxation. One important consequence of the NO-mediated increase in intracellular cyclic GMP is the activation of PKG, which catalyzes the phosphorylation of various proteins in smooth muscle. Another important target of this kinase is the myosin light-chain phosphatase, which is activated on binding PKG and leads to dephosphorylation of the myosin light chain and thereby promotes vasorelaxation. Phosphorylation of the myosin light chain regulates the maintenance of the contractile state in smooth muscle. The pharmacological and biochemical effects of the nitrovasodilators appear to be identical to those of an endothelium-derived relaxing factor now known to be NO. Although the soluble
isoform of guanylyl cyclase remains the most extensively characterized molecular "receptor" for NO, it is increasingly clear that NO also forms specific adducts with thiol groups in proteins and with reduced glutathione to form nitrosothiol compounds with distinctive biological properties. The enzyme mitochondrial aldehyde dehydrogenase has been shown to catalyze the reduction of nitroglycerin to yield bioactive NO metabolites, providing a potentially important clue to the biotransformation of organic nitrates in intact tissues.

3. Mechanism of Relief of Symptoms of Angina Pectoris. The nitrate-induced relief of anginal pain has been assigned to a decrease in cardiac work secondary to the fall in systemic arterial pressure. The ability of nitrates to dilate epicardial coronary arteries, even in areas of atherosclerotic stenosis, is modest. The primary effect of nitrates in chronic stable angina is a reduction in myocardial work, and thus in myocardial oxygen demand as shown by majority of evidence.

In contradiction to the above mechanism, high doses of organic nitrates can reduce blood pressure to such an extent that coronary flow is compromised; reflex tachycardia and adrenergic enhancement of contractility may also occur. These effects can prevail over the beneficial action of the drugs on myocardial oxygen demand and can aggravate ischemia. Also, sublingual nitroglycerin administration may produce bradycardia and hypotension probably due to activation of the Bezold-Jarisch reflex.

(B) CA2+ Channel Antagonists

1. Introduction
Voltage-sensitive Ca2+ channels (L-type or slow channels) bring about the entry of extracellular Ca2+ into smooth muscle and cardiac myocytes and sinoatrial (SA) and atrioventricular (AV) nodal cells in response to electrical depolarization. Ca2+ is a trigger for contraction, in smooth muscle and cardiac myocytes, although by different mechanisms. Ca2+ channel function is inhibited by Ca2+ channel antagonists also known as Ca 2+ entry blockers. Inhibition of Ca2+ channel function causes relaxation in vascular smooth muscle, especially in arterial beds. The negative inotropic and chronotropic effects in the heart can also be produced by these drugs.

2. Mechanisms of Action: An increased concentration of cytosolic Ca2+ causes increased contraction in cardiac and vascular smooth muscle cells. The entry of extracellular Ca2+ is more important in initiating the contraction of cardiac myocytes (Ca2+-induced Ca2+...
The release of Ca2+ from intracellular storage sites also contributes to contraction of vascular smooth muscle, particularly in some vascular beds. Cytosolic Ca2+ concentrations may be increased by various contractile stimuli. Thus many hormones and neurohormones increase Ca2+ influx through so-called receptor-operated channels, whereas high external concentrations of K+ and depolarizing electrical stimuli increase Ca2+ influx through voltage-sensitive, or "potential operated," channels. The Ca2+ channel antagonists produce their effects by binding to the a1 subunit of the L-type Ca2+ channels and reducing Ca2+ flux through the channel.

Voltage-sensitive channels contain domains of homologous sequence that are arranged in tandem within a single large subunit. In addition to the major channel-forming subunit (termed a1), Ca2+ channels contain several other associated subunits (termed a2, b, g, and d). Voltage-sensitive Ca2+ channels have been divided into at least three subtypes based on their conductances and sensitivities to voltage. The channels best characterized to date are the L, N, and T subtypes; P/Q and R channels also have been identified. Only the L-type channel is sensitive to the dihydropyridine Ca2+ channel blockers. Large divalent cations such as Cd2+ and Mn2+ block a wider range of Ca2+ channels. All approved Ca2+ channel blockers bind to the a1 subunit of the L-type Ca2+ channel, which is the main pore-forming unit of the channel. This 200,000- to 250,000-dalton subunit is associated with a disulfide-linked a2d subunit of approximately 140,000 daltons and an intracellular b subunit of 55,000 to 72,000 daltons. The a1 subunits share a common topology of four homologous domains (I, II, III, and IV), each of which is composed of six putative transmembrane segments (S1-S6). The a2d and b subunits modulate the a1 subunit. The phenylalkylamine Ca2+ channel blockers bind to transmembrane segment 6 of domain IV (IVS6), the benzothiazepine Ca2+ channel blockers bind to the cytoplasmic bridge between domain III (IIIS) and domain IV (IVS), and the dihydropyridine Ca2+ channel blockers bind to transmembrane segment of both domain III (IIIS6) and domain IV (IVS6). These three separate receptor sites are linked allosterically.

(C) Beta-Adrenergic Receptor Antagonists

1. Introduction

β-Adrenergic receptor antagonists are effective in reducing the severity and frequency of attacks of exertional angina and in improving survival in patients who have had an MI. In contrast, these agents are not useful for vasospastic angina and, if used in isolation, may worsen the condition. Most β-adrenergic receptor antagonists apparently are equally effective in the treatment of exertional angina. Timolol, metoprolol, atenolol, and propranolol have
been shown to exert cardioprotective effects. The effectiveness of b adrenergic receptor antagonists in the treatment of exertional angina is attributable primarily to a fall in myocardial oxygen consumption at rest and during exertion, although there also is some tendency for increased flow toward ischemic regions.

The decrease in myocardial oxygen consumption is due to a negative chronotropic effect (particularly during exercise), a negative inotropic effect, and a reduction in arterial blood pressure (particularly systolic pressure) during exercise. Not all actions of b adrenergic receptor antagonists are beneficial in all patients.

The decreases in heart rate and contractility cause increases in the systolic ejection period and left ventricular end-diastolic volume; these alterations tend to increase O2 consumption. However, the net effect of b adrenergic receptor blockade is usually to decrease myocardial O2 consumption, particularly during exercise.

Nevertheless, in patients with limited cardiac reserve who are critically dependent on adrenergic stimulation, b adrenergic receptor blockade can result in profound decreases in left ventricular function. Despite this, several b adrenergic receptor antagonists have been shown to reduce mortality in patients with congestive heart failure. Numerous b adrenergic receptor antagonists are approved for clinical use in the United States.

However, the use of these anti-anginal agents is limited by their frequent and sometimes severe side effects:

1. β-blocker side effects include fatigue, depression, cold extremities, symptomatic bradycardia, sexual dysfunction and worsening of respiratory symptoms in asthma and chronic obstructive pulmonary disease.62
2. Calcium-channel blockers may cause peripheral oedema, hypotension, constipation, headache and flushing.62
3. Long-acting nitrates may cause light-headedness or headaches. Prolonged use of nitrates can also result in tolerance to the therapeutic effects and possible rebound vasoconstriction and angina when discontinued.62
4. Although not life-threatening, some mild side effects are common causes for poor treatment compliance with the antianginal medicines. The frequency of use of “combination therapy” to prevent or control angina symptoms also adds to the side effect burden. Adverse effects such as leg oedema, negative inotropy and hypotension frequently account for poor compliance, which in turn may exacerbate heart failure in patients with associated impaired ventricular function. These adverse effects
associated with current anti-anginal treatment together with poor symptom control results in a persistent reduction in quality of life.62

ß-blockers, and to some extent calcium-channel blockers, represent the cornerstone in management of stable angina.

Patient compliance and physician use of these treatments, as well as that of nitrates, may however be limited by contra-indications, development of tolerance or common side effects. Conversely, ß-blockers have a confirmed track record and therefore the risk of adverse effects with ß-blockers should be put into context of their established benefits. Due to the advantages regarding precautions and side-effects, ß-1 selective agents are preferred over non-selective ß-blockers. Chronic obstructive pulmonary disease (COPD) and asthma are relative contraindications to ß-blocker use and caution is advised, but some authors believe that ß-blockers should not necessarily be withheld in patients with mild-to-moderate well-controlled asthma.

Nevertheless, some patients do develop wheezing and bronchospasm with ß-blockers, which then requires dosage decrease or withdrawal of treatment.

In view of these side effects, a new pharmacological target was sought as a mechanism of lowering HR.

In view of the current data on the efficacy and safety of ivabradine, there appears to be an important clinical role for the drug in patients with chronic stable angina. In these patients, ivabradine markedly improves all exercise tolerance test parameters and significantly decreases the number of angina attacks.71, 72, 73
2.2.4 Ivabradine Hydrochloride

(A) Chemical Name

3-((3-{[(7S)-3,4-Dimethoxybicyclo[4,2,0]octa-1,3,5-trien-7-yl] methyl} methylamino) propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one, hydrochloride.

(B) Molecular formula

C_{27}H_{36}N_{2}O_{5}, HCl;

(C) Molecular weight (hydrochloride): 505.05

(D) Chemical Structure

![Chemical Structure Image]

(E) Indications

Treatment of coronary artery disease
Symptomatic treatment of chronic stable angina pectoris in coronary artery disease adults with normal sinus rhythm. Ivabradine is indicated:
- in adults unable to tolerate or with a contra-indication to the use of beta-blockers
- or in combination with beta-blockers in patients inadequately controlled with an optimal beta-blocker dose and whose heart rate is > 60 bpm.

Treatment of chronic heart failure
Ivabradine is indicated in chronic heart failure NYHA II to IV class with systolic dysfunction, in patients in sinus rhythm and whose heart rate is ≥ 75 bpm, in combination with standard therapy including beta-blocker therapy or when beta-blocker therapy is contraindicated or not tolerated.
(F) **Dosage and Method of Administration**

**Treatment of coronary artery disease**

The usual recommended starting dose of ivabradine is 5mg twice daily. After three to four weeks of treatment, the dose may be increased to 7.5mg twice daily depending on the therapeutic response. If, during treatment, heart rate decreases persistently below 50 beats per minute (bpm) at rest or the patient experiences symptoms related to bradycardia such as dizziness, fatigue or hypotension, the dose must be titrated downward including the possible dose of 2.5mg twice daily (one half 5mg tablet twice daily). Treatment must be discontinued if heart rate below 50 bpm or symptoms of bradycardia persist.

**Treatment of chronic heart failure**

The treatment has to be initiated only in patient with stable heart failure. It is recommended that the treating physician should be experienced in the management of chronic heart failure. The usual recommended starting dose of ivabradine is 5mg twice daily. After two weeks of treatment, the dose can be increased to 7.5mg twice daily if resting heart rate is persistently above 60 bpm or decreased to 2.5mg twice daily (one half 5 mg tablet twice daily) if resting heart rate is persistently below 50 bpm or in case of symptoms related to bradycardia such as dizziness, fatigue or hypotension. If heart rate is between 50 and 60 bpm, the dose of 5mg twice daily should be maintained.

If during treatment, heart rate decreases persistently below 50 beats per minute (bpm) at rest or the patient experiences symptoms related to bradycardia, the dose must be titrated downward to the next lower dose in patients receiving 7.5mg twice daily or 5 mg twice daily.

If heart rate increases persistently above 60 beats per minute at rest, the dose can be up titrated to the next upper dose in patients receiving 2.5 mg twice daily or 5mg twice daily.

Treatment must be discontinued if heart rate remains below 50 bpm or symptoms of bradycardia persist.

**Special population**

**Elderly**

In patients aged 75 years or more, a lower starting dose should be considered for these patients (2.5mg twice daily i.e. one half 5mg tablet twice daily) before up-titration if necessary.
Chapter 2.2

Review of Literature - Ivabradine

Renal impairment
No dose adjustment is required in patients with renal insufficiency and creatinine clearance above 15 ml/min.

No data are available in patients with creatinine clearance below 15 ml/min. Ivabradine should therefore be used with precaution in this population.

Hepatic impairment
No dose adjustment is required in patients with mild hepatic impairment. Caution should be exercised when using ivabradine in patients with moderate hepatic impairment. Ivabradine is contra-indicated for use in patients with severe hepatic insufficiency, since it has not been studied in this population and a large increase in systemic exposure is anticipated.

Paediatric population
The safety and efficacy of ivabradine in children aged below 18 years have not yet been established.

No data are available.

Method of administration
Tablets must be taken orally twice daily, i.e. once in the morning and once in the evening during meals.

(G) Mechanism of action
Ivabradine is a pure heart rate lowering agent, acting by selective and specific inhibition of the cardiac pacemaker \( I_f \) current that controls the spontaneous diastolic depolarisation in the sinus node and regulates heart rate. The cardiac effects are specific to the sinus node with no effect on intra-atrial, atrioventricular or intraventricular conduction times, nor on myocardial contractility or ventricular repolarisation.

Ivabradine can interact also with the retinal current \( I_h \) which closely resembles cardiac \( I_f \). It participates in the temporal resolution of the visual system, by curtailing the retinal response to bright light stimuli. Under triggering circumstances (e.g. rapid changes in luminosity), partial inhibition of \( I_h \) by ivabradine underlies the luminous phenomena that may be occasionally experienced by patients. Luminous phenomena (phosphenes) are described as a transient enhanced brightness in a limited area of the visual field.
(H) Pharmacokinetics

1. Absorption and bioavailability
Ivabradine is rapidly and almost completely absorbed after oral administration with a peak plasma level reached in about 1 hour under fasting condition. The absolute bioavailability of the film-coated tablets is around 40%, due to first-pass effect in the gut and liver.
Food delayed absorption by approximately 1 hour, and increased plasma exposure by 20 to 30%. The intake of the tablet during meals is recommended in order to decrease intra-individual variability in exposure.

2. Distribution
Ivabradine is approximately 70% plasma protein bound and the volume of distribution at steady state is close to 100 l in patients. The maximum plasma concentration following chronic administration at the recommended dose of 5 mg twice daily is 22 ng/ml (CV=29%). The average plasma concentration is 10 ng/ml (CV=38%) at steady state.

3. Biotransformation
Ivabradine is extensively metabolised by the liver and the gut by oxidation through cytochrome P450 3A4 (CYP3A4) only. The major active metabolite is the N-desmethylated derivative (S 18982) with an exposure about 40% of that of the parent compound. The metabolism of this active metabolite also involves CYP3A4. Ivabradine has low affinity for CYP3A4, shows no clinically relevant CYP3A4 induction or inhibition and is therefore unlikely to modify CYP3A4 substrate metabolism or plasma concentrations. Inversely, potent inhibitors and inducers may substantially affect ivabradine plasma concentrations.

4. Elimination
Ivabradine is eliminated with a main half-life of 2 hours (70-75% of the AUC) in plasma and an effective half-life of 11 hours. The total clearance is about 400 ml/min and the renal clearance is about 70 ml/min. Excretion of metabolites occurs to a similar extent via faeces and urine. About 4% of an oral dose is excreted unchanged in urine.

5. Linearity/non linearity
The kinetics of ivabradine is linear over an oral dose range of 0.5 – 24 mg.
6. Special populations

**Elderly**: no pharmacokinetic differences (AUC and Cmax) have been observed between elderly (≥ 65 years) or very elderly patients (≥ 75 years) and the overall population.

**Renal insufficiency**: the impact of renal impairment (creatinine clearance from 15 to 60 ml/min) on ivabradine pharmacokinetic is minimal, in relation with the low contribution of renal clearance (about 20 %) to total elimination for both ivabradine and its main metabolite S 18982.

**Hepatic impairment**: in patients with mild hepatic impairment (Child Pugh score up to 7) unbound AUC of ivabradine and the main active metabolite were about 20% higher than in subjects with normal hepatic function. Data are insufficient to draw conclusions in patients with moderate hepatic impairment. No data are available in patients with severe hepatic impairment.

(I) Cardiovascular Effects of Ivabradine

1. **Coronary Blood Flow**
   
   
   If inhibition with ivabradine does not directly interfere with coronary vasomotion because the current is not present on vascular smooth cells unlike beta - blockers (they decrease the diameter of large coronary arteries and blood flow through the combined blockage of vascular β receptors and unopposed α vasomotor tone). Furthermore, ivabradine promotes a redistribution of transmural coronary blood flow by limiting the reduction in subendocardial flow during myocardial ischaemia; this effect is due especially to the reduction in heart rate and to the prolongation of diastolic perfusion time of the coronary vascular bed. There is, also an important difference between ivabradine and beta -blockers on diastolic perfusion time because Ivabradine can reduce heart rate without significant changes in myocardial inotropy whereas beta-blockers cannot.

2. **Heart Rate Reduction**

   Ivabradine reduces heart rate in a dose dependent manner and it is independent of the pathophysiological status, since this heart rate slowing effect remains unchanged in animals or humans with reduced myocardial contractility or relaxation, such as in congestive heart failure. Because If channels are activated by a use dependent mechanism by hyperpolarization and by direct binding of cAMP, the reduction in heart rate induced by ivabradine is more marked at higher than at lower heart rates. This reduction, as showed by Vilaine, is limited to
18%-20% of basal heart rate either during rest or exercise even thought ivabradine reduced heart rate in a dose-dependent manner when it is given in the range of therapeutic dose. Furthermore, ivabradine can reduce heart rate in patients in whom beta-blockers are ineffective and this reduction is an additive effect when concomitantly administered with beta-blockers, at least when a residual sympathetic tone is still significant in these patients. Ivabradine does not induce a significant prolongation of the QT interval and does not modify the PR interval at doses that clearly reduce heart rate.

3. Myocardial Ischaemia, Myocardial Infarction and Endothelial Dysfunction

Monnet in an experimental animal model of myocardial ischaemia, induced by a combination of a coronary stenosis and physical exercise on treadmill, showed that, even beta blocker (atenolol) an ivabradine at doses that reduced heart rate, redistributed myocardial flow towards the subendocardium and reduced myocardial dysfunction during the ischemic time; nevertheless while atenolol worsened myocardial stunning during the recovery period, ivabradine decreased the severity and duration of stunning in the same conditions. The main difference between these two drugs was related to the β1 adrenergic blockade - mediated negative inotropic effect of atenolol. On the contrary ivabradine reduces myocardial stunning through its ability to reduce heart rate selectivity as well as its inability to alter myocardial inotropy.

An important effect of reduction in heart rate on myocardial structure is the effect of ivabradine on prevention of loss of coronary vessels within the remaining viable part of the failing myocardium, a property that is probably closely linked to the observed angiogenesis in healthy animals receiving long - term treatment with a drug that decreases heart rate [32]. Mulder showed that a mechanism involved in this prevention of coronary rarefaction could be the augmented levels of hypoxia - inducible factor 1α and associated growth factors through the increase in left ventricular diastolic diameter and thus in myocardial stretch, which is a trigger for multiple factors involved in angiogenesis. This effect of long - term heart rate reduction with ivabradine can preserve (or improve) the coronary reserve associated with a decrease in perivascular collagen in the surviving myocardium thus preventing the progressive degradation of the cardiac function in heart failure. Furthermore ivabradine, different from beta-blockade, has the unique property of not only improving ischaemic and postischemic regional myocardial function but also reversing post-ejection wall thickening (a typical sign of asynchrony of ventricular contraction and relaxation) to wall thickening during ejection and thus making this contraction available for cardiac output.