REVIEW OF LITERATURE
PEPTIC ULCER

A peptic ulcer is a mucosal lesion of the stomach or duodenum in which the acid and pepsin play major pathogenic roles. The major forms of peptic ulcer are gastric ulcer and duodenal ulcer, both of which are chronic diseases often caused by Helicobacter pylori. The term peptic ulcer also encompasses gastric ulcers and duodenal ulcers associated with stress or the ingestion of drugs, most commonly aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Ulcer associated with Zollinger-Ellison Syndrome (ZES), caused by gastrin secreting islet cell tumors is also considered a form of peptic ulcer. Whether an ulcer develops, depends on the balance between aggressive factors (principally gastric acid and pepsin) and factors that participate in mucosal defense or resistance to ulceration. Peptic ulcer develops when gastroduodenal mucosal defenses are unable to protect the epithelium from the corrosive effects of acid and pepsin. The proteolytic effects of pepsin in concert with the corrosive properties of secreted gastric acid contribute to the tissue injury that produces peptic ulcer. Gastric acid catalyses the cleavage of inactive pepsinogen molecules to proteolytically active pepsins and also provides the low pH for pepsin activity.

EPIDEMIOLOGY

Peptic ulcers are remitting, relapsing lesions that are most often diagnosed in middle-aged to older adults, but they may first become evident in young adult life. They may often appear without obvious precipitating influences and may then, after a period of weeks to months of active disease, heal with or without therapy. Even with healing, however, the propensity to develop peptic ulcers remains. Hence “once a peptic ulcer patient, always a peptic ulcer patient”. Thus it is difficult to obtain accurate data on the prevalence of active disease. Based on autopsy studies and population surveys, the best estimate indicates a prevalence of 6 to 14% for men and 2 to 6% for women. The male to female ratio for duodenal ulcers is about 3:1 and for gastric ulcers about 1:5 to 1:2. Women are affected most often at or after menopause.
It is now well established that *Helicobacter pylori* is a major acquired factor in the pathogenesis of duodenal ulcer disease. The infection is found in more than 95% of patients with duodenal ulcer and numerous studies show that eradicating it markedly decreases the ulcer relapse\textsuperscript{41-44}. Patients with duodenal ulcer have the following abnormalities of acid secretion\textsuperscript{45}:

1) threefold increase in basal acid output  
2) six fold increase in acid response to gastrin releasing peptide  
3) increased maximal acid response to exogenous gastrin  
4) increased ratio of basal acid output to maximal gastrin stimulated output and  
5) increased ratio of maximal gastrin releasing peptide-stimulated output to maximal gastrin stimulated output.

**GASTRIC ACID SECRETION**

**Regulation of gastric acid secretion:**

1) *Central regulation:*

Pavlov\textsuperscript{46} established the concept that the CNS participates in the initiation of acid secretion. Although it is generally recognized that the sight, smell, taste or thought of food can stimulate acid secretion, it is well appreciated that the strongest central stimulus is hypoglycemia\textsuperscript{47}.

Central structures identified as key participants in the regulatory process include the dorsal medullary nucleus of the vagus (DMX) and the hypothalamic nucleus tractus solitarius (NTS). The final integration of central stimuli appears to occur in the DMX, which supplies stimulatory efferent fibres to the stomach via the vagus nerve\textsuperscript{48,49}. Vagus efferents to the stomach arise also from the nucleus ambiguus (NA), but these appear to be primarily involved in regulating motility rather than secretion\textsuperscript{50}. Destruction of DMX eliminates central stimulation of acid secretion\textsuperscript{51}, whereas electrical stimulation of the DMX results in a strong secretory response\textsuperscript{52,53}. The DMX does not appear to initiate
stimulation itself but integrates central sensory input arising primarily from the hypothalamus and visceral sensory input from NTS\textsuperscript{54}.

Several sites in the hypothalamus have been identified as having potential stimulatory and inhibitory influences on acid secretion. The ventromedial hypothalamus (VMH) appears to exert a tonic inhibitory influence, since destruction of the VMH enhances secretion\textsuperscript{55}, while electrical stimulation of VMH suppresses secretion\textsuperscript{56}. The influence of the VMH is exerted indirectly by inhibiting the stimulatory signals arising from the lateral hypothalamus (LH) and adjacent medial forebrain bundle (MFB). The LH and MFB serve as the primary structures responsible for glucoprival stimulation of the acid secretion\textsuperscript{57}. Inhibition of secretory responses to administration of 2-deoxyglucose by electrical stimulation of the VMH\textsuperscript{58} thus would be mediated through LH and MFB. Both direct and indirect connections from LH and MFB to DMX have been identified\textsuperscript{59}.

The NTS also has been found to respond directly the glucose deprivation\textsuperscript{54}, with a strong stimulation of acid secretion mediated through DMX. In addition to a glucoprival response, the NTS receives major inputs from taste fibres and visceral afferents. The former is probably responsible for initiating acid secretion due to taste of food. Visceral afferents inputs to the NTS arise primarily from synapses in the inferior ganglion of the vagus. It is noteworthy that more than 95\% of the fibres in the vagus nerve are afferent rather than efferent\textsuperscript{60}. The abundance of sensory input to the CNS is critical to the continuous central regulation of gastric function and integration of CNS and peripheral mechanisms.

Sensory information from the stomach is relayed to the CNS by both vagus afferent fibres and sympathetic afferent fibres\textsuperscript{61}. The sensory receptors of the stomach consist primarily of unmyelinated nerve endings that detect mechanical, e.g., touch and distension, chemical and thermal stimuli.

Vagal afferent fibres are found in the smooth muscle layers and the mucosa of the stomach. The receptors in the muscle layers are primarily tension or stretch receptors
capable of detecting motility changes. Although these receptors detect and regulate motility of the muscle layers, they are also involved in the vasovagal reflexes associated with distension-dependent secretory activity, an important element in the peripheral regulation of acid secretion.

II) Peripheral regulation:

Mechanisms intrinsic to stomach are itself capable of initiating and regulating acid secretion. These peripheral mechanisms include neural, hormonal, paracrine and autocrine elements.

a) Acetylcholine release:

The only known source of acetylcholine (ACh) that can act directly on the parietal cell is from the postganglionic nerve fibres of the enteric nervous system. Accordingly, the regulation of ACh release depends on the synaptic connections and transmitters controlling enteric nerve activation. In addition to the direct stimulation of parietal cells, ACh release from enteric nerve fibres serves to regulate the activity of several endocrine cell types and thus exerts an indirect influence on the parietal cell. The efferent fibres of the vagus nerve do not innervate the parietal cells directly but can synapse with ganglion cells of the enteric nervous system.

There is great numerical disparity between efferent vagal fibres and ganglia of the enteric nervous system. Some 200 vagal fibres synapse with an estimated 10 million ganglia, supports the concept that the CNS serves to modulate enteric nervous system regulatory mechanisms rather than exerting any direct control on the parietal cell function. Essentially all of the cell bodies of enteric neurons are found in the two plexuses, the myenteric (Auerbach’s) plexuses and submucosal (Meissner’s) plexus.
b) Histamine release:

Initially it was proposed that histamine is released from mucosal mast cells\textsuperscript{64}, but later it was proposed that gastric histamine is released from specialized endocrine cell of the stomach, the ECL cell\textsuperscript{65}. The release of histamine from the ECL cell is regulated by a complex set of mechanisms involving neurocrine, endocrine, paracrine and autocrine pathways. Figure 1A and 1B presents a simplified version of the known mechanisms involved in regulating the ECL cell.
Figure 1A: Model for regulation of enterochromaffin-like (ECL) cell function. Illustrated are ECL cell (E), fundic D cell (D), active pumps (P), and some innervation pathways. Both ECL and parietal cell are shown having cholecystokinin (CCK)-B receptors, and D cell is shown as having probably a calcitonin gene-related peptide (CGRP) receptor. Muscarinic receptor on parietal cell is an M₁ receptor, whereas muscarinic receptor on ECL is unknown subtype (M?). ECL cell has an inhibitory H₁ receptor, whereas parietal cell has an stimulatory H₂ receptor. Also ECL is stimulated to release histamine by β-adrenergic innervation mediated by cAMP and has an inhibitory ST-2 somatostatin receptor.
Figure 1B: Regulation of G-cell function. Shown are G-cell and antral D cell, where food stimulates release of gastrin from G-cell and acid inhibits G-cell function and stimulates release of somatostatin (SS) from D-cell. Stimulation of G cell also depends on the release of gastrin releasing peptide (GRP) and acetylcholine (ACh) from innervation of this region of the stomach. Muscarinic receptor on D cell is to inhibit somatostatin release presumably via an M₂ or M₁ subtype, whereas ACh stimulates release of gastrin.
c) Gastrin release: Before the recognition of ECL cells as intermediate for gastrin stimulation of acid secretion, it was widely held that gastrin was the major peripheral regulator of acid secretion, and much attention was focussed on the mechanisms regulating gastrin release.

i) Antral \textit{G} Cells: In the adult, gastrin is found primarily in the \textit{G} cells of the gastric antrum and duodenum with small amounts located in the pituitary and some vagal nerve fibres. The fetal and neonatal pancreas produces gastrin, and this may be the source of neonatal hypergastremia. The predominant form of gastrin found in the circulation is a heptadecapeptide.

ii) Regulation of the \textit{G} cell: Physiologically gastrin is released from the antral \textit{G} cells by the presence of food in the stomach. At least three stimulatory pathways, associated with ingestion of food, have been shown to be involved in the release of gastrin, central neural activation, distension of the antrum, and specific chemical components of the food. At the cellular level these pathways regulate release of gastrin through the actions of ACh, gastrin releasing peptide (GRP), somatostatin (SS) and direct chemical effects of H\textsuperscript{+} and amino acids. Additional cellular effectors such as adenosine, galanin, and epinephrine have been postulated on the basis of \textit{in vitro} studies, but their physiological significance remains uncertain. Gastrin is released in response to oropharyngeal and central stimuli via the vagus nerve. Accordingly, gastrin release resulting from either sham feeding or hypoglycemia is abolished by selective antral vagotomy. Paradoxically, selective fundic vagotomy leads to an enhanced vagal release of gastrin, suggesting that the vagus initiates an inhibitory action by fundus. Both the antral release and the fundic inhibitory reflex are inhibited by atropine indicating that muscarinic synapses are involved in both the pathways.

d) Control of gastric acidity:

The interaction of the three gastric endocrine cell, ECL cell, \textit{G} cell, and \textit{D} cell, serves to regulate the release of histamine. Histamine together with ACh, and possibly gastrin, in
turn activates the parietal cell to secrete acid. Despite the obvious complexity of the mechanisms involved, the overall goal of these processes is to regulate the acidity of the gastric contents. The implication of the goal is that the regulatory mechanisms must be able to detect the intragastric pH and respond appropriately. The only known mechanism corresponding to this requirement is the suppression of gastrin release by pH < 3.0°.

III) Cellular regulation:

a) Parietal cell receptors: The parietal cell appears to possess a variety of receptors for both stimulatory and inhibitory modulators. The majority of these receptors have been defined only on the basis that specific ligands affect parietal cell function.

i) Histamine receptor: The role of histamine in stimulating acid secretion has been one of the more controversial topics in this field, since the development of selective antagonists for the H₂ histamine receptor77. However, overwhelming evidence has accumulated to identify histamine as the most important stimulus of the parietal cell. Histamine is a potent and efficacious stimulus for acid secretion in vivo78 and in vitro79-82. The stimulatory action of histamine mediated through the H₂ receptor is well established79,81-85. Histamine stimulation of acid secretion is inhibited competitively by selective H₂ receptor antagonists but is not inhibited by agents acting on other receptors types, e.g., cholinergic or adrenergic agents indicating that histamine acts directly on the parietal cell. In contrast, H₂ receptor antagonists inhibit at least partly acid secretion stimulated by cholinergic agents and gastrin, suggesting that these secretagogues act in part through histamine86,87.

ii) Acetylcholine receptor: The non-selective cholinergic antagonist atropine is well known for its ability to inhibit acid secretion in vivo62. Indeed, extracts of Belladonna had been used to treat dyspepsia since the Roman Empire, and its major component, atropine was a primary medical treatment for peptic ulcer before development of H₂ receptor antagonists. Because there are multiple sites for the action of atropine within the peripheral regulatory pathways, it is not possible, based solely on in vivo inhibition to
conclude that the parietal cell contains a cholinergic receptor. The observation that H₂ receptor antagonists inhibit most, but not all, of the cholinergic stimulation of acid secretion¹⁶,¹⁷ suggested that the action of ACh both in vivo and in vitro may be indirect.

*In vitro*, particularly in the presence of H₂ receptor antagonists, cholinergic stimulation of acid secretion is weak and often transient¹⁸. Portion of the cholinergic stimulation which is not blocked by H₂ receptor antagonist is blocked by atropine⁷⁶. These results suggest that there is some direct action of ACh on the parietal cell in addition to the observed interaction with the histamine. Pharmacological characterization of cholinergic stimulation of acid secretion *in vitro* indicates that the parietal cell contains M₃ subtype muscarinic receptor⁸⁹,⁹⁰.

iii) *Gastrin receptor*: The presence of a receptor for gastrin on the parietal cell has been the subject of considerable controversy. The central point in this controversy is the total absence of marginal stimulation of acid secretion by gastrin in presence of H₂ receptor antagonists⁹¹,⁹². This has led most investigators to conclude that gastrin does not act directly on the parietal cell but stimulates secretion only through the release of histamine from ECL cells⁹³. Favoring a direct action of gastrin on the parietal cell, are reports indicating the presence of gastrin binding sites on the gastric mucosal membranes⁹³ and enriched preparations of parietal cells⁹⁴. These binding sites are characterized as gastrin type, or CCK-B sites in that they show equal affinity for gastrin and sulfated CCK.

iv) *Miscellaneous receptors*: A variety of agents have been reported to stimulate or inhibit acid secretion by a direct action on parietal cell. Many of these e.g., cAMP derivatives as well as forskolin, do not act through cellular receptors while others are suggested to reduce the presence of parietal cell receptors. Although no direct evidence for action on parietal cell exists, the ability of some substances to inhibit histamine stimulation of acid secretion, a direct action on the parietal cell argues for the presence of parietal cell receptor. Even in these cases caution is necessary to interpret the inhibition as being direct rather than due to the release of other inhibitors. With this caveat in mind, the parietal cell appears to contain receptors for SS⁹⁵,⁹⁶, prostaglandin⁹⁷-¹⁰⁰, and epidermal
growth factor (EGF)\textsuperscript{101}. Each of these has been shown to inhibit histamine stimulation of acid formation in isolated cell preparations.

**GASTRIC CYTOPROTECTION**

The term cytoprotection was first introduced by Robert\textsuperscript{102} in 1979. He used this term to refer protection by prostaglandins against experimentally induced acute gastric lesions, in doses, which do not affect gastric secretion in rats. Now the term ‘cytoprotection’ is used in a broader sense to mean protection against gastric mucosal injury by a mechanism other than inhibition or neutralization of gastric acid.

**Mechanism Of Cytoprotection:**

Though the concept of cytoprotection has come to stay, there are diverse opinions to the exact mode of action of cytoprotective agents. Various mechanisms have been suggested:

1. *Increase in mucus secretion:* The gastric mucus barrier is an important protective factor against gastric and duodenal diseases\textsuperscript{103}. The mucus gel structure in patients with gastric ulcer has been found to be abnormal in that it contains less glycoprotein\textsuperscript{104} and several cytoprotective agents have been shown to increase mucus gel thickness like carbenoxolone and prostaglandins\textsuperscript{105}. The gastric mucus gel thickness is also affected by *Helicobacter pylori* infection\textsuperscript{106}. Morris *et al*\textsuperscript{107} have demonstrated by electron microscopy that the unstressed rat gastric mucosa is only partially covered by an interconnected but discontinuous layer of mucus ‘ropes’ ‘sheets’ and ‘mats’ thus allowing ulcerogenic agents direct access to surface epithelial cells. This finding also goes against the generally accepted protective role of mucus. However, several studies have suggested that mucus may play an important role in protecting the mucosa after the initial insult by providing a thick ‘cap’ over the rapidly migrating epithelial cells favoring a rapid reepithelialization of the mucosa\textsuperscript{108}.
2. Increase in bicarbonate secretion: Flemstrom\textsuperscript{109} first demonstrated the existence of bicarbonate secretion from fundic and antral mucosa. Vagal stimulation increases both acid and alkali secretion. This ‘alkaline tide’ during hydrogen ion secretion increases bicarbonate delivery to the surface epithelium. However, the rate of bicarbonate secretion is only 5 to 10\% of the maximal acid output\textsuperscript{110}. Thus bicarbonate alone cannot lower sufficiently the hydrogen ion concentration but it can complement the action of mucus, forming what is known as the ‘mucus-bicarbonate barrier’\textsuperscript{111}. It has been seen that in duodenal ulcer, there is a defective bicarbonate response to an acid load\textsuperscript{112}. However, though some prostaglandins cause an increase in bicarbonate secretion\textsuperscript{113}, other cytoprotective prostaglandins do not\textsuperscript{114}, thus casting doubts on the importance of bicarbonate secretion as a mechanism of cytoprotection.

3. Strengthening of the gastric mucosal barrier: Many studies have provided evidence that surface epithelial cells have intrinsic barrier properties and play an important role in the first line defense of the stomach. Davenport \textit{et al}\textsuperscript{115} proposed that the apical membrane or tight junctions between epithelial cells are relatively impermeable to hydrogen ions and therefore form a physical barrier to back diffusion of acid. They called this the ‘gastric mucosal barrier’. Few studies\textsuperscript{116} have shown the existence of surface active phospholipids which form a hydrophobic lining on the luminal surface of the gastric epithelium and retard the passage of water soluble ions such as hydrogen ions. NSAIDs have been shown to eliminate hydrophobicity and disrupt the mucosal barrier to hydrogen ions. On the other hand, cytoprotective agents like prostaglandins increase the concentration of surface-active phospholipids\textsuperscript{117}.

4. Increase in mucosal blood flow: Several studies have demonstrated that vascular injury to subepithelial capillaries with an increased vascular permeability and circulatory stasis is an early pathogenic factor in experimental gastric lesion. These changes lead to functional impairment of gastric microcirculation, the decrease in mucosal blood flow correlating with the extent of haemorrhagic erosions\textsuperscript{118}. Increase in mucosal blood flow has been shown to protect against mucosal damage\textsuperscript{119}. The mucosal microcirculation is extremely important in maintaining oxygenation and supplying nutrients. The anatomical
design of the gastric vasculature is such that the 'alkaline tide' from secreting oxyntic cells is readily available to the basal aspect of surface epithelial cells\textsuperscript{20}. Thus if blood flow is adequate there can be an almost unlimited supply of bicarbonate neutralization of back diffused hydrogen ions. In addition enhanced blood flow ensures that the absorbed injurious agent is diluted with subepithelial capillaries. However, some studies raise doubts about the importance of mucosal blood flow in cytoprotection. For example PGE\textsubscript{2a} a vasoconstrictor has been shown to exert a gastric cytoprotective effect similar to that of the vasodilator PGE\textsubscript{2}\textsuperscript{102}. Further agents like histamine and ACh have been shown to increase gastric mucosal blood flow and yet cause gastric ulceration\textsuperscript{121}. Some studies have shown no correlation between gastric cytoprotection and blood flow. For example the ACE inhibitor captopril which is known to increase gastric mucosal blood flow\textsuperscript{122} does not affect ethanol induced gastric lesions\textsuperscript{123} while the nonselective \(\beta\)-antagonist propranolol which decreases blood flow\textsuperscript{124} has a marked gastroprotective effect\textsuperscript{125}.

5. Decrease in gastric motility: Various studies have suggested that changes in gastric motility may play a role in the development and the prevention of experimental gastric lesions\textsuperscript{126}. It has been consistently observed that gastric injury caused by necrotizing agents occurs as band-like lesions, at the crest of mucosal folds and is preceded by violent gastric contractions. As the lesions occur at the site of the greatest mechanical stress, mucosal compression by gastric hypercontraction probably accounts for necrosis and ulceration of epithelium\textsuperscript{126}. As the formation of mucosal folds relates closely to muscle action, especially circular muscle, an inhibiting effect on gastric motility may protect the gastric mucosa through flattening of the folds. This will lead to an increase in the mucosal surface area exposed to ulcerogens and thereby reduce the volume of irritant on the specific site of the mucosa (rugal crests). Studies using prostaglandins, mast cell stabilizers and sulphahydryl compounds have confirmed that inhibition of gastric motility is associated with their cytoprotective action in the rat\textsuperscript{127}. 
6. Increased release of endogenous mediators of gastric cytoprotection:

a) Prostaglandins: Prostaglandins were the first endogenous compounds implicated in gastric cytoprotection. The importance of endogenous prostaglandins in mucosal defense mechanism is evident from the observation that NSAIDs damage gastric mucosa. Since prostaglandins increase mucosal blood flow\textsuperscript{128} this has been suggested to be responsible for their gastroprotective effect. However various other mechanisms have also been postulated like dilution of noxious agent by prostaglandin stimulated mucus secretion\textsuperscript{129}, stimulation of basal bicarbonate secretion\textsuperscript{130}, increase in the concentration of surface active phospholipids\textsuperscript{118}, stimulation of cAMP\textsuperscript{131}, stabilization of lysosomes\textsuperscript{132}, decrease in gastric motility and dissolution of gastric mucosal folds\textsuperscript{126} and maintenance of mucosal sulphahydryl groups\textsuperscript{133}. Prostaglandins probably also have repair function by stimulating rapid resolution of disrupted surface epithelium, although prostaglandins are major mediators for the action of many drugs, few reports indicate that gastroprotection can also occur in the absence of prostaglandins\textsuperscript{134}. It has been shown that prior exposure of gastric mucosa to mild irritants protect its damage by more noxious agents. This adaptive cytoprotection is mediated by prostaglandins\textsuperscript{135}.

b) Sulphahydryls: Szabo et al\textsuperscript{133} observed that the naturally occurring sulphahydryl (SH)-containing amino acids, L-cysteine and methionine as well as sulphahydryl containing drugs protect rats from ethanol induced gastric lesions whereas sulphahydryl blocking drugs counteract the cytoprotective effect of PGE\textsubscript{2}. They proposed that endogenous sulphahydryls might be one of the mediators of cytoprotection. Various mechanisms have been suggested: Synthesis of prostaglandins as well as prostaglandin receptor action is dependent on endogenous sulphahydryls\textsuperscript{136}. In addition, by influencing membrane permeability or production of free radicals they may be indirectly involved in mucosal defense\textsuperscript{137}. On the other hand, Robert et al\textsuperscript{138} reported that depletion of endogenous sulphahydryls paradoxically had gastric protective effect.

c) Epidermal growth factor: This polypeptide, a potent inhibitor of acid secretion, is found in salivary glands as well as other sources like duodenal mucosa and pancreas\textsuperscript{139}. 

Perhaps its effect is mediated through endogenous sulphahydryl group rather than prostaglandins or alkali secretion\(^{137}\). In addition, other studies\(^{140}\) have shown its efficacy in preventing stress induced ulcers and in healing chronic duodenal ulcers in rats.

7. *Scavenging of free radicals*: The involvement of oxygen-derived free radicals, specially the superoxide radical in ischaemic gastric mucosal damage has been suggested but the exact mechanism is not yet defined. Probably free radicals result in lipid peroxidation and damage to intracellular components\(^{141}\). Antioxidants like vitamin E and selenium have been shown to have a protective effect on the gastric mucosa against stress and chemically induced lesions\(^{142,143}\).

8. *Decreased release of endogenous mediators of gastric injury*: It has been shown that at least part of the injurious action of ethanol on gastric microcirculation is due to the release of mediators. Mast cell stabilizers like disodium cromoglycate and doxantrazol and \(\mathrm{H}_1\) receptor antagonists decrease ethanol induced haemorrhagic mucosal damage\(^{144}\). Further, ethanol induced gastric lesions are also less in mice genetically deficient in mast cells\(^{145}\). In addition to mast cells and vasoactive amines, leukotrienes have been proposed as endogenous mediators of acute gastric mucosal damage. Leukotrienes have been shown to induce gastric vasoconstriction\(^{146}\) and to increase vascular permeability\(^{147}\). Mucosal levels of leukotrienes are increased after exposure to ethanol\(^{148}\). In addition, inhibition of \(\mathrm{LTC}_4\) and \(\mathrm{LTD}_4\) in the gastric mucosa protects against damage by noxious agents.

Since the two products of arachidonic acid pathway prostaglandins and leukotrienes have opposite effect on gastric mucosa, it is possible that the balance between production of prostaglandins and leukotrienes may play an important role in mucosal integrity. There is experimental evidence to indicate that decreased synthesis of leukotrienes may be more significant as compared to increased levels of prostaglandins\(^{149}\).

9. *Stimulation of cellular repair and growth*: It is well established that rapid epithelial resolution of the damaged mucosal surface takes place by migration of cells from deep
within the gastric pits, which recover the denuded basal lamina\textsuperscript{150}. Following injury with agents like ethanol, aspirin and hypertonic saline, mucosal reepithelization occurs within as short a time as 30 minutes\textsuperscript{151}. It should be noted that an intact basal lamina is vital for the cells to migrate during this repair process. The integrity of the basal lamina is maintained by a medium with high pH\textsuperscript{152}. On the other hand, if the luminal pH is low (acid) reepithelization is hampered\textsuperscript{153}.

To conclude, different mechanisms have been proposed for gastric cytoprotection, their relative importance and interdependence are not very clear, indicating that gastric cytoprotection may be multifactorial phenomenon.
PROLACTIN

HISTORICAL PERSPECTIVE

Among the hormones of the anterior pituitary, PRL with more than 185 functions documented among various vertebrate species, is by far the most versatile. PRL was first discovered in 1928, based upon its ability to cause lactation in pseudopregnant rabbits. The suckling induced release of PRL, a universal response in mammals, has emerged as a classic experimental model for the study of neuroendocrine interactions. In the late 1980's, the cloning and characterization of Pit-1 as a tissue specific transactivator of PRL gene transcription enhanced the understanding of pituitary cell development and PRL gene regulation.

BIOSYNTHESIS

1) Pituitary PRL

The capacity of the pituitary to synthesize hormones predates histological differentiation of the pituitary cells into lactotrophs (PRL secreting cells) and somatotrophs (growth hormone secreting cells). In all species studied, growth hormone (GH) secretion predates PRL secretion. Data have accumulated supporting the concept that GH and PRL secreting cells present in the adult animal may be inter convertible. This has been suggested by the observation of marked increase in dual secreting mammosomatotrophs in male rat pituitaries exposed to estrogen associated with a commensurate decrease in GH-secreting cells.

The morphology of the lactotrophs have been best described in the rat where PRL containing cells are sparsely distributed in the lateroventrical portion of the anterior lobe and are present as band adjacent to the intermediate lobes. Their shapes are heterogeneous, appearing as either polyhedral or angular but at times rounded or oval.
Pituitary preprolactin like all secreted proteins is synthesized and its signal peptide is removed on the membrane-bound ribosomes of the rough endoplasmic reticulum. From there processed PRL is transported to the golgi, where it is glycosylated to a variable extent and packaged into secretory granules. PRL was originally thought to be secreted by two pathways, the regulated secretory granule pathway described above and a constitutive pathway, characterized by lack of secretory granule formation and a rapid transport to the cell surface. The constitutive pathway was considered to be the bulk flow default pathway, whereas the secretory granule pathway was considered to be the pathway responding to secretagogues\textsuperscript{162}.

2) Brain:

The first observation that PRL is produced in the brain was by Fuxe \textit{et al}\textsuperscript{163} who found PRL immunoreactivity in hypothalamic axon terminals. PRL immunoreactivity was subsequently found in the telencephalon in the cerebral cortex, hippocampus, amygdala, septum\textsuperscript{164}, caudate putamen\textsuperscript{165}, brain stem\textsuperscript{164}, cerebellum\textsuperscript{166}, spinal cord\textsuperscript{167}, choroid plexi and the circumventricular organs\textsuperscript{168}.

\textit{Hypothalamus:} PRL immunoreactivity is found within numerous hypothalamic areas in a variety of mammals\textsuperscript{167-173}. Within the rat hypothalamus, PRL immunoreactivity is detectable in the dorsomedial, ventromedial\textsuperscript{170}, supraoptic and paraventricular nuclei\textsuperscript{174}. Several approaches have been taken to prove that PRL found in the hypothalamus is synthesized locally, independent of PRL synthesis in the pituitary gland. Indeed, hypophysectomy has no effect on the amount of immunoreactive PRL in the male hypothalamus and only diminishes but does not abolish the quantity of immunoreactive PRL in the female rat hypothalamus\textsuperscript{164}.

It has now been established that the primary structure of PRL of the hypothalamic and pituitary origin is identical\textsuperscript{175}. Although the role of PRL of hypothalamic origin is not apparent, it has been speculated that PRL of central origin may exert its effect as neurotransmitter, neuromodulator or a central cytokine regulating vascular growth and/or
glial functions. To ascribe a role for PRL of central origin is troublesome, in part, because it is difficult to differentiate between the effects of PRL of pituitary versus hypothalamic origin in the CNS. One cause of these difficulties is that pituitary PRL from the circulation bypasses the blood brain barrier and enters the CNS through the choroid plexi of the brain ventricles. Aside from passage from the blood to the cerebrospinal fluid by way of the choroid plexus, pituitary PRL may also reach the brain by retrograde blood flow from anterior pituitary to the hypothalamus. Therefore, the actions of PRL in the CNS can be due to the hormone of the pituitary or hypothalamic origin.

3) Decidual PRL:

In addition to its major site of synthesis in the pituitary, human PRL is also synthesized in the decidua basalis of the pregnant uterus. PRL is present in amniotic fluid at levels 100-fold higher than in maternal or fetal blood. PRL produced by the decidual cell appears to be 50% glycosylated, whereas that produced by the pituitary is only 10% glycosylated. The PRL found in the amniotic fluid is heavily glycosylated.

4) Mammary gland and Milk:

PRL has been detected in epithelial cells of the lactating mammary gland as well as in the milk itself.

REGULATION OF SECRETION

1) Physiological Observations

1) Normal circadian secretion: Like all pituitary hormones, PRL is secreted episodically, with a distinctive 24-hour pattern. In normal human subjects, there are about 14 pulses of PRL secretion in 24 hours, approximately one each 95 minutes. Superimposed upon this pattern is a bimodal 24-hour pattern of secretion, with a major nocturnal peak beginning after sleep onset and peaking in mid sleep. Minimal levels (the smallest spikes)
occur around noon followed by a lesser peak of secretion in the evening\textsuperscript{182,183}. The enhancements of secretion during the night is due to increase in the amplitude of each pulse, unaccompanied by an increase in pulse frequency\textsuperscript{184}. PRL secretion remains pulsatile in patients with prolactinomas, whereas circadian variation is abolished. Furthermore, rat anterior pituitaries transplanted under the pituitary capsule of hypophysectomized rats release PRL in pulses of 8 to 10 minute intervals. Because hypothalamic connections have been severed, this short periodicity appears to be intrinsic to the lactotroph\textsuperscript{185}. The data from both human and rat studies support the concept that these short pulses are not controlled by the hypothalamus but arise within the gland\textsuperscript{186}.

2) \textit{Secretion during the menstrual cycle, pregnancy and lactation}: Serum PRL levels are generally higher in women than in men. This reflects the effects of estrogen. The daytime peak of PRL secretion is more pronounced during the luteal phase of the menstrual cycle\textsuperscript{187}. During pregnancy, maternal serum PRL levels begin to rise during the first trimester and increase steadily throughout pregnancy, resulting in about a 10-fold increase by term, presumably due to high levels of estrogen during pregnancy\textsuperscript{188}. In contrast to maternal serum levels, the levels of PRL in amniotic fluid peak at 17 to 25 weeks of gestation then decline to a lower plateau at 36 weeks\textsuperscript{189}. Serum PRL levels fall during labor by about 50%, reaching a nadir about two hours prior to delivery. After delivery, serum PRL levels rise markedly, peaking about two hours postpartum and then falling again six hours later\textsuperscript{190}. The postpartum period is characterized by physiological hyperprolactinemia, which progressively drops toward normal nulliparous levels over a period of four weeks despite continued suckling\textsuperscript{191}.

3) \textit{Stress}: Human PRL secretion has been shown to increase after many types of stress, including general anesthesia, surgery, exercise, and insulin induced hypoglycemia. In each case the stress causes a significantly greater PRL increase in women than men. Following general surgery, levels as high as five-fold over basal have been reported\textsuperscript{192}. It has been postulated that the stress induced increase in PRL is partially mediated by the opiate peptides, particularly $\beta$-endorphin\textsuperscript{193}. Neuronal histamine and arginine vasopressin have been implicated as mediators in other studies\textsuperscript{194}, whereas melanocyte-stimulatig
hormone (MSH) has been implicated as an inhibitor of stress induced increase in PRL\textsuperscript{195}.

II) Endocrine Regulation

1) Estrogen: Estrogen regulates the secretion of PRL in many different species. Surveys of human pituitaries at autopsy have demonstrated that estrogen increases the number of PRL-secreting cells\textsuperscript{196}. In line with this, it has long been known that high estrogen levels induce prolactinomas in certain strains of rats\textsuperscript{197}.

2) Insulin: Physiological doses of insulin stimulate PRL expression in GH3 cells; PRL mRNA levels increase 3 to 10-fold and secretion is accelerated\textsuperscript{198}. This effect is also detectable in primary pituitary cells\textsuperscript{199} and in decidua\textsuperscript{200}. The direct mitogenic effect of PRL on the islet $\beta$-cell and its proposed role in the maintenance of normal glucose levels during pregnancy suggest a feedback loop on the islets\textsuperscript{201}.

III) Neuroendocrine Regulation

Several examples of neuroendocrine control of PRL secretion have been discussed above: the sleep related, stress and suckling induced surges of PRL. The suckling stimulus represents a neuroendocrine reflex and is a popular experimental model: the magnitude of PRL response is closely coupled to the intensity of the stimulus\textsuperscript{202}. An example of highly complex neuroendocrine reflex unique to PRL is the release of PRL in response to stimulation of the uterine cervix in the rat. Either artificial cervical stimulation or normal mating results in a twice-daily surge of PRL release, peaking at about three fold over baseline. This twice daily pattern continues for up to 13 days after the stimulus and in the absence of fertilization induces a state known as pseudopregnancy\textsuperscript{203}. Although the exact molecular mechanisms controlling these responses are incompletely understood, a variety of PRL releasing factor and PRL inhibiting factors have been discovered that may be of importance.
PRL-inhibiting factors (PIF's)

1) Dopamine:
Dopamine, a catecholamine present in the hypophyseal portal blood at levels sufficient to inhibit PRL release\(^{204}\), acts as the major physiological PIF via a direct action on the pituitary. If dopamine or its agonists are administered intravenously to humans, PRL levels falls in normal individuals as well as in most patients with hyperprolactinemia\(^{205}\). Although other PIF's have been identified, current evidence supports dopamine as physiologically significant PIF.

The CNS contains several dopaminergic pathways that differ in distribution and function, of these tuberoinfundibular dopaminergic (TIDA) system is one that primarily regulates PRL secretion. Dopamine has a series of specific isoreceptors, but only the D\(_2\) receptor subtype is present on the anterior pituitary cells\(^{206}\). The number of D\(_2\) receptors is up regulated by decrease in the level of dopamine reaching the anterior pituitary and appears to be down regulated by estrogen to a lesser extent\(^{207}\).

Prolactin releasing factors (PRF's)

The existence of the suckling and copulomimetic induction of the acute PRL release, the stress response, and the proestrus PRL surge all argue for the existence of PRF's. Loss of tonic inhibition of PRL release by dissociation of dopamine from its receptor is the most parsimonious explanation. However, it cannot explain all observations, and the dissociation of dopamine from its receptor is likely to work in conjunction with PRF's not alone\(^{208}\).

1) Thyrotrophin Releasing Hormone (TRH):
In humans, TRH administered intravenously causes PRL secretion, even at the lowest doses capable of inducing a TRH response\(^{209}\). Although levels of TRH in hypophyseal portal blood have been reported to increase in parallel with PRL in several studies\(^{210}\).
TRH is a PRF, but its role in the physiologically important PRL surges remains elusive.

2) Vasoactive Intestinal Peptide (VIP):
VIP and its cosynthesized peptide, peptide-histidine-isoleucine (PHI), stimulates PRL release from isolated pituitary glands\(^{211,212}\). VIP and PHI act through a shared binding site to increase PRL mRNA levels, perhaps secondary to the induction of cAMP. The level at which VIP is active remains controversial; some report activity at nanomolar concentrations, others at micromolar again leaving confusion regarding its physiological significance.

3) Serotonin:
Serotonin stimulates PRL release in vivo but not in vitro. Two mechanisms have been proposed: reduction in the activity of TIDA neurons, or stimulation of the release of PRF(s)\(^{213}\). 5-HT administration decreases dopamine synthesis in the median eminence with an associated rise in serum PRL\(^{214}\), but paradoxically serotonin still stimulates PRL release when dopamine is infused at high levels\(^{215}\).

4) Oxytocin:
As early as 1944, Peterson\(^{216}\) speculated that OXT released during suckling in the rat might stimulate the simultaneous secretion of PRL. Later, Benson and Folley\(^{217}\) and McCann's group\(^{218}\) presented evidence for a physiological role of OXT in the control of PRL release. Meitus and Hopkins\(^{219}\), however, challenged these claims, and the debate over the PRL-releasing action of OXT was initiated. Several investigators then attempted to demonstrate in vitro PRL release, as monitored by bioassay in response to OXT exposure with mixed success\(^{220}\). The advent of radioimmunoassay greatly facilitated the examination of OXT's possible PRL releasing activity. It was reported that the ability of OXT to stimulate, in a dose related fashion, PRL release, does not alter the release of other hormones like luteinizing hormone, thyroid stimulating hormone, growth hormone or follicle stimulating hormone from rat hemipituitaries and dispersed anterior pituitary cell in vitro\(^{221}\). This effect can be seen with doses of OXT similar to the amount of OXT present in hypophyseal portal plasma\(^{222}\) and it is specific for OXT, as the closely related
structural homologs vasopressin\textsuperscript{223} and vasotonin\textsuperscript{224} did not alter PRL release in these systems.

6) Additional PRF's:
A large list of additional putative PRF's has been reported in recent years. Among these are \(\beta\)-endorphin, met and leu-enkephalin, dynorphin, \(\alpha\) and \(\beta\)-neoendorphin, bombesin, substance P, neurotensin, histamine, melatonin, bradykinin, epidermal growth factor, fibroblast growth factor, tumor necrosis factor-\(\alpha\), \(\alpha\)-subunit of luteinizing hormone, gastrin, ACh, and others\textsuperscript{202,203}.

Paracrine and Autocrine Regulation

Data supporting cell to cell interaction in the regulation of PRL secretion have been accumulating. Initially it was shown that GnRH stimulates PRL release, but only if lactotrophs are co-cultured with gonadotrophins\textsuperscript{225}. The mediator for this effect may be ANG II because ANG II is localized in the gonadotroph and is released in response to GnRH, and high affinity angiotensin binding sites have been detected in the lactotrophs\textsuperscript{226}. ANG II stimulates PRL secretion \textit{in vivo} and \textit{in vitro}. ANG II is a extremely potent PRF effective at 1 nM levels\textsuperscript{227}. Pituitary folliculostellate cells inhibit PRL secretion when co-cultured with lactotrophs. The identity of the mediator is unknown\textsuperscript{228}.

PROLACTIN RECEPTOR:

The PRL receptor (PRL-R) is a single membrane-bound protein that belongs to class I of the cytokine receptor superfamily\textsuperscript{229}. Just like their respective ligands, PRL and growth hormone receptors share several structural and functional features despite their low (30\%) homology\textsuperscript{230}. Each contains an extracellular, transmembrane, and intracellular domain\textsuperscript{231}. The gene encoding the human PRL-R is located on chromosome 5 and contains at least 10 exons\textsuperscript{229}. Transcriptional regulation of the PRL-R gene is accomplished by three different promoters, tissue-specific promoter II for the liver, and
promoter III is "generic" present in both gonadal and intragonadal tissues\textsuperscript{232}. Numerous PRL-R isoforms have been described in different tissues\textsuperscript{233}. These isoforms are result of transcription starting at alternative initiation sites of the different PRL-R promoters as well as alternative splicing of non coding exon transcripts\textsuperscript{232}. Although the isoforms vary in the length and composition of their cytoplasmic domains, their extracellular domains are identical\textsuperscript{234}. The three major PRL-R isoforms described in the rats are the short (291 amino acids), intermediate (393 amino acids), and long (591 amino acids) forms\textsuperscript{234}. In mice, one long and three short forms have been described\textsuperscript{235}. In addition to the membrane bound receptors, soluble PRL receptor binding proteins were also described in mammary epithelial cells\textsuperscript{236} and milk\textsuperscript{237}. These soluble forms contain 206 NH\textsubscript{2}-terminal amino acids of the extracellular domain of the PRL-R\textsuperscript{238}. The soluble PRL binding proteins are also products of the same PRL-R gene, but it is still uncertain whether they are results of alternative splicing of the primary transcripts or products of proteolytic cleavage of the mature receptor (or both)\textsuperscript{234}.

**HYPERPROLACTINEMIA**

Hyperprolactinemia is the most common hypothalamic pituitary disorder encountered in clinical endocrinology. The causes of pathological hyperprolactinemia are diverse, and treatment depends upon identification of precise cause. In humans, pathological hyperprolactinemia is defined as a consistently elevated serum PRL level (greater than 20 ng/ml) in the absence of pregnancy or postpartum lactation\textsuperscript{239}. Causes of hyperprolactinemia are listed in the Table.
ETIOLOGIES OF PATHOLOGICAL HYPERPROLACTINEMIA.

1. Hypothalamic Disease
   A. Tumor (e.g., craniopharyngioma, third ventricle cyst, glioma, hamartoma)
   B. Infiltrative disease (e.g., sarcoidosis, giant cell granuloma, tuberculosis, eosinophilic granuloma)
   C. Pseudotumor cerebri
   D. Cranial radiation

2. Pituitary disease
   A. Prolactinoma (microadenoma, macroadenoma)
   B. Acromegaly
   C. Cushing's disease
   D. Glycoprotein producing tumor (Lh, FSH, TSH, α-subunit)
   E. Other tumors (metastatic, intrasellar germinoma, meningioma, nonsecretory pituitary tumor)
   F. Pituitary stalk section (trauma)
   G. Empty sella
   H. Infiltrative disease (lymphocytic hypophysitis, giant cell granuloma, sarcoidosis)

3. Drugs
   A. Neuroleptics (perphenazine, fluphenazine, thorazine, promazine, trifluoperazine, haloperidol, chlorpromazine)
   B. Dopamine receptor blockers (metaclopramide, sulpiride, domperidone)
   C. Antidepressants (amoxapine, imipramine)
   D. Antihypertensives (α-methyl dopa, reserpine, verapamil)
   E. Estrogens
   F. Opiates
   G. Cimetidine (intravenous)

4. Primary hypothyroidism

5. Chronic Renal Failure

6. Cirrhosis

7. Neurogenic (spinal cord lesions, chest wall lesions, breast stimulation)

8. Stress (physical, psychological)

9. Idiopathic

PROLACTIN AND STRESS

As described earlier, PRL is released in high quantity in response to stress that is physical and psychological in nature. Swingle et al. first described the occurrence of pseudopregnancy in female rats after the application of stressor stimuli. Later, it was found that stress promotes milk secretion, suggesting the possible involvement of PRL. In 1965, Grosvenor described in detail the stress induced depletion of PRL from the pituitary.

The teleologic significance of the release of the other hormones in stress has been recognized and is bound to the maintenance of the physiological standards or to restore body homeostasis. The adaptation to stress however is often accompanied by "endogenous" damage (e.g., gastric ulcers) that is caused by overall activation of nervous, endocrine and autonomic responses.

PRL, which does not recognize a specific target organ in the periphery, exerts positive effects in the mechanisms of coping with stress. There is much data showing that this hormone affects behavioral, endocrine and autonomic changes elicited by stress.

The influence of PRL on stress induced behavioral changes has been studied in adaptive and nonadaptive situation. Rats with hyperprolactinemia induced by pituitary homograft under the kidney capsule exhibit facilitated acquisition of active avoidance response in the shuttle box and in the pole jumping test situations. This effect is either reduced by dopamine or opioid receptor antagonists and does not decrease in long term hyperprolactinemic rats.

Hyperprolactinemic rats exhibit also reduced pain sensitivity when they are tested for behavioral responsiveness to electrical foot shock. This effect has been confirmed in other tests of analgesia measurements.
Novelty-induced grooming behavior is particularly sensitive to PRL. Both endogenous hyperprolactinemia induced by pituitary homografts and intracerebroventricular (i.c.v) administration of PRL enhance novelty induced grooming in rat. It is worth mentioning that the enhanced grooming of hyperprolactinemic rats seems to depend on the actual presence of this hormone (or of its biologically active fragments) in the brain. In fact i.c.v administration of anti-prolactin serum suppresses the excessive grooming of animals with high plasma levels of PRL. Basal levels of the plasma corticosterone in hyperprolactinemic rats bearing pituitary homografts under the kidney capsule have been found to be higher than those animals with normal levels of PRL. This finding is in agreement with other studies showing an increase in the secretory activity of adrenal glands in rats with hyperprolactinemia of various origin. The endocrine change appears to be accompanied by hypertrophy of adrenal glands, as observed in postmortem examination of hyperprolactinemic rats.

When physical stress is applied (e.g., forced swim in cold water), hyperprolactinemic rats show a suppression of the three fold increase in plasma corticosterone levels observed in control animals given the same type of stress stimulus.

The effect of endogenous hyperprolactinemia on core temperature has been studied in rats before and after the application of restraint stress. Hyperprolactinemia is accompanied by small but significant decrease in core temperature of freely moving rats, and this effect can be totally reversed by the administration of the opioid receptor antagonist naloxone. In animals with normal levels of plasma PRL, the injection of naloxone is also followed by hypothermic effect. Both animals with hyperprolactinemia and those injected with naloxone failed to show any increase in normal core temperature after application of restraint stress.
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The effects of prolactin on stress induced gastric ulcers

A number of findings have demonstrated that PRL may exert a protective action against typical "endogenous" damage caused by stress that is, gastric ulcers. In a preliminary study, it was found that hyperprolactinemia as induced by pituitary homographs under the kidney capsule, is accompanied by inhibition of the development of gastric ulcers by cytoprotection in the model of cold plus restraint stress20.

The role of central dopamine transmission in PRL cytoprotection has been studied in more detail. It was found that injection of microdoses of 6-hydroxydopamine into the corpus striatum, leading to the destruction of the dopamine nerve endings in the area, is followed by total abolition of PRL cytoprotective action on stress induced gastric ulcers. The same has been found after injection of 6-OHDA into nucleus accumbens. These findings suggest that this effect of PRL is central in origin and involves both nigrostriatal and mesolimbic dopaminergic system235.

Thoman et al254 have found that hyperprolactinemic lactating rats exhibit marked resistance to stress induced changes in body temperature and diminished secretory activity of adrenal glands under stress conditions.

PROLACTIN BINDING SITES IN THE BRAIN

PRL has a wide range of effects on brain functions including maternal behavior, grooming behavior, reproductive functions, and sleep-wake cycles and stress responses. PRL has also been shown to regulate the electrical activity of neurons, neurotransmitter release, enzyme activities and specific gene expression in neurons10,255. Some actions of PRL on brain function have been demonstrated to be achieved by direct interaction with specific brain regions, particularly within the hypothalamus. PRL stimulation of tuberoinfundibular dopamine neurons in the arcuate (Arc) and periventricular hypothalamic nuclei (Pe) of the hypothalamus has been well characterized256. PRL stimulates tyrosine hydroxylase (TH) mRNA and TH neuronal activities in the Arc, and
also increases dopamine turnover\textsuperscript{257-260}. Some other examples of direct effects of PRL on specific parts of the hypothalamus are induction of maternal behavior by an action on the medial preoptic area of female rats\textsuperscript{25}, increased food intake by acting on the ventromedial hypothalamic nuclei (VMH) in the ring dove\textsuperscript{26}, and alteration of the sleep wake cycle by an action on the rat dorsolateral hypothalamus\textsuperscript{27}.

**PROLACTIN AND GASTROINTESTINAL TRACT**

PRL is reported to have modulatory actions on the gastrointestinal tract\textsuperscript{261-277}. The most important of these actions are the stimulation of small intestinal ion/saccharide/amino acid transport\textsuperscript{264,265} and islet cell insulin secretion\textsuperscript{277}. PRL receptors have been identified in the gastric mucosa\textsuperscript{278-280}. The exact role of these receptors is not clearly known. PRL binding sites are also present in esophageal epithelium and surface epithelial cells of the duodenum and jejunum\textsuperscript{176}.
**OXYTOCIN**

**HISTORICAL PERSPECTIVE**

The neurohypophysial hormone OXT was first neuropeptide to have its structure determined in the year 1953\(^2\), although the peripheral endocrine effects had been described 60 years ago. Indeed the initial observation of the uterotonic action of posterior pituitary extracts was reported by Dale\(^2\) in the pregnant cat in 1906, and in 1910, Ott and Scott\(^2\) demonstrated the milk ejection activity of such extracts. For a long time, only these two effects were known.

Until 1950s, knowledge of OXT advanced mainly in the fields of morphology and biochemistry, more or less in association with the other neurohypophysial hormone vasopressin. Rapid progress was made in the early 1970s and gave rise to two international colloquies, one on the neurohypophysis, held in Cambridge in 1982\(^2\), and other dealing specifically with OXT, held in Quebec in 1984\(^2\).\(^\)\(^\)

**BIOSYNTHESIS**

The OXT stored in and released from the posterior pituitary gland is produced in neurons whose cell bodies are located in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus. Because of their size these cells are often referred to as magnocellular neurons. Magnocellular neurons from both the nuclei project to posterior pituitary gland. Apart from these magnocellular neurons, there are parvocellular neurons, which project from these nuclei to terminal fields within the brain. Stimulation of paraventricular and supraoptic nuclei cause the release of OXT in the posterior pituitary gland through magnocellular neurons and within brain through parvocellular neurons\(^2\),\(^3\).\(^3\)

OXT is synthesized as a 20-kd peptide molecule termed prooxyphysin from the primary structure pro-pressophysin\(^2\), these precursors are present in the neurohypophyseal tissue
and are transported along the axon and stored in the nerve terminals in the neurohypophysis. Several secretory pathways designated for OXT have been identified.

1. Hypothalamic pathways.
   a. The magnocellular PVN-neurohypophyseal system secretes OXT into general circulation via terminals in the neural lobe.
   b. The PVN-median eminence pathway secretes into the portal circulation, where concentration of OXT are 50-fold higher than in the plasma.

2. Extrahypothalamic pathways.
   a. Within the CNS, an extensive network of OXT fibres is found throughout the brain. The OXT fibres are predominant in the caudal brain region and spinal cord, where they may integrate via the autonomic system in response to stress and the suckling reflex.
   b. In the reproductive tract, OXT have been found in the human ovary, follicular fluids and oviductal tissue. The concentrations in the tissues and in follicular fluids are approximately 400-fold and 30-fold higher than in the plasma. The concentrations of the OXT is 6 times higher in the corpus luteum than in ovarian tissues without the corpus luteum.

REGULATION OF SECRETION:

I. Physiological Release of Oxytocin:

1. Parturition:

OXT is an important stimulator of myometrial contraction late in the labor and in the homeostasis at the placental site of the delivery. Plasma OXT levels are unchanged until the expulsive phase of the labor. The primary stimulus for the release of maternal OXT during labor seems to be vaginal distension or the Ferguson reflex. Estrogen induces an increase in OXT receptors in myometrial and decidual tissue of the pregnant women, and
OXT receptors in both of these tissues reach maximum concentrations near the term \(^{290}\). This may account for the increase in spontaneous myometrial contraction and the increased sensitivity to OXT during late pregnancy even in the absence of the increased plasma OXT levels. During the second stage of the labor, OXT release may play a synergistic role in the expulsion of the fetus by virtue of its ability to release prostaglandins \(^{289}\).

Although fetal plasma OXT levels are elevated during the first stage of the labor, infusion of OXT to the fetus induces uterine contractions only with pharmacological doses; therefore normal levels of the fetal OXT probably have little influence on labor \(^{289}\).

2. Milk Let-Down:
During nursing, stimulation of nerve endings in the nipple induces OXT release. This neurogenic reflex is transmitted through the spinal cord, midbrain and hypothalamus. Where it triggers OXT release from the neurohypophysis \(^{288}\). Of particular significance is the episodic release of OXT even in anticipation of suckling \(^{291}\). This psychogenic flow is suppressed when fear, anger or other stresses are encountered, thereby inhibiting OXT release \(^{288}\).

II. Endocrine and Neuroendocrine regulation:

The release of the OXT involves multiple regulatory sites and mechanisms. Central or hypothalamic control involves cholinergic and noradrenergic neurotransmitters as well as several neuropeptides, as follows.

1. Acetylcholine:
\(\text{ACh}\) releases OXT via the nicotinic receptors. Application of the \(\text{ACh}\) into the supraoptic neurons markedly accelerates their firing rate, and nicotine or tobacco smoking induces antidiuresis by acute increments of vasopressin along with OXT in plasma \(^{292,293}\).
2. **Noradrenaline:**

Noradrenergic influence on the secretion of OXT seems to involve a stimulatory $\alpha$-adrenergic and an inhibitory $\beta$-adrenergic pathway. There is a direct innervation of magnocellular neurons by adrenergic fibres arising from the locus coeruleus\textsuperscript{294}. The firing rate of the magnocellular neurons is reduced by exposure to an $\alpha$-adrenergic antagonist and enhanced by a $\beta$-adrenergic antagonist, such as propranolol. However, recent reports indicate that blockade of either $\alpha$- or $\beta$ receptors in the PVN or SON prevents the suckling induced release of OXT. Further, OXT release is increased in non-suckled rats by central application of either $\alpha$ or $\beta$ agonist indicating that both $\alpha$ and $\beta$ receptor mediate OXT release\textsuperscript{295}.

There are strong evidences to support that norepinephrine is an important excitatory neurotransmitter in the regulation of OXT secretion. For example, noradrenergic fibres arising predominantly from the region of the nucleus tractus solitarius in the medulla innervate magnocellular neurons\textsuperscript{296}. Moreover, depletion of hypothalamic norepinephrine decreases the release of OXT in response to foot shock\textsuperscript{297}, peripheral administration of CCK and hypertonic saline\textsuperscript{298} and suckling during lactation\textsuperscript{299}. Peripheral administration of an $\alpha$-adrenergic antagonist significantly increases the latency of the milk ejection reflex\textsuperscript{300} which is mediated by OXT, and completely prevents the suckling induced increase in plasma OXT concentration\textsuperscript{301}.

3. **Opioid peptides:**

Opioid peptides are also involved in the regulation of OXT secretion. The neurohypophysis receives opioid peptide containing nerve fibres from the arcuate nucleus and the nucleus tractus solitarius. It also possesses opioid receptors of the $\kappa$-subtype, which are important regulators of the neurohypophyseal hormone secretion at the level of the nerve terminal\textsuperscript{302, 303}.

Dynorphin, a $\kappa$ receptor agonist, inhibits OXT secretion by an action on axonal terminals within the neurohypophysis. Naloxone, an opiate receptor antagonist, markedly enhances the release of OXT induced by electrical stimulation\textsuperscript{304}. 
4. Activin-Containing Neurons:
Activin is localised in the nucleus tractus solitarius (NTS), a major recipient of visceral sensory information with projections to the PVN. That activin conveys inputs to oxytocinergic neurons is suggested by eliciting OXT secretion in response to infusion of purified activin into the PVN and anti-activin sera into the PVN attenuates the suckling induced OXT secretion. Thus, the NTS as an ascending somatosensory pathway may represent the circuitry involving activin mediated OXT secretion. This is compatible with the observation that gastrointestinal stimuli (such as nausea) induce OXT secretion and firing of oxytocinergic neurons.

5. Estrogen:
An appreciable number of OXT producing cells in the PVN contain estrogen binding sites, and estrogen induces an increased sensitivity to OXT by augmenting OXT receptors. It is likely that these estrogen-receptive OXT cells mainly project to the posterior lobe. The elevated immunoreactive OXT in men or women treated with estrogen are actually not authentic OXT but an OXT precursor intermediate, OXT-glycine. Thus estrogen influences intraneural post-translational processing of OXT precursor. The physiologic role, if any, of this estrogen induced change in processing precursor hormone remains to be determined. Serum concentrations of OXT increase during the following phase to a peak at midcycle and then decrease early in the luteal phase.

6. Angiotensin II (ANG II):
Intracerebroventricularly (i.c.v.) administered ANG II at a dose of 100 ng is reported to cause a large increase in plasma oxytocin levels. The elevation of oxytocin secretion in response to ANG II depends largely on activation of cyclo-oxygenase and production of prostaglandins. The oxytocin response to angiotensin is reported to be completely abolished by pretreatment with losartan providing evidence of AT1 receptor involvement in mediation of the ANG II-stimulating effect on OXT secretion.
6. Cholecystokinin (CCK):

Administration of CCK to rats causes a dose-dependent increase in the plasma levels of OXT. The effect of CCK on OXT secretion was blunted after vagotomy. CCK is reported to cause a stimulation of OXT secretion by stimulating common central mechanisms involving both the magnocellular and parvocellular neurons in the PVN of the hypothalamus.

7. Relaxin:

Relaxin is a potent inhibitor of OXT release. Earlier data suggests that relaxin may be involved during labor. In several species the myometrium is quiescent shortly before parturition. At this time high titres of relaxin are reported to be present in the plasma and there is evidence that the hormone has a direct inhibitory action on the uterine muscle. Recent reports indicate that relaxin may act through the central ANG II system to inhibit the release of vasopressin and OXT.

OXYTOCIN RECEPTORS

Specific receptors for OXT in human myometrium have been identified, and differences in receptor density at various stages of labor have also been noted. OXT has dual effects on the uterus. It regulates the contractile properties of myometrial cells and elicits prostaglandin production by endometrial/decidual cells. At least in animal models, these effects are mediated by two distinct receptor subtypes, suggesting that OXT antagonists designed as tocolytic agents for blockade of preterm labor must block both the uterotonic and prostaglandin-releasing effects of OXT, and thus must block both the receptor subtypes. A human OXT receptor has been cloned. Receptor occupancy has been coupled to activation of phospholipase C and release of intracellular Ca²⁺ by inositol-1,4,5-triphosphate as well as direct or depolarisation induced activation of voltage sensitive Ca²⁺ channels, but the precise signalling mechanisms that mediate the diverse effects of the OXT in the hypothalamus, pituitary and the uterus have not been clarified.
OXYTOCIN ANTAGONIST-ATOSIBAN

Atosiban is a nonapeptide desamino OXT analog that has been shown to be a competitive OXT antagonist and to inhibit OXT induced uterine contraction in *in vitro* and *in vivo* models. Atosiban has been shown to be effective in reducing spontaneous preterm uterine activity in humans. Recently, atosiban has been shown to be effective in the treatment and maintenance of preterm labor. Trials of this agent in intact animals and women as a tocolytic agent for preterm labor have shown atosiban to be devoid of cardiac effects. This is in contrast to other tocolytic agents, which have profound hemodynamic effects. Atosiban is reported to reduce a number of effects of OXT when given by either subcutaneously or intracerebroventricularly. Atosiban is known to block the effect of OXT on pancreatic hormone release, pain threshold, blood glucose, intraoral glucose intake, and sweat glands.

The half life of atosiban is around 18 ± 3 min in pregnant women with premature uterine contractions. Atosiban is shown to have minimal placental transfer when administered to pregnant women at term. Other activities of atosiban are not well explored.

OXYTOCIN AND STRESS

Repeated administration of OXT to rats causes an effect profile that in part differs from that induced by single injections. These effects cannot be attributed to the direct effects of OXT, due to its short half life, but to the activation of secondary mechanisms. The effects are exerted centrally because they may be induced by i.c.v administration of OXT. With subcutaneous administration, 1,000-fold higher doses are required to induce the same effects. However, because 1-2% of a dose of OXT given peripherally passes through the blood brain barrier to reach the CNS, the effects in response to subcutaneous administration are also likely to be exerted centrally.

A 5-day period of OXT treatment (1 mg/kg s.c) causes the following effects. First, systolic and diastolic pressures are lowered by ≈15 mm Hg without affecting pulse rate.
In males blood pressure is normalized 10 days after the last OXT treatment, whereas in females differences between saline treated controls and OXT treated animals persist\(^3\). Second, the withdrawal latency in the tail flick test is increased and gradually declines to pretreatment values within 10 days. The sustained effect induced by repeated administration of OXT cannot be antagonized by the OXT antagonists as in the case of acutely administered OXT induced elevation of withdrawal latency. Instead, naloxone temporarily inhibits the enhanced delay in withdrawal latency, suggesting that the activity of an endogenous opiate system has somehow been increased\(^3\). Third, corticosterone levels are significantly lower and CCK levels are significantly higher than in saline treated controls\(^3\). Fourth, female rats experience a slow, spontaneous weight gain\(^3\). This effect occurs without increase in food intake, suggesting that the effect is due to metabolic changes favoring the storage of energy.

**OXYTOCIN BINDING SITES IN THE BRIAN**

OXT nerve terminals are not confined to the median eminence and posterior pituitary gland but are located throughout the CNS, even reaching the lower spinal cord\(^3\). The presence of these extrahypothalamic pathways raises the possibility that, besides its hormonal action, OXT may act as neurotransmitter or neuromodulator in neuron-neuron interaction. Certainly, OXT applied iontophoretically to the hippocampal neurons, a region of the brain where OXT has been localized\(^3\), increases their firing pattern and does so by affecting receptors that show pharmacological similarities to those of the uterus\(^3\). Other regions where OXT is known to bind include the central amygdala, olfactory nucleus and nucleus tractus solitarius\(^3\).
OXYTOCIN AND GASTROINTESTINAL TRACT

The paraventricular nucleus and the OXT fibres are liable to be involved in the vasovagal reflexes other than those affecting the cardiovascular system, particularly in controlling motility and gastric secretions.

In rats, after lesion of a paraventricular nucleus, the increase in gastric secretion in response to stimulation of the ipsilateral afferent vagal fibres is suppressed\textsuperscript{345}. Applying various neuropeptides (bombesine, neuropeptide Y) to the paraventricular nucleus generally inhibits gastric secretion\textsuperscript{346,347}. Electrical stimulation of the paraventricular nucleus or of the pituitary stalk inhibits gastric motility\textsuperscript{348}. Sakaguchi and Ohtake\textsuperscript{348} suggest an action via paraventriculoneurohypophysial axons running towards the brain stem. These are OXT fibres that might be at the origin of the inhibition of gastric motility in response to systemic injections of CCK or lithium chloride, a nauseous agent\textsuperscript{349}. Other authors reveal more directly the participation of the central OXT fibres. Stimulating the paraventricular nuclei increases gastric secretion\textsuperscript{350}, enhances the development of acute gastroduodenal ulceration\textsuperscript{351} and has a biphasic effect on gastric motility, with a strong increase followed by a weak decrease\textsuperscript{352}. Some of these effects are blocked by injecting an OXT antagonist into the dorsal motor nucleus of the vagus nerve: the increase in gastric secretion is suppressed\textsuperscript{350} as is the increase in the second phase of the response to paraventricular nucleus stimulation. On the other hand, the increase in motility observed in the first phase of the response to paraventricular nucleus stimulation is amplified\textsuperscript{352}

Injecting OXT (10 pmol, i.e., 10 ng) into the dorsal motor nucleus of the vagus nerve increases gastric secretion and reduces motility\textsuperscript{352}. These authors showed that for gastric secretion the effect is specific: it is blocked by OXT antagonist and is not reproduced by either vasopressin or OXT when injected into area prostema. The nature of the afferent pathway has been partly established by applying OXT to the dorsal motor nucleus of the vagus nerve.
The increase in gastric secretion is blocked by peripheral injections of atropine. The decrease in motility is entirely suppressed by vagotomy but only partially by atropine, which does not prevent the decrease in gastric motility after paraventricular nucleus stimulation, although it does block the preceding phase, namely the increase in gastric motility.

However, few other authors reported that OXT decreases gastric acid secretion contradicting to the reports discussed above. It was reported that electrical stimulation of paraventricular nuclei could reduce gastric secretion in rats. In addition, it was shown nanomolar quantities of OXT injected into the paraventricular nucleus produced reduction in gastric acid secretion. Recently Petersson et al. reported that repeated administration of OXT by subcutaneous route reduced plasma concentration of vagally controlled hormones such as gastrin, CCK and insulin.