Melatonin in the gastrointestinal tract

FANNY SÖDERQUIST
Abstract

Melatonin is recognised as the pineal hormone regulating sleep and circadian rhythm. It has also been identified in peripheral tissues (mainly in animals) and thought to display a variety of actions, including anti-inflammatory properties, regulation of gastrointestinal (GI) functions, glucose homeostasis and beneficial effects in different tumour types. Patients with irritable bowel disorder commonly exhibit psychiatric co-morbidity and disturbances of the gut-brain axis have been proposed to play a role in these disorders. The focus of this thesis was to study melatonin and melatonin receptors in the normal human GI tract, the pancreas and small intestinal neuroendocrine tumours. The thesis also explores the complex relationship between GI symptoms and underlying psychiatric traits in the context of elevated levels of peripheral melatonin during waking hours.

In paper I-II, tissue samples from the normal human GI tract and pancreas and tumour tissue from small intestinal neuroendocrine tumours were analysed for expression of melatonin and melatonin receptors using immunohistochemistry. For tumour patients, melatonin was also analysed in plasma and set in relation to symptoms and outcome. In paper III-IV, a cohort of young adults (18-25 years) seeking psychiatric care was examined for GI symptoms, melatonin levels in saliva, depressive symptoms and anxiety traits. Psychiatric assessments were performed using structured or semi structured interviews. Depressive symptoms were measured using the self-rating version of the Montgomery-Åsberg Depression Rating Scale; GI symptoms were measured using the Gastrointestinal Symptoms Rating Scale for Irritable Bowel Syndrome; and personality traits were evaluated using the Swedish Universities Scales of Personality.

Melatonin and melatonin receptors were widely expressed in the normal human gut and pancreas (paper I) but even in small intestinal neuroendocrine tumours known to produce serotonin (paper II). The intensity of the melatonin immunoreactivity in tumour tissue was found to correlate with lower proliferation index. After treatment, plasma levels of melatonin were reduced in tumour patients. Young adult patients seeking psychiatric care reported more GI symptoms than healthy controls, regardless of the currently active psychotropic medication. The level of GI symptoms was associated with severity of depressive symptoms and trait anxiety (paper III). Higher postprandial levels of melatonin were associated with the GI symptoms of bloating and pain (paper IV).

In summary, these findings demonstrate the widespread presence of melatonin in the human gut and confirm a link between melatonin, psychiatric health and GI symptoms.

Keywords: melatonin, MT1, MT2, gastointestinal tract, small intestinal neuroendocrine tumours, IBS, depression, anxiety, personality and affective disorder

Fanny Söderquist, Department of Neuroscience, Psychiatry, University Hospital, Akademiska sjukhuset, Uppsala University, SE-751 85 Uppsala, Sweden.

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ISSN 1651-6206
urn:nbn:se:uu:diva-396347 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-396347)
Lindrig sömnlöshet
ökar genialiteten.

Brokiga iakttagelser
Edith Södergran, 1919

To my family
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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<th>Definition</th>
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<tr>
<td>AANAT</td>
<td>Arylalkylamine N-acetyltransferase</td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AUDIT</td>
<td>Alcohol Use Disorders Identification Test</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CgA</td>
<td>Chromogranin A</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
</tr>
<tr>
<td>CRHR1</td>
<td>Corticotropin-releasing hormone receptor 1</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P</td>
</tr>
<tr>
<td>DSM</td>
<td>Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>DUDIT</td>
<td>Drug Use Disorders Identification Test</td>
</tr>
<tr>
<td>EC</td>
<td>Enterochromaffin</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ENS</td>
<td>Enteric nervous system</td>
</tr>
<tr>
<td>FGID</td>
<td>Functional gastrointestinal disorder</td>
</tr>
<tr>
<td>GAD</td>
<td>Generalised anxiety disorder</td>
</tr>
<tr>
<td>GDP</td>
<td>Guanosine diphosphate</td>
</tr>
<tr>
<td>GEO</td>
<td>Gene Expression Omnibus</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GSRS-IBS</td>
<td>Gastrointestinal Symptom Rating Scale-IBS</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>HIOMT</td>
<td>Hydroxyindole-O-methyltransferase</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>IDO</td>
<td>Indolamine 2,3 dioxygenase</td>
</tr>
<tr>
<td>IF</td>
<td>Immunofluorescence</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IPANs</td>
<td>Intrinsic primary afferents</td>
</tr>
<tr>
<td>IR</td>
<td>Immunoreactivity</td>
</tr>
<tr>
<td>KYNA</td>
<td>Kynurenic acid</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery-Åsberg Depression Rating Scale</td>
</tr>
<tr>
<td>MDD</td>
<td>Major depressive disorder</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>M.I.N.I.</td>
<td>Mini-International Neuropsychiatric Interview</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Ribonucleic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MT1</td>
<td>Melatonin receptor type 1</td>
</tr>
<tr>
<td>MT2</td>
<td>Melatonin receptor type 2</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PsTAT</td>
<td>Psychic trait anxiety T-score</td>
</tr>
<tr>
<td>PTSD</td>
<td>Post-traumatic stress disorder</td>
</tr>
<tr>
<td>QoL</td>
<td>Quality of life</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>ROR/RZR</td>
<td>Retinoid orphan receptors/retinoid Z receptors</td>
</tr>
<tr>
<td>SCID-I</td>
<td>Structural Clinical Interview for DSM IV axis I</td>
</tr>
<tr>
<td>SCN</td>
<td>Suprachiasmatic nucleus</td>
</tr>
<tr>
<td>SI-NET</td>
<td>Small intestinal neuroendocrine tumour</td>
</tr>
<tr>
<td>SSP</td>
<td>Swedish Universities Scale of Personality</td>
</tr>
<tr>
<td>SST</td>
<td>Stress susceptibility T-score</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitors</td>
</tr>
<tr>
<td>STAT</td>
<td>Somatic trait anxiety T-score</td>
</tr>
<tr>
<td>TDO</td>
<td>Tryptophan 2,3 dioxygenase</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative colitis</td>
</tr>
</tbody>
</table>
Introduction

What is a hormone?

Hormones are chemical messengers that help the organs and cells to communicate. There are many types of hormones: proteins, peptides, amino acids, steroids, fatty acid derivatives, ions and gases. All nucleated cells have the genetic information required to produce hormones (1).

The target receptor, the type of cell that expresses it and its location all determine the effect of the hormone and the same hormone can exert a variety of effects in different tissues. Ligand binding activates the receptor, which, in turn, sets off a cascade of intracellular signalling pathways resulting in changed behaviour of the cell.

The terms autocrine, paracrine and endocrine signalling describe three levels of signalling, based on where the target cell is located. Autocrine signalling implies that the hormone sends its message to the same cell from which it was secreted (e.g., cancer cells producing growth factors that stimulate their survival and proliferation) (2). In paracrine signalling the receiver of the hormonal message is neighbouring cells from where the hormone was secreted (e.g., neurotransmitters such as acetylcholine). Endocrine signalling refers to systemic effects of hormones; for example, insulin is produced in the pancreatic islets, secreted to the bloodstream and the target cells are adipocytes, hepatocytes and myocytes (3).

Historically, descriptions of different hormonal effects have been simplified explanations in keeping with the idea that one hormone has one action. This long-standing belief, however, is an oversimplification given the increasing knowledge of the growing number of diverse effects exerted by known hormones. For instance, it has recently been shown that insulin has other target cells in the brain and the effects extend far beyond glucose homeostasis and may even influence cognitive function (for a review, see (4)).

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1 The paragraph "What is a hormone?" has previously been published in the licentiate thesis Söderquist F. Melatonin and its receptors in the normal human gastrointestinal tract, pancreas and in small intestinal neuroendocrine tumours. 2017.
Melatonin, general aspects

Melatonin is a small amphiphilic indoleamine, commonly known as the hormone involved in sleep and circadian rhythm in vertebrates. Its precursor is tryptophan via the synthesis of serotonin (Figure 1). Melatonin was first discovered when Aaron B. Lerner and his colleagues isolated it from the bovine pineal gland in 1958. It was named melatonin for its ability to lighten the skin of frogs (5).

In the pineal gland production of melatonin follows a circadian rhythm. Signals from the suprachiasmatic nucleus stimulate the release of noradrenaline, which then activates adrenoreceptors on pinealocytes causing a rise in enzyme activity of arylalkylamine N-acetyltransferase (AANAT), the rate-limiting step in melatonin synthesis (1). This pineal system is effectively inhibited by light and melatonin release from the pineal gland is therefore a nocturnal event, with plasma levels steeply increasing soon after sunset to peak about 02:00, remain elevated throughout the night and then decline to lower levels by morning. During the daytime, plasma levels of melatonin are low and believed to be of a peripheral origin (6). Light exposure during the night results in lower plasma levels of melatonin (7).
Figure 1. Synthesis of melatonin from tryptophan (left) and kynurenine pathways (right). Enzymatic reactions catalysed by tryptophan hydroxylase (TPH1, TPH2), amino acid decarboxylase (AAD), arylalkylamine N-acetyltransferase (AANAT) and 5-hydroxyindole O-methyltransferase (HIOMT). Enzymatic reactions driving the kynurenine pathway are indoleamine dioxygenase (IDO), tryptophan-2,3-dioxygenase (TDO) and formamidase. Adapted from licentiate thesis (8).

Besides the pineal gland, melatonin is present in various peripheral tissues and organs. The two enzymes needed for melatonin synthesis from serotonin have been identified in retina, stomach, gut, spleen, liver, heart, skeletal muscle, bone marrow, testes, ovaries, placenta, skin and immune cells (9, 10). However, most studies are conducted in animals and studies in humans are needed to confirm the animal findings. Recent studies have proposed that melatonin may be produced within mitochondria as a protection against the high concentrations of free radicals produced in mitochondria. Acting as a powerful antioxidant is also the most basic function of melatonin (11). Hence, melatonin could be synthesised in all mitochondria-containing cells.
Circadian synthesis of melatonin begins at 3-5 months in a newborn infant and before endogenous production, melatonin is provided via breast milk (12). Ageing is associated with lower nocturnal melatonin amplitude and this decline appears to occur at approximately 40 years of age (13).

Owing to its size and solubility, melatonin can easily pass cell membranes, including the blood-brain barrier and thus is also present in essentially all biological fluids: cerebrospinal fluid, saliva, bile, amniotic fluid, synovial fluid and breast milk (9). When administered, melatonin is rapidly absorbed and distributed. Varying results for half-life elimination time have been reported, but ranges from 30-60 minutes (min) regardless of oral or intravenous administration (14, 15). The main route of degradation is via the liver and the CYP P450 enzymes, converting melatonin to 6-hydroxymelatonin, which is then conjugated with sulphate or glucuronide before secreted in the urine (16, 17).

Melatonin receptors and interaction sites

There are two known membrane bound receptor subtypes for melatonin in humans and other mammals: receptor type 1 (MT₁) and type 2 (MT₂). They are both guanine nucleotide-binding regulatory protein (G-protein)-coupled receptors. The binding of melatonin results in intracellular signalling via second messengers (18). Both MT₁ and MT₂ receptors display a wide variety of signal transduction pathways in different tissues and cell types (19). A third receptor for melatonin named MT₃ has been identified as the quinone reductase 2, which at least in part appears to be membrane-associated (20). Studies investigating the effect of melatonin administration on intestinal motility have also suggested that melatonin may act through the serotonin receptor 5-HT₃, delaying gastric emptying and counteracting the actions of serotonin in the gut (21, 22). Melatonin can also bind to and interact with other intracellular proteins, including calmodulin, calreticulin and tubulin (23-25). Finally, melatonin can bind to nuclear receptors of the retinoid-related orphan nuclear hormone receptor (ROR/RZR) subfamily, which acts as transcription factors upon ligand binding (26, 27). Moreover, some of the actions of melatonin are receptor independent, including its free radical scavenging properties (11).

Melatonin and the brain

Melatonin’s sleep-promoting effects have been extensively studied. In psychiatry melatonin and agomelatin, a melatonin receptor agonist, are commonly used to treat mood disorders and sleep disturbances. Alterations of the normal circadian secretory pattern of melatonin have been demonstrated in
several psychiatric disorders. However, the direction of this association is unclear and it is not fully understood whether circadian disturbances are a cause or a consequence of the psychiatric condition (28).

Altered circadian rhythms and lower night-time melatonin levels have been demonstrated in neurodegenerative disorders, including Alzheimer’s, Huntington’s and Parkinson’s disease. Moreover, in patients with depression evening melatonin levels in saliva are decreased (29-31). Of note, in the context of melatonin’s anti-inflammatory properties, neuroinflammation has been proposed as a contributor to the pathogenesis in all of these conditions (32).

Melatonin may also be involved in cognitive functions and behaviour. Middle-aged patients with cognitive impairment had a lower median nocturnal melatonin response compared with healthy controls. Moreover, in patients with affective disorders lower melatonin levels in the evening correlated with poorer performance in verbal memory tasks (33, 34). A study using knock-out mouse models found that MT₁ deletion increased anxiety-like behaviour, whereas deletion of the MT₂ receptor resulted in anhedonia and generalised social avoidance, signifying depressive-like behaviour (35).

**The gut-brain axis**

The brain and the gut share common signalling pathways that can be roughly divided into neural, endocrine and immunological routes of communication. This complex system is commonly referred to as the gut-brain axis. The system has implications for the function of the whole body in that it is linked to metabolism and the immune system.

The endocrine routes of gut-brain interactions involve the hypothalamic-pituitary-adrenal (HPA) axis and the release of gut peptides from enteroendocrine cells in the intestinal wall. The activation of the HPA axis, resulting in elevated levels of cortisol, affects several aspects of gastrointestinal (GI) function. For example, the corticotropin-releasing hormone (CRH) is an important modulator of the gut-brain axis and mediates the stress response in both the brain and the gut (36) Activation of the CRH receptor 1 (CRHR1) appears to be involved in the colonic response to stress, with increased stress-related defecation in mice (37). The CRH-associated stimulation of colonic transit involves parasympathetic activation of colonic serotonin secretion and the binding to 5-HT₃ receptors (38). In addition to the centrally active CRH, peripheral administration of the CRH stimulates colonic motility and secretion, which appears to be CRHR1 dependent (39).

Enteroendocrine cells of the GI mucosa produce various substances that regulate motility and secretion, as well as different gut peptides that affect hunger and satiety and regulate food intake and insulin release. The most common enteroendocrine cells are the enterochromaffin (EC) cells, which
produce and secrete serotonin and stain positive for chromogranin A (CgA) (40).

The intestinal microbiota is also highly interactive with the gut-brain axis, influencing both neural and endocrine signalling, but also modulating the immune response. Short chain fatty acids (SCFAs) produced by intestinal bacteria can, for instance, stimulate EC cells to increase serotonin production (41). In addition, many of the common neurotransmitters in our nervous system can be synthesised by commensal bacteria (42). Moreover, intestinal macrophages located in the muscularis externa can, by the influence of commensal microbiota, cause changes in smooth muscle contractions of the colon (43). The influence of the gut microbiota on the gut-brain axis, however, is beyond the scope of this thesis.

Melatonin signalling in peripheral tissue

MT\textsubscript{1} and MT\textsubscript{2} melatonin receptors have been identified in several organs and tissues primarily in animal studies (44-46). In humans, MT\textsubscript{1} and MT\textsubscript{2} have been identified in different parts of the brain, with notable differences in distribution between the two receptors (47-50). The MT\textsubscript{1} receptor, identified in immune cells, has been more extensively investigated (51). Few studies, however, have investigated the receptor expression in the normal GI tract of humans. In one human study binding sites in the colon, caecum and appendix were identified using iodinated melatonin. Another human study found expression of MT\textsubscript{1} in colon adenocarcinoma but also identified the MT\textsubscript{1} receptor in normal adjacent tissue of the colon using immunohistochemistry (52, 53). Table 1 summarises the present knowledge of MT\textsubscript{1} and MT\textsubscript{2} receptor distribution in normal human tissue.

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>Receptor</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>MT\textsubscript{1}, MT\textsubscript{2}</td>
<td>PCR, IHC</td>
<td>(47, 49, 50, 54, 55)</td>
</tr>
<tr>
<td>Retina</td>
<td>MT\textsubscript{1}, MT\textsubscript{2}</td>
<td>PCR, IHC (MT\textsubscript{1})</td>
<td>(56)</td>
</tr>
<tr>
<td>Parotid glands</td>
<td>MT\textsubscript{1}, MT\textsubscript{2} (weakly)</td>
<td>IHC</td>
<td>(57)</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>MT\textsubscript{1}, MT\textsubscript{2}</td>
<td>PCR</td>
<td>(58, 59)</td>
</tr>
<tr>
<td>Immune system</td>
<td>MT\textsubscript{1}, MT\textsubscript{2}</td>
<td>PCR</td>
<td>(51, 60)</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>MT\textsubscript{1}, MT\textsubscript{2}</td>
<td>PCR</td>
<td>(61)</td>
</tr>
<tr>
<td>Breast</td>
<td>MT\textsubscript{1} (alpha-cells), MT\textsubscript{2} (beta-cells)</td>
<td>IHC</td>
<td>(55)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>MT\textsubscript{1}, MT\textsubscript{2} (beta-cells)</td>
<td>PCR</td>
<td>(62, 63)</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>MT\textsubscript{1} (colon)</td>
<td>PCR, western blot, IF</td>
<td>(64)</td>
</tr>
<tr>
<td>GI tract</td>
<td>[125I]iodomelatonin binding sites, MT\textsubscript{1} (colon)</td>
<td>Autoradiography, PCR, IHC, western blot</td>
<td>(52, 53)</td>
</tr>
<tr>
<td>Kidney</td>
<td>MT\textsubscript{1}, MT\textsubscript{2}</td>
<td>PCR</td>
<td>(65)</td>
</tr>
</tbody>
</table>
Consequences of peripheral melatonin

Melatonin in the gastro-pancreatic system

As early as 1975, Kvetnoy and colleagues reported the presence of melatonin in the human GI tract (71). From studies in animals, the cells responsible for the synthesis of gut melatonin are the EC cells (72, 73). The EC cells are neuroendocrine cells that line the intestinal mucosa and are the main source of gut-derived serotonin. They function as sensory cells, responding to various stimuli from the lumen. For example, changes in pH, glucose and amino acids cause release of serotonin, which, can activate neural reflexes that result in changes in mucosal blood flow, secretion and gut motility (74).

The amount of melatonin in the GI tract easily surpasses the amount in the pineal gland. It has also been demonstrated that melatonin produced in the gut most likely attributes to daytime circulating levels, as oral administration of melatonin or its precursor tryptophan increases circulating levels of melatonin. An increase in melatonin in the portal blood was seen before that in the systemic circulation and the effect was almost completely eliminated by ligation of the portal vein, indicating that the GI tract was the source of this rise in circulating levels (6, 75). Pinealectomy reduces nighttime levels of circulating melatonin, but it does not affect levels in the GI tract, suggesting that gut melatonin is independent of pineal production (76).

Variations in GI melatonin levels appear to be related to fasting and food intake, with both resulting in increased formation of melatonin in the GI tract in response to food restriction, as well as postprandial elevations of melatonin in human saliva (77, 78). Moreover, receptor expression appears to be highly variable with nutritional status. In rats up-regulation of MT1 mRNA transcripts increased after short-term fasting (79).

Melatonin has been demonstrated to increase intracellular calcium (Ca$^{2+}$) in human and rat enterocytes, possibly through the MT$_2$ receptor, as the effect was eliminated when adding a predominantly MT$_2$-selective antagonist (80). Another study has identified MT$_1$ immunoreactivity (IR) in the human colon (53). The actions of gut melatonin include regulation of intestinal motility, protection of intestinal mucosa by reducing the paracellular permeability and glucose homeostasis. Understanding the complex systems that control intestinal motility is challenging, largely because many substances are

<table>
<thead>
<tr>
<th>Ovary</th>
<th>MT$_1$, MT$_2$</th>
<th>PCR</th>
<th>(66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterus</td>
<td>MT$_1$, MT$_2$</td>
<td>PCR, in situ hybridisation</td>
<td>(67)</td>
</tr>
<tr>
<td>Placenta</td>
<td>MT$_1$, MT$_2$</td>
<td>IHC</td>
<td>(68)</td>
</tr>
<tr>
<td>Prostate</td>
<td>MT$_1$, MT$_2$</td>
<td>IHC</td>
<td>(69)</td>
</tr>
<tr>
<td>Skin</td>
<td>MT$_1$, MT$_2$</td>
<td>PCR</td>
<td>(70)</td>
</tr>
</tbody>
</table>

Abbreviations: GI = gastrointestinal, MT$_1$/MT$_2$ = melatonin receptor type 1/type 2, PCR = polymerase chain reaction, IHC = immunohistochemistry, IF = immunofluorescence.
involved. Nevertheless, it has been proposed that melatonin and serotonin co-operate through a feedback system, in which melatonin appears to act as a physiological antagonist of serotonin, mainly dampening motility (81, 82).

Melatonin also appears to influence glucose homeostasis and melatonin receptors have been identified in the human pancreas, with MT1 mRNA located largely in the glucagon-secreting cells of the pancreas, whereas MT2 mRNA seems to be primarily located in the insulin-producing cells (62, 63). For example, melatonin can affect transcription factors involved in insulin secretion (83) and mutations in the gene encoding the MT2 receptor, resulting in impaired melatonin signalling increases the risk of type 2 diabetes (63).

In summary, both melatonin and its receptors are widely expressed in different organs and melatonin in the gut appears to play an important role in many regulatory functions. However, studies demonstrating the expression of melatonin and melatonin receptors in the human GI tract and pancreas are scarce.

Immunomodulatory functions of melatonin

Perhaps the first and most important function of melatonin during evolution is as a powerful antioxidant, acting as a scavenger of free radicals (11, 84). In contrast to other well-known antioxidants, which generally neutralise one free radical per molecule, melatonin is far more powerful. The reason for this expansive anti-oxidative effect is the cascade of metabolites generated when melatonin interacts with reactive oxygen or nitrogen species. Many of these metabolites also have radical scavenging capacities, some even more powerful than melatonin (85). In addition, melatonin can reduce oxidative stress indirectly by influencing the expression and activity of other antioxidant enzymes (86).

Melatonin also acts as a modulator of immune functions and human lymphocytes produce large amounts of melatonin (87). According to a recent review, melatonin may act as a stimulatory factor on the immune system in immunosuppressive conditions and, in contrast, augment anti-inflammatory effects in acute inflammation (88). Melatonin and its precursor, serotonin, are abundant in the GI tract and both are important modulators of immune responses. For example, endocrine cells lining the gut can modulate the activity of immune cells. By interaction with EC cells, CD4+ T cells can increase serotonin production, and conversely, serotonin can regulate inflammation, activating immune cells to produce and secrete inflammatory mediators (89). Pro-inflammatory cytokines (such as TNF and IFN-\(\gamma\)) increase intestinal paracellular permeability, which appears to play a role in the pathophysiology of irritable bowel syndrome (IBS) and linked to more severe GI symptoms (90-92).
The immunomodulatory properties of serotonin seem to be chiefly stimulatory, where administration of serotonin receptor antagonists can attenuate intestinal inflammation (93). In contrast, significant protective effects against stress-induced damage to the intestinal mucosa have been attributed to melatonin. Luminal melatonin strengthens tight junctions and reduces mucosal paracellular permeability in the duodenum (94), which prevents bacterial translocation and leakage of inflammatory substances. Moreover, melatonin binding to MT₂ stimulates bicarbonate secretion in the duodenum, thereby protecting the mucosa from the acidic luminal contents propagated from the stomach (95). Finally, in rats melatonin administration reduced the damages seen in small intestinal microvasculature after exposure to systemic inflammation, possibly by limiting local immune cell recruitment (96). The authors proposed a therapeutic benefit of melatonin in mild systemic inflammation.

The synthesis of melatonin is dependent on the availability of its precursors, tryptophan and serotonin. Immune activation induces indolamine 2,3-dioxygenase (IDO), a key enzyme in the tryptophan metabolism, driving tryptophan towards the kynurenine pathway, away from serotonin and melatonin production (97). Both activation of IDO and the tryptophan 2,3 dioxygenase (TDO) decrease serotonin availability, and consequently, less melatonin can be synthesised (98). The metabolism of tryptophan via the kynurenine pathway results in kynurenine and other metabolites involved in immunomodulatory processes linked to depression and suicidal behaviour (99). Metabolites of the kynurenine pathway include quinolinic acid (QUIN), which is associated with neurodegenerative changes and such disorders as multiple sclerosis, Alzheimer’s disease and major psychiatric disorders. On the other hand, the metabolite kynurenic acid (KYNA) is neuroprotective, counteracting the negative effects of QUIN (100). The enzyme converting kynurenine to KYNA is up-regulated by physical exercise, which can help explain the mechanisms underlying the positive effects of physical exercise in depression (101).

In summary, low levels of melatonin, either due to a shift in tryptophan metabolism or disturbed melatonin signalling due to impaired receptor function, may be linked to symptoms of depression and anxiety.

Melatonin and cancer

When it was concluded that the risk of developing cancer is higher in shift workers exposed to light at night, melatonin and its involvement in cancer development became a subject of discussion. The increased risk is thought to be at least partly due to the suppression of nocturnal production of melatonin in the pineal gland (102). The anti-tumorigenic effects of melatonin have been investigated in several types of cancer. The overall findings demon-
strate that melatonin exhibits strong oncostatic effects, including anti-proliferative (103, 104) and pro-apoptotic actions (105).

Many studies have investigated the influence of melatonin in mammary cancer and the oncostatic effect seen appears to be related to the regulation of different hormone receptors. However, melatonin also looks to inhibit cancer growth by affecting several signalling pathways. For instance, in prostate cancer melatonin causes up-regulation of the cell cycle regulatory protein p27kip1, which could inhibit tumour proliferation (106). In human MCF-7 breast cancer cells melatonin decreases cell proliferation by increasing the expression of p53 and p21WAF1 proteins, which induce cell cycle arrest (107). One recent study demonstrated that melatonin could inhibit cytochrome C release from mitochondria, an initiator signal for apoptosis, via MT1 receptors on the mitochondrial membrane (108). The opposite is found in haematological malignancies, where melatonin appears to induce apoptosis by activation of the intrinsic and extrinsic pathway (109).

Small intestinal neuroendocrine tumours

EC cells are hormone-producing cells located throughout the GI tract. When these cells become malignant, they form neuroendocrine tumours, which also produce hormones. Small intestinal neuroendocrine tumours (SI-NETs) are characterised by their low proliferation rate and long survival expectancy and yet high propensity to metastasise to the mesentery and liver. The tumours are rare, with an incidence of 1.12 per 100 000 inhabitants in Sweden (110). However, a recent study using autopsy records discovered that the number might be higher, with subclinical disease and multiple primary tumours present before metastatic progression (111).

SI-NETs produce and secrete serotonin and other bioactive agents such as tachykinins (112), causing the carcinoid syndrome, a set of symptoms that include diarrhoea, vomiting, cutaneous flush, carcinoid heart disease and bronchial constriction. At the time of diagnosis, metastases have generally already formed.

The diagnosis of SI-NETs is based on the analysis of CgA and urinary 5-hydroxyindoleacetic acid (U-5-HIAA), radiology and histopathological analysis (113). Surgery is the primary treatment for SI-NETs, which delay the recurrence of the disease and reduce symptoms. When the tumour has spread, surgery is still beneficial to reduce hormone levels and prevent complications of fibrosis (e.g., intestinal obstruction and bowel ischemia) (114). Medical treatment includes somatostatin analogues, interferon-alpha, or both and aims to ameliorate symptoms caused by hormone production and delay progression of tumour growth (115-117).

SI-NETs constitute a form of hormone-producing tumours resulting in major effects on gut function and symptoms. What is noteworthy is that although melatonin is only two enzymatic reactions from serotonin, the produc-
tion of melatonin in these tumours is yet to be investigated. If present, this could be an important disease model for understanding the effects of melatonin in malignancy and on GI function.

Functional gastrointestinal disorders

Functional GI disorders (FGIDs) are a group of disorders without clear pathogenesis. They are disorders that are common and costly for society (and the individual), often causing considerable impact on health-related quality of life (QoL) (118). IBS is the most common FGID, with a pooled global prevalence of 11.2%, with most studies reporting a higher prevalence in women than in men (119). Symptoms of IBS include recurrent abdominal pain and bloating in combination with altered stool consistency and frequency. The diagnosis of IBS is based on the Rome Criteria, with recurrent abdominal pain, at least one day per week for the past 3 months and associated with two or more of the following: 1) related to defecation, 2) associated with a change in frequency of stool and 3) associated with a change in the form of stool (120).

Currently, there are no clinically accepted biomarkers for IBS, although several molecules have been investigated. In the context of visceral hypersensitivity, Chromogranin A, faecal calprotectin, some SCFAs and β-defensin 2 have been reported to be altered in IBS patients compared with controls (121). Changes in bile acid balance and altered colon transit have also been proposed as possible future biomarkers for significant gut dysfunction in IBS (122).

Peripheral factors reported to be involved in the pathophysiology of IBS are increased gut permeability, changes in enteroendocrine activity, dysregulation of the immune system and altered gut microbiota composition, all affecting the bi-directional communication between the gut and the brain commonly referred to as the gut-brain axis (the biochemical signalling that occurs between the GI tract and the central nervous system) (90, 123).

Of potential relevance for melatonin is that higher levels of postprandial serotonin levels in plasma have been reported in patients with diarrhoea-predominant IBS and in patients with post-infectious IBS following gastroenteritis. Patients with constipation-predominant IBS, however, had lower levels of serotonin compared with controls (124).

Melatonin has been studied in the treatment of IBS. Although the dosage of melatonin administered and the methodology of the studies vary widely, some studies have primarily found amelioration of abdominal pain and QoL in patients with IBS (125, 126).
Psychiatric co-morbidity and functional gastrointestinal disorders

Psychiatric co-morbidity is high in patients with FGIDs and up to 60% of patients who seek care for FGIDs also suffer from psychiatric disorders, predominantly depression and anxiety (127). In a Hong Kong study based on telephone surveys with questionnaires, generalised anxiety disorder (GAD) was five times more common in respondents with IBS and respondents with both IBS and GAD had higher functional impairment than those suffering from only one of the disorders (128).

Disturbances of the HPA axis are common in people with FGIDs, who typically present with high anxiety traits and psychiatric disorders. (127, 129). IBS patients have a higher increase in ACTH than controls after CRH administration and variations in the CRHR1 gene have been linked to high anxiety traits and an increased risk of developing stress-induced psychopathology (130, 131). Another proposed mechanism is via the CRH, mast cell activation and the release of proteases (132). In addition, stress can cause alterations in gut permeability, which further supports the link between the brain and gut in FGIDs.

Most studies have investigated the prevalence of psychiatric disorders in patients with IBS, but less research has been done from a psychiatric perspective, especially in younger patients in which the somatic disorder panorama is different from an older population. In this respect it is of interest to separate different types of GI symptoms in relation to anxiety traits but also current psychiatric diagnosis and depressive symptoms.

Finally, as described above, previous work indicates that melatonin signalling may be disturbed in some patients with depression and anxiety. The next question is whether different patterns of melatonin production during the day are related to specific symptoms of GI dysfunction in young adult patients with psychiatric morbidity.
Aims

The overall aim of this thesis was to study the relationship between melatonin and its receptors in the human GI tract and gut function and psychiatric disorders. The specific aims of each paper were:

I To characterise the tissue expression of melatonin and its receptors in the normal human GI tract and pancreas.

II To characterise the expression of melatonin and its receptors in small intestinal neuroendocrine tumours and further investigate possible links between circulating levels of melatonin, GI symptoms, prognostic factors and treatment response.

III To investigate the frequency and severity of IBS symptoms in young adults with mood or anxiety disorders.

IV To examine the possible associations between daytime levels of melatonin and IBS symptoms in young adults with mood or anxiety disorders.
Materials and methods

Design
The four studies of this thesis were cross-sectional observational studies.

Setting
Paper I
Human tissue, representing different parts of the GI tract, was acquired from resection margins from surgical material removed due to various malignancies in adult patients. Normal macro- and microscopic appearance of the tissue was a prerequisite for inclusion in the study.

Gene expression data for key enzymes in tryptophan metabolism, serotonin and melatonin receptors from small intestine and pancreas were extracted from the Gene Expression Omnibus (GEO).

Paper II
Tumour tissue, representing serial sections of primary tumours and metastases from patients with SI-NETs was acquired from the Laboratory of Pathology and Cytology, Uppsala University Hospital in Sweden. Patients were treated at the Department of Endocrine Oncology, Uppsala University Hospital in Sweden.

Plasma samples from patients with SI-NETs were collected before and after medical or surgical treatment and promptly stored at -80°C until assayed.

Medical records for patients with SI-NETs were examined and clinical data on age at time of diagnosis, body mass index (BMI), smoking history, diabetes, psychiatric medication, Ki67 and levels of U-5HIAA and CgA at the time of surgery or plasma sampling collected. Treatment response was classified as disease regression, stabilisation or progression, measured by biochemical or radiological examination at follow-up.
Paper III and IV

Data used in paper III and IV were collected as part of the Uppsala Psychiatric Patient Samples (UPP) cohort, a project designed to collect biological material from patients seeking psychiatric care at the Department of General Psychiatry, Uppsala University Hospital. For study III, data was collected between 2013 and 2017 from patients 18-25 years old with primarily mood and anxiety disorders; for paper IV, data were collected from the same cohort but between 2012 and 2014.

Study population and patient samples

Paper I

Tissue material from the GI tract from 39 individuals and pancreatic tissue from 3 individuals were analysed (17 purchased from Asterand, Detroit, MI, USA and 25 from the Department of Pathology, Uppsala University Hospital, Uppsala, Sweden). Biopsies represented stomach (n=12), small intestine (n=11), appendix (n=3) and large intestine (n=13). No additional clinical data for the patients were known and thus not included in the analyses.

Paper II

Fifty-two patients were included in the study. For histology assessment, only cases with paired sections of primary tumour and metastasis were included (n=26).

For plasma analyses, patients who completed plasma collection at both time points (before and after treatment) were included (n=43). Seventeen patients were included in both tissue and plasma analyses (Figure 2).
Paper III

From the UPP cohort, which comprised patients, age 18-25 seeking psychiatric outpatient care, 682 patients were eligible for this study. Patients with established inflammatory bowel disorder, endometriosis and Anorexia nervosa were excluded. In addition, patients were excluded who did not undergo a structured psychiatric assessment or when questionnaires were incomplete or improperly completed. In total, 491 (72%) patients were included in the study.

Healthy controls under the age of 30 years (n=139) were recruited from the staff of Uppsala University Hospital and students at Uppsala University. Of these, 85 (61%) were included in the statistical analyses (Figure 3).

Paper IV

For paper IV, the study population originated from the same UPP cohort as for paper III. From 621 eligible patients, 264 (43%) agreed to participate in this study and 102 (39%) of these patients completed both saliva sampling and validated questionnaires for GI symptoms. Six patients were excluded from the analyses because they did not fulfil criteria for any DSM-IV axis I diagnosis.
Microarray expression analysis

For paper I, raw data from human small intestinal epithelium, of which it is estimated that 25% of the captured cells are endocrine, and from human normal pancreas tissue were extracted from GEO (133). The gene expression series is summarised in Table 2.

Data were imported into Expression Console software provided by Affymetrix (http://www.affymetrix.com) and normalised with the robust multi-array average method (134). Log2 expression signals were then extracted. Using the statistical computing language R (http://www.r-project.org), gene expression data for melatonin and serotonin receptors as well as key enzymes in melatonin synthesis were analysed in the data from small intestinal epithelium, whole pancreas and pancreatic islets. Table 4 presents a summary of the genes analysed. Expression signals for parathyroid hormone were used as a negative control.

Differences in gene expression between samples from colon biopsies in patients with ulcerative colitis (UC) and healthy controls were analysed using the Linear Models for Microarray Analysis (Limma) R package on GEO2R (http://www.ncbi.nlm.nih.gov/geo/info/geo2r.html).
Table 2. Summary of the gene expression series from the Gene Expression Omnibus data archive used in the microarray analyses. Adapted from licentiate thesis (8).

<table>
<thead>
<tr>
<th>GEO series</th>
<th>Tissue</th>
<th>Platform</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSE9576</td>
<td>Small intestinal epithelium</td>
<td>Affymetrix Human Genome U133 Plus 2.0 Array (GEO Accession GPL570)</td>
<td>(135)</td>
</tr>
<tr>
<td>GSE16515</td>
<td>Pancreas</td>
<td>Affymetrix Human Genome U133 Plus 2.0 Array (GEO Accession GPL570)</td>
<td>(136)</td>
</tr>
<tr>
<td>GSE15471</td>
<td>Pancreas</td>
<td>Affymetrix Human Genome U133 Plus 2.0 Array (GEO Accession GPL570)</td>
<td>(137)</td>
</tr>
<tr>
<td>GSE3842</td>
<td>Pancreas</td>
<td>Affymetrix Human Gene 1.0 ST Array (GEO Accession GPL6244)</td>
<td>(138)</td>
</tr>
<tr>
<td>GSE11223</td>
<td>Colon</td>
<td>Agilent-012391 Whole Human Genome Oligo Microarray G4112A</td>
<td>(139)</td>
</tr>
</tbody>
</table>

Table 3. Summary of genes analysed using microarray analysis. Adapted from licentiate thesis (8).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Main function</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPH1</td>
<td>Tryptophan hydroxylase 1</td>
<td>Converts Trp to 5-OH-Trp</td>
</tr>
<tr>
<td>TPH2</td>
<td>Tryptophan hydroxylase 2</td>
<td>Converts Trp to 5-OH-Trp</td>
</tr>
<tr>
<td>TDO2</td>
<td>Tryptophan-2,3-dioxygenase</td>
<td>Converts Trp to N’-formyl kynurenine</td>
</tr>
<tr>
<td>IDO1</td>
<td>Indoleamine dioxygenase</td>
<td>Converts Trp to N’-formyl kynurenine</td>
</tr>
<tr>
<td>DDC</td>
<td>Amino acid decarboxylase</td>
<td>Converts 5-OH-Trp to 5-HT</td>
</tr>
<tr>
<td>AANAT</td>
<td>Arylalkylamine N-acetyltransferase</td>
<td>Converts 5-HT to N-acetyl serotonin</td>
</tr>
<tr>
<td>HIOMT</td>
<td>5-hydroxyindole O-methyltransferase</td>
<td>Converts N-acetyl serotonin to melatonin</td>
</tr>
<tr>
<td>MTNR1A</td>
<td>Melatonin receptor type 1</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>MTNR1B</td>
<td>Melatonin receptor type 2</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>5-HTR1</td>
<td>5-HT receptor 1</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>5-HTR2</td>
<td>5-HT receptor 2</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>5-HTR3</td>
<td>5-HT receptor 3</td>
<td>Ligand-gated ion channel</td>
</tr>
<tr>
<td>5-HTR4</td>
<td>5-HT receptor 4</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>5-HTR5</td>
<td>5-HT receptor 5</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>5-HTR6</td>
<td>5-HT receptor 6</td>
<td>G-protein coupled receptor</td>
</tr>
<tr>
<td>5-HTR7</td>
<td>5-HT receptor 7</td>
<td>G-protein coupled receptor</td>
</tr>
</tbody>
</table>
Immunohistochemistry

Tissue specimens representing the normal human GI tract and tumour tissue were stained for melatonin, MT₁ and MT₂ receptors and serotonin. Tissue sections from human skin served as positive controls for MT₁ and MT₂ and antibody specificity tests were performed for melatonin, MT₁ and MT₂. For double immunofluorescence staining, tissue sections were incubated with a cocktail of two primary antibodies. Detailed information on immunohistochemical methods, antibodies and positive/negative controls is provided in paper I.

Histological assessment – Paper I

Two independent observers examined each tissue specimen microscopically and assessed immunoreactivity (IR) for melatonin, MT₁ and MT₂ receptors. Intensity was classified into four categories: negative, weak, medium or strong. IR localisation in different cell types was documented.

Co-localisation studies of sections with double immunofluorescence staining were performed using a Zeiss 510 confocal microscope.

Histological assessment – Paper II

Tumour tissue specimens were examined microscopically by three independent observers and IR intensity for melatonin, MT₁ and MT₂ was classified after study I. The intraclass correlation coefficient between observers was 0.955, indicating high reliability. Tissue specimens were also analysed using Image J software and the intraclass correlation coefficient between computerised and manual scoring was high (0.934).

Hormone collection and analysis

Analysis of melatonin in plasma – Paper II

Plasma samples were collected at two time points (before and after treatment). Sampling was performed in the morning from fasting patients. Plasma levels of melatonin were measured using competitive radioimmunoassay (Melatonin direct RIA, LDN, Norhorn, Germany).

Analysis of melatonin in saliva – Paper IV

Patients collected saliva samples at home at six time points for one day: when waking up, 30 min after waking up, at 11:00, 30 min after lunch, at 22:00 and bedtime. Salivary melatonin levels were measured at the Depart-
ment of Clinical Chemistry at Uppsala University Hospital, using the competitive enzyme-linked immunosorbent assay technique (ELISA) (Direct Salivary Melatonin Elisa EK-DSM. Bühlmann Laboratories AG.Schönenbuch. Switzerland). The three time points representing daytime melatonin measurements (30 min after waking up, at 11:00, 30 min after lunch) were selected for statistical analysis.

Figure 3. Illustration of saliva samples and ELISA with the three daytime measurement time points selected for analyses in paper IV.

Psychiatric assessment and questionnaires – Paper III and IV

A trained doctor in psychiatry or a clinical psychologist performed the psychiatric assessment by structured or semi structured interviews, using the Structural Clinical Interview for DSM-IV Axis I Disorders – Clinical Version (SCID-I) or the Mini-International Neuropsychiatric Interview (M.I.N.I.). In conjunction with the psychiatric assessment, a physical examination was performed and BMI recorded.

Self-assessment – Paper III and IV

Montgomery-Åsberg Depression Rating Scale

The self-rating version of the Montgomery-Åsberg Depression Rating Scale (MADRS-S) was used to rate depressive symptoms. The MADRS-S is a nine-item diagnostic questionnaire with items rated on a six-point Likert-scale. The items include mood, feeling of unease, sleep, appetite, ability to concentrate, initiative, emotional involvement, pessimism and zest for life. The overall score ranges from 0-54 points.

Gastrointestinal Symptoms Rating Scale for IBS

The Gastrointestinal Symptoms Rating Scale for IBS (GSRS-IBS) was used to measure GI symptoms. The GSRS-IBS is a validated self-assessment in-
instrument for the evaluation of IBS symptoms (140). The instrument contains 13 questions on a seven-point Likert-scale (1-7), spanning from no symptoms to very severe symptoms during the past week. The overall score ranges from 13-91. The questions are grouped into five symptom clusters depicting pain, bloating, constipation, diarrhoea and satiety.

**Swedish Universities Scales of Personality**

The Swedish Universities Scales of Personality (SSP) instrument was used to estimate personality traits at the baseline assessment. The SSP is a revised version of the Karolinska Scales of Personality (KSP), a self-rating questionnaire with improved psychometric properties. The instrument contains 91 items divided into 13 scales (141). Normative T-scores for the extracted scales of somatic trait anxiety (STAT), psychic trait anxiety (PsSTAT) and stress susceptibility (SST) were used for data analysis in paper III.

**Statistics**

**Paper II**

Data were analysed using the Statistical Program Package for the Social Sciences (SPSS Inc., Chicago, IL, USA). Statistical analysis of the two cohorts, where 1) tumour tissue and 2) plasma samples were available, was conducted separately and they were not compared with each other.

Survival was calculated from time of diagnosis to death (or, if still alive, to 25 April 2015) and analysis was performed using the Mantel-Cox log-rank test. The Wilcoxon signed-ranks test was applied for comparison of IR intensity between primary tumour and metastasis and for change in biomarker response between samplings.

The Spearman’s test (rho) was used to calculate pairwise correlations between IR intensity or plasma levels of melatonin and clinical parameters. Sex differences in plasma levels of melatonin were analysed using the Mann-Whitney U test.

**Paper III**

The Mann-Whitney U test was performed to compare GSRS-IBS scores between patients and healthy control, followed by the Kruskal-Wallis one-way analysis of variance for comparison between three groups (patients with psychotropic medication, psychotropic medication-free patients and controls).

A principal component analysis (PCA) was performed using normalised z-scores of the individual items of the MADRS-S and GSRS-IBS but also the scales of STA, PsTA, SS and BMI to identify principal components,
which accounted for most of the variance in the dataset. A two-step cluster analysis method was then performed to look for subgroups in the patient population.

Spearman’s rank test (rho) was carried out to assess pairwise correlations between GSRS-IBS scores, MADRS-S scores and the STAT, PsTAT and SST SSP subscales. To control for possible confounding factors a generalised linear model was constructed using the covariates sex, BMI and ongoing bulimia nervosa. A second model was then performed that also included the three scales representing trait anxiety.

**Paper IV**

Generalised linear model analyses were conducted for salivary melatonin at the three time points in relation to GSRS-IBS scores. Sex, BMI, antidepressive medication and oral contraception were included as potential confounders. The Bonferroni correction method was applied to adjust for multiple comparisons. Post-hoc explorations of correlations between the GSRS-IBS subscales and salivary melatonin were performed using the Spearman’s rank test.
Summary of results

Melatonin and melatonin receptor expression (Paper I)

Normal human GI tract
Positive IR for melatonin was found in EC cells throughout the GI tract. Co-localisation studies showed cellular localisation of melatonin in the cytoplasm that was only partially overlapping with serotonin. In addition, diffuse melatonin IR was seen in the cytoplasm of enteric epithelial cells and mononuclear immune cells of the lamina propria.

MT₁ IR was negative in EC cells throughout the gut, whereas positive IR for MT₂ was found in EC cells in 5 of 12 of the gastric sections and all of the sections from the small intestine and large intestine and appendix. In large intestinal epithelial cells both MT₁ and MT₂ IR were strong, with cellular localisation in the cytoplasm. Positive IR for both receptors was also seen in the myenteric plexus and vascular structures, with generally more positive sections and higher intensity for MT₂ compared with MT₁. In the submucosal plexus only positive IR for MT₂ IR was seen.

The gene expression analysis supported these results in that mRNA levels of MT₂ were higher than MT₁ in small intestinal epithelium enriched for EC cells.

Normal human pancreas
Melatonin IR was identified in endocrine cells of pancreatic islets, where also MT₂ IR was positive in a subset of endocrine cells. MT₁ IR varied between sections, with medium IR in endocrine cells in one section, whereas the other two tissue sections were negative for MT₁.

In the gene expression analysis mRNA for the gene encoding MT₂ was higher than for MT₁ in pancreatic tissue, which supports the IHC results.

Melatonin and melatonin receptors in SI-NETs (Paper II)

Tissue expression
Positive melatonin IR was seen in all tumour tissue sections, with varying IR intensity (weak to strong) in primary tumours and in metastases. MT₁ IR was
weak or negative in tumour tissue, whereas MT$_2$ IR was positive in all sections representing primary tumours and in 92% of sections representing metastases. IR intensity of MT$_2$ was lower in metastases compared with primary tumours ($r=0.53; p=0.007$).

**Plasma levels**

The median level of melatonin in morning plasma from patients with SI-NETs was 26.0 pg/L [4.5–220.0] before treatment and 23.0 pg/L [8.9–90.7] after treatment. Patients with disease regression or stabilisation according to biochemical or radiological response had lower levels of plasma melatonin at the second sampling ($r=0.356; p=0.038$).

**Relationship between melatonin tissue intensity, plasma levels and clinical parameters**

Higher melatonin IR intensity in primary tumours correlated with lower Ki67 ($r=-0.446; p=0.022$) and to less reported symptoms of diarrhoea ($r=-0.484; p=0.012$).

A positive correlation between higher levels of melatonin in plasma and increased nausea or vomiting was noted at both sampling time points ($\rho=0.337; p=0.027$ and ($\rho=0.413; p=0.006$). High melatonin levels at the second sampling (i.e. post-treatment) also correlated with the symptom of flush ($\rho=0.353; p=0.020$).

**GI symptoms and depressive symptoms (Paper III)**

In this study 491 patients (mainly with mood or anxiety disorders) and 85 healthy controls were included. Patients had higher MADRS-S scores (median 23 vs. 4; p<0.001), and GSRS-IBS scores (median 30 vs. 22; p<0.001) than controls. There were no differences in total GSRS-IBS scores between patients receiving psychotropic medication and psychotropic medication-free patients.

Using PCA, six factors were identified, which accounted for 63.9% of the variation in the dataset. They were characterised according to the items that loaded the highest on each construct and were labelled as follows: slow bowel, fast bowel, depressive symptoms, trait anxiety, disturbed appetite and BMI. A cluster analysis resulted in six clusters and the characteristics of the six cluster groups are shown in Figure 5.

Higher MADRS-S total scores correlated with higher GSRS-IBS total scores and the association remained significant after controlling for sex, BMI and ongoing bulimia in a generalised linear model. The GSRS-IBS total
scores also correlated with all three SSP subscales analysed: STAT (rho=0.313; p<0.001), PsTAT (rho=0.147; p=0.001) and SST (rho=0.233; p<0.001). The correlation with STAT showed the largest effect size of the three subscales. In a second generalised linear model we discovered that all studied factors were independent predictors of higher GSRS-IBS total score.

Figure 4. Chord diagram of the different cluster groups for the six factors extracted from the principal component analysis. Abbreviations: MADRS-S = Montgomery-Åsberg Depression Rating Scale self-rating version, GSRS-IBS = Gastrointestinal Symptoms Rating Scale for Irritable Bowel Syndrome, STAT = Somatic trait anxiety T-score, PsTAT = Psychic trait anxiety T-score, SST = Stress susceptibility T-score, BMI = body mass index.

Melatonin levels and GI symptoms (Paper IV)
Daytime melatonin levels in saliva were analysed and compared with reported GI symptoms in 96 young adult patients seeking psychiatric care for
mainly mood or anxiety disorders. The median levels of salivary melatonin were 6.9 (2.7–15) 30 min after waking up, 3.3 (1.8–5.8) at 11:00 hours and 2.8 (1.8–4.7) 30 min after lunch. The median total GSRS-IBS score was 31 (range=13–78).

Higher levels of melatonin in saliva after lunch correlated with higher total GSRS-IBS scores, a relationship that remained significant after controlling for possible confounding factors ($p=0.015$, $q=0.045$) and bloating and pain contributed most to this association.
Discussion

The present studies have demonstrated the widespread presence of melatonin and melatonin receptors in the normal human gut, pancreas, and for the first time, in SI-NETs. In these tumour patients melatonin levels in plasma and IR in the tumours correlated with clinicopathological features. Moreover, young adult patients seeking psychiatric care commonly report GI symptoms, which are independently associated with sex, depressive symptom severity, trait anxiety and BMI. In agreement with our hypothesis of a role for melatonin in GI symptoms from paper II a link between daytime melatonin levels and GI symptoms was identified in young adults with psychiatric morbidity.

Melatonin and melatonin receptors in normal human gut and pancreas

In paper I we demonstrated positive IR for melatonin in EC cells, a finding concurring with previous knowledge from animal studies in which the enzymes needed for melatonin synthesis have been identified in EC cells (71, 142, 143). Microarray gene analyses also supported this finding and together these results strongly suggest the production of melatonin in EC cells in humans. In addition, a diffuse positive IR for melatonin was seen throughout the gut, with localisation in the cytoplasm of epithelial cells and the mucus layer lining the epithelium. A tendency towards higher intensity was observed in the aboral direction, indicating an accumulation of melatonin. Melatonin is amphiphilic and, unlike serotonin, not stored in secretory vesicles. It can easily cross cell membranes, which could explain the scattered distribution reported in IHC analyses. In addition to synthesis in EC cells, melatonin is produced by microorganisms and the intestinal microbiota may further contribute to melatonin accumulation in the large intestine (144). Finally, knowing that vegetables and meat contain melatonin, part of the melatonin noted in the mucosal lining may originate from ingested food. (6, 145).

The distribution of MT1 and MT2 melatonin receptors was somewhat different. MT2 IR was seen in EC cells, whereas MT1 IR was negative. In the epithelium IR for both receptors was positive, with a generally higher intensity for MT2. MT1 IR localisation in epithelial cells of the colon is in agreement with a study of human colonic mucosa (53). Beyond this, human stud-
ies are limited and receptor expression has mainly been investigated in animals. A study in rats demonstrated MT$_1$ mRNA in the sub-epithelial layers of the GI tract, with generally low levels of mRNA in the epithelium (79). The reason for this discrepancy may be related to differences in feeding behaviour and diet, as melatonin levels vary with fasting and food intake, possibly regulating receptor expression through a feedback mechanism. IHC studies of rat intestine found intense expression of MT$_2$ in colonic smooth muscle, but not in the villi or the crypts of the mucosa (146). The finding of MT$_2$ in the human colonic epithelium may, in addition to species-specific differences, be caused by the use of different antibodies. However, we show that the machinery for melatonin synthesis is actively expressed in the human gut and thus likely not only absorbed from food containing melatonin.

Some of the central functions of gut melatonin include protecting the mucosal barrier (e.g., by reducing paracellular permeability and increasing bicarbonate secretion) (94, 95). These effects appear to be receptor-mediated given that the effect was abolished when adding a melatonin receptor antagonist. In vascular structures IR for both receptors was positive and melatonin has recently been shown to reduce inflammatory damage to the microvasculature in the small intestine (96).

In response to inflammation induced by lipopolysaccharide administration to rodents an increase in the AANAT enzyme has previously been demonstrated in macrophages in mice resulting in melatonin synthesis (147). The radical scavenging properties of melatonin, protecting lipids, proteins and nuclear DNA from oxidative stress, are receptor-independent, merely requiring the close presence of melatonin where the radical is formed (148). Moreover, in patients with UC, both an increased number of EC cells and higher levels of HIOMT in colonic mucosa have been reported (149). In paper I, however, there were no significant differences in gene expression of key enzymes in melatonin synthesis or melatonin receptors in tissues from patients with UC compared with controls.

Melatonin appears to be involved in glucose homeostasis. In paper I pancreatic endocrine cells demonstrated positive IR for melatonin and MT$_2$. Gene expression analysis supported the finding of a dominating MT$_2$ receptor expression, with higher levels of MTNR1B (encoding MT$_2$) compared with MTNR1A (encoding MT$_1$), which, however, is not consistent with previous studies in which the relationship was found to be reversed (62). A recent study investigating the expression of MT$_1$ and MT$_2$ melatonin receptors in human pancreas found receptor expression in alpha, beta and delta cells, with the highest receptor density in alpha cells (150). Mutations in the MT$_2$ receptor that cause impaired melatonin signalling, and decreased melatonin secretion increase the risk of type 2 diabetes (151, 152). One study in rats demonstrated that the administration of a MT$_1$/MT$_2$ agonist ameliorated impaired glucose metabolism induced by chronic stress and high-fat diet. The authors concluded that the mechanism for this was through normalisation of
the HPA axis function, evidencing important regulatory receptor-mediated actions of melatonin in glucose homeostasis (153).

Melatonin and melatonin receptors in SI-NETs

In paper II the presence of melatonin in SI-NETs has been described for the first time. Our analysis also revealed that all tumour sections (primary tumour and metastasis), showed positive IR for melatonin. Higher IR intensity was significantly associated with a lower Ki67. Ki67 is a widely used marker for proliferation and the discovered association could indicate anti-proliferative effects of melatonin in these tumours. The anti-tumorigenic effects of melatonin in other non-endocrine cancers are more extensively studied and include several mechanisms and signalling pathways that result in proliferation suppression, induction of apoptosis, anti-metastatic actions and a drive towards cell differentiation. The oncostatic effects of melatonin have recently been reviewed (106, 154). Most studies, however, have investigated the effect of melatonin administration in different cancers, rather than the endogenous melatonin effect. In prostate cancer, for instance, melatonin administration stopped cell cycle progression, reduced cell growth and stimulated cellular differentiation (155).

Melatonin receptor expression in different types of solid tumour varies and its clinical implications may be of varying importance. In GI neuroendocrine tumours the influence of melatonin has, to our knowledge, not been investigated. In ovarian cancer, MT1 receptor expression was identified but there was no correlation between receptor expression and prognostic factors (156). In mammary and prostate cancer, however, a growth inhibitory effect of melatonin has been demonstrated and linked to the MT1 receptor (106, 157). Therefore, a more dominant expression of MT1 could have been expected. The MT2 receptor has not been as extensively investigated in the context of tumour progression, although one study in mice found oncostatic effects in colon cancer, probably mediated via the MT2 receptor but also via nuclear receptors of the ROR/RZR family (158). In addition, the growth-inhibiting effects of melatonin in melanoma are presumably also mediated through the MT2 receptor (159). In paper II we investigated SI-NETs and found that MT2 IR was rich in primary tumours and generally lower in metastases. Down regulation of receptors may indicate a reduced sensitivity to the anti-proliferative effects of melatonin with increasing malignant progression.

Daytime levels of melatonin are primarily of peripheral origin and the gut constitutes a major source of extrapineal melatonin (78). With possible melatonin production in SI-NETs, higher circulating levels of melatonin could have been expected. However, as mentioned earlier, melatonin is not confined to the bloodstream but passes freely across membranes. Moreover,
clearance through the liver is high, which could explain why levels in plasma remain modest (75). In tumour patients with disease regression or stabilisation plasma a reduction in levels of melatonin was noted at the second sampling (after treatment), possibly because of a reduction in tumour burden and less tumour-derived melatonin.

The most prominent symptom of SI-NETs is diarrhoea, with watery stools up to 10 times per day. This symptom is mainly caused by the overwhelming release of serotonin. Melatonin affects motility and its actions appear to be largely inhibitory, with a longer colonic transit time (160). In rats melatonin administration attenuated the stress-induced increase of faecal output and decreased the dry weight of stool (161). In paper II patients with high melatonin IR in tumour sections reported less difficulty with diarrhoea, speculatively related to a larger inhibitory effect of locally produced melatonin. In addition, circulating levels of melatonin correlated positively with more nausea or vomiting. Theoretically, slower intestinal transit can cause nausea because blocking of 5-HT₃ receptors prolongs colonic transit time and 5-HT₃ antagonists are used as an anti-emetic, relieving symptoms of nausea (162, 163). Other possible explanations for the symptom of nausea are high serotonin levels. Yet, in this study there was no correlation between the levels of U-5-HIAA at the time of sampling and nausea. Circulating levels of melatonin also correlated with the symptom of flush. Other active substances, such as tachykinins, are known to be involved in flush (112). Because it has been shown to have vasodilatory effects by binding to the MT₂ receptor in arterial smooth muscle, melatonin may also influence circulatory changes (164).

Melatonin is often used in psychiatric care, to treat depression and sleep disturbances. The hypothesis that high circulating levels of melatonin could have an influence on mental health and sleep in these patients was also tested. The use of medication for depression, anxiety or sleep disturbances was explored in patient medical records, but no associations were identified. Furthermore, the study design did not allow for reliable evaluation of more complex psychiatric symptoms and information concerning mental health was not routinely requested or recorded.

**GI symptoms and psychiatric comorbidity**

In paper III we investigated self-reported GI symptoms in young adult patients seeking psychiatric care, predominantly for mood and anxiety disorders. Patients more commonly reported GI symptoms than healthy controls and higher GI symptom burden correlated with depressive symptom severity, which is in accord with previous studies (129, 165-167). Patients with IBS often have psychiatric co-morbidity and implications of disturbed signalling pathways between the brain and the gut, which largely affects the HPA axis, have been proposed as a common pathogenesis. Pa-
Patients with IBS appear to have an exaggerated stress response compared with controls, with both elevated levels of basal cortisol and stress-induced cortisol levels (168). Early life stress, as modelled by maternal separation in rodents shows long-term alterations in gut functions, including a compromised intestinal mucosal barrier and bacterial translocation (169). Activation of the HPA axis also results in increased CRH production, which is implicated in stress-induced gut dysfunction.

Surprisingly, there was no difference in GI symptom burden between patients receiving psychotropic medication and those without. Both SSRI and other antidepressants have been proposed to treat IBS. A recent review found that treatment with antidepressants, particularly tricyclic antidepressants, improved IBS symptoms, especially in patients with diarrhoea-predominant IBS (170). However, SSRI and other antidepressants have well-known GI side effects. Thus any positive effects may have been cancelled out in analyses of the total patient group.

In a PCA and cluster analysis six cluster groups were identified demonstrating a heterogeneity in both psychiatric and GI symptoms. One of the cluster groups did not appear to have high GI symptoms, whereas all the others scored high on either symptoms of fast or slow bowel or disturbed appetite.

**Daytime melatonin and GI symptoms**

In paper IV a link between daytime levels of salivary melatonin and GI symptoms was identified. Melatonin levels after lunch correlated with higher GSRS-IBS scores and more specifically to the symptoms of bloating and pain. A possible explanation for this link may be through the motility regulating actions of melatonin that can be both stimulatory and inhibitory depending on the concentration of melatonin (81). Daytime levels of melatonin are believed to be of peripheral origin, and because the gut constitutes a major source, circulating levels likely reflect the levels of the GI tract. The reliability of salivary melatonin as a reflection of plasma levels has previously been confirmed (171). An increased number of EC cells have been reported in patients with IBS compared with controls (172). This increased EC cell level could theoretically result in higher levels of melatonin during the day. Earlier studies have reported that melatonin levels in the GI tract are related to fasting and food intake, with both causing an elevation in melatonin levels (77, 78). However, studies in humans and in different animal species have reported ambiguous results, which may be a consequence of differences in feeding behaviour, the extent of food restriction and measuring methods.

The correlation between melatonin and pain was somewhat surprising seeing that previous studies have indicated a beneficial effect of melatonin administration and abdominal pain, rectal pain sensitivity, and extra colonic
symptoms in patients with IBS (126, 173). In these studies, however, melatonin administration was at night, when food processing is less active.

Because both depression and functional GI disorders (e.g., IBS) are related to increased inflammation, melatonin elevation may be secondary to immune activation and low-grade inflammation in this cohort.

Methodological considerations and limitations

The work included in this thesis has several limitations. In paper I and II most of the results are based on immunohistochemical analyses. A recognised limitation in immunohistochemical methods is the risk of cross-reactivity in which the antibody, instead of binding to the intended antigen, binds to something similar. Melatonin is a small molecule, similar to serotonin, and therefore cross-reactivity is challenging. To ensure reliability of the method we performed antibody neutralisation tests in which the antibody for melatonin was preincubated with both melatonin and serotonin. The antibody did show partial IR for serotonin, indicating cross-reactivity. To further minimise this limitation, co-localisation studies were performed. Only partial overlap in melatonin and serotonin IR was seen, indicating that the antibody indeed binds to melatonin. In addition, there was no correlation between IR for serotonin and melatonin in serial sections, denoting separate antibody targets.

Paper I aimed to characterise the expression of melatonin and melatonin receptors in normal human GI tract. However, the biopsies from normal tissue may have been influenced by local immunological processes. This possibility may have arisen because tissue specimens were obtained from patients undergoing surgery for different types of cancer. Given melatonin’s important functions in both cancer and modulation of inflammation, this might constitute an important confounding factor. Pancreatic tissue was only available from three individuals and the expression showed substantial variation. The small sample size prevents any firm conclusions on receptor expression.

In paper II correlation analysis between melatonin IR or plasma levels of melatonin and clinical parameters (e.g., symptoms) were performed. The study design, however, was not optimal for this analysis because information about symptoms was extracted from medical records and not standardised in rating scales. The validity of this information is therefore questionable and should be considered exploratory.

The GSRS-IBS in paper III and IV was used to evaluate GI symptoms in psychiatric patients, which is somewhat outside the intended scope. The GSRS-IBS is originally designed to identify the most bothersome symptoms and evaluate response to treatment in patients with IBS. Still, in these studies the scale was chosen considering that it covers a variety of symptoms and
the possibility to classify GI symptoms into symptom clusters in patients not primarily seeking care for GI symptoms, but with an anticipated high prevalence of GI symptoms.

In paper II and IV melatonin levels, local in tissue, plasma and saliva were analysed. From these results, the origin of the melatonin measured is not possible to determine and several alternative or contributing sources of melatonin have to be considered. As an illustration, both plants and animals contain melatonin and intake of melatonin- or tryptophan-rich foods could contribute to higher levels (174). In paper II plasma samples were collected after overnight fasting, which minimises the interaction of dietary melatonin. In paper IV this interaction is probably higher, and a limitation is the lack of information for the term “lunch”. Patients collected saliva after lunch, but no information of what they ate or how much was available. The cross-sectional design (paper II and IV) used in these studies restricts in establishing cause-effect relationships between melatonin and GI symptoms.

Clinical implications and future perspectives

Communication between the gut and brain is a rapidly growing field of research. It is a complex system that is difficult to grasp and possibly even more difficult to study.

Many of the studies on melatonin in the GI tract have been conducted in animals. While animal models are necessary for practical reasons, to be able to study aetiological factors and mechanisms, the direct translation of animal results to humans cannot be assumed i.e. animal trial results may not always translate to humans. Of special importance in this context are differences in circadian rhythm, diet, feeding behaviour and microbiota composition (175, 176). Paper I in this thesis constitutes basic research, but is an essential piece of the puzzle. Indeed, some variations in melatonin receptor expression from previous animal studies were found (79). Further exploration of the melatonin system in other clinical patient groups may add nuances to our understanding of its regulation and function.

In paper II, melatonin was identified in SI-NETs, which are known to produce serotonin. Based on previous research on EC cells, it is likely that the tumour cells also produce melatonin (72, 73). These tumours could therefore be used as a model to study the effects of increased local melatonin production and accumulation. Moreover, contradictory results have been demonstrated on the role of melatonin in regulation of apoptosis in different studies. In various types of cancer, melatonin can induce apoptosis, whereas in normal tissues (such as neuronal mitochondria and umbilical endothelium) melatonin reduces apoptosis (105, 108, 177, 178). In this respect it is of interest that small intestinal neuroendocrine tumours generally display very low levels of apoptosis (179). In addition, a lower intensity of MT2 receptor
expression was seen in metastases compared with primary tumours, which may also account for changes related to malignant progression and impaired melatonin signalling. This indicates that the melatonin system may be a possible treatment target worth further investigation.

In paper I and II membrane-bound receptors were studied. Nuclear receptors of the ROR/RZR family may play a role in mediating the antiproliferative effects of melatonin (180). Their function and impact in healthy tissue and melatonin-containing tumours are yet to be investigated.

Behaviour and mood are influenced by numerous factors. It is central to identify subgroups of patients that may benefit from more targeted treatment, and for this purpose, novel biomarkers are needed. One of the challenges when studying IBS and mood disorders is that both are wide concepts with large differences within the patient groups. Therefore it is essential to identify the underlying causes of both disorders and to find subgroups that are more alike. In paper III cluster analysis revealed different cluster groups characterised by varying levels of psychiatric and GI symptoms.

The link between depression, anxiety and somatic symptoms requires further investigation and if any conclusions are to be stated regarding causality, long-term observation time and prospective studies are needed. In addition, repeated measurements of possible biomarkers (in this case melatonin) following treatment or intervention are desirable. Considering the pronounced anti-inflammatory actions of melatonin, measurements of inflammation with respect to melatonin levels and GI symptoms may further identify subgroups of patients with possibly different underlying pathology.

Some of the common pathogenic mechanisms of functional GI disorders and depression include inflammation and changes in microbiota composition. Some bacteria can produce melatonin but also other factors that may have secondary effects on melatonin pathways (181). The administration of probiotics has shown hopeful results in improving not only symptoms of IBS but also in reducing depressive symptoms (182). These results, however, are still in the very early stages and remote from stable evidence-based treatments.

Mutations in genes affecting melatonin synthesis and signalling have been linked to autism spectrum disorders (183, 184). In future studies vulnerability factors, such as genetic variants affecting melatonin synthesis, gut dysfunction and metabolic alterations, should be studied in relation to clinical symptom subgroups that extend beyond autism. Possible future biomarkers need to be evaluated concerning not only gut function and GI symptoms but also psychiatric symptoms in order to develop strategies for screening and treatment.
Ethics

The projects included in this thesis all obtained ethical approval by the Regional Ethics Committee in Uppsala (Dnr 2007/143/1 with decisions 2007-06-13 and 2012-09-14, Dnr 2012/81, Dnr 2012/81/1, Dnr 2013/219). All study patients signed a written informed consent. For study I, written informed consent was obtained for material collection, but not for this specific study on normal tissue in that the data were analysed anonymously and samples cannot be traced back to individuals. The Regional Ethics committee waived the need for consent in this case in accordance with Swedish law.
Conclusions

Melatonin and its receptors are widely expressed in the human gut and pancreas: in EC cells, epithelial cells, in immune cells, vascular structures and muscular cells. This widespread expression is consistent with the multiple proposed roles of gut melatonin, including regulation of motility, gut permeability, immunomodulation and influence of glucose homeostasis and metabolic control.

Neuroendocrine tumours derived from EC cells display positive melatonin IR and higher melatonin IR intensity is associated with lower proliferation index, supporting the previous findings of melatonin as an oncostatic agent. Melatonin IR intensity and circulating levels of melatonin in patients with SI-NETs are associated with GI symptoms and treatment response.

Young adult patients seeking psychiatric care report more GI symptoms than controls, regardless of ongoing psychotropic medication. Moreover, GI symptom burden is associated with depressive symptom severity and anxiety traits. In the same cohort salivary melatonin levels after lunch are associated with increased GI symptoms, especially bloating and pain.

Taken together, the present results confirm a link between endogenous melatonin regulation and IBS symptoms in patients with psychiatric disorders.
Sammanfattning på svenska

Bakgrund
Melatonin är ett hormon som produceras i tallkottkörteln och som är involverat i reglering av sömn och dygnssytem. Melatonin finns också i många perifera organ och vävnader och har betydligt fler funktioner utöver sömnreglering. Förekomsten av melatonin och melatoninreceptorer har framför allt studerats hos djur och mag-tarmkanalen har föreslagits vara den största källan till perifert producerat melatonin och mer specifikt enterokromaffincellerna, som också producerar serotonin. När enterokromaffinceller blir maligna, bildas hormonproducerande tumörer som visserligen är ovanliga, men som ger stora bekymmer med symptom i form av diarré på grund av kraftig frisättning av serotonin.

En av de viktigaste egenskaperna hos melatonin är dess antiinflammatoriska effekt och förmågan att binda fria syreradikaler, något som har varit avgörande evolutionärt, till exempel för energiproduktion i mitokondrier, där stora mängder fria radikaler bildas. Melatonin är också viktig för reglering av glukosmetabolismen och har visat sig ha positiva effekter vid olika typer av cancer. I mag-tarmkanalen reglerar melatonin motorik och sekretion, samt skyddar tarmslemhinnan, som utgör en viktig barriär för att motverka uppkomsten av låggradig inflammation.

Olika processer i mage och tarm kan genom signalering till hjärnan ge förändringar i psykiskt mående och beteende, något som på engelska brukar kallas ”gut-brain axis” och avser kommunikationen mellan tarmen och hjärnan. Kommunikationen består av neurologiska, endokrina och immunologiska signaler. Uppkomsten av funktionella tarmsjukdomar som irriterabla tarm eller IBS tros delvis bero på störningar i detta kommunikationssystem och patienter med IBS lider oftare av depression och ångest än kontroller.

Syfte
Syftet med de olika delarbetena i den här avhandlingen har varit följande:

I. Att undersöka uttrycket av melatonin och melatoninreceptorer i normal tarm och bukspottkörtel från människa.
II. Att undersöka uttrycket av melatonin och melatoninreceptorer i tumörvävnad från tunntarmskarzinoider, hormonproducerande tumörer, som utgår från enterokromaffinceller, samt att studera sambandet mellan melatonininnivåer, mag-tarmbesvär och prognostiska faktorer.

III. Att undersöka förekomsten av olika typer av mag-tarmbesvär hos unga patienter med psykiatriska sjukdomar, framför allt depression och ångest jämfört med kontroller och vidare att undersöka om det är någon skillnad mellan patienter som har psykiatrisk medicinering och de som inte har det.

IV. Att undersöka sambandet mellan melatonininnivåer i saliv på dagtid och besvär från mag-tarmkanalen.

Metod

I studie I och II, användes immunohistokemi för att identifiera och påvisa uttryck av melatonin och melatoninreceptorer i vävnadsbiopsier från människa. Biopsierna i studie I utgjordes av normal tarm och bukspottkörtel från patienter som opererats av annan anledning. I studie II analyserades biopsier från tunntarmskarzinoidtumörer. För tumörpatienter mättes även nivåer av melatonin i plasma med en metod som kallas RIA vid två tillfällen. Melatonininnivåer jämfördes med kliniska parametrar så som besvär från mag-tarmkanalen och sjukdomsut普法.

I studie III och IV undersöktes patienter, 18-25 år som sökte vård på psykiatrimottagningen för unga vuxna, avseende förekomsten av mag-tarmbesvär, melatonininnivåer i saliv, depressiva symptom och ångestbenägenhet. Melatonin i saliv mättes med ELISA och patienterna fyllde i skattningsskalor för mag-tarmbesvär, depressiva symptom och olika personlighetsdrag som ångestbenägenhet.

Resultat och slutsats

I. Uttryck av melatonin och melatoninreceptorer identifierades i stora delar av mag-tarmkanalen och i flera olika typer av celler, bland annat enterokromaffinceller, epitelceller, immunceller, muskelceller och celler som omger blodkärl. Uttryck av melatonin och dess receptorer påvisades också i bukspottkörteln, med varierande intensitet.

II. Uttryck av melatonin och åtminstone en av receptorerna för melatonin identifierades i tumörvävnad från hormonproducerande tunntarmskarzinoider. Det fanns ett samband mellan högre intensitet av melatonin i tumörvävnad och lägre prolifer-
tion. Det fanns också samband mellan högre nivåer av melatonin i plasma och mindre diarrésymtom. Melatoninnivåer var lägre efter behandling hos patienter med stabil eller minskad tumörbörda.

III. Unga patienter med psykiatriska sjukdomar, framför allt depression och ångest hade mer mag-tarmbesvär än friska kontroller, oavsett om de hade pågående behandling med psykiatriska läkemedel eller inte.

Sammantaget visar dessa studier att melatonin och dess receptorer finns i stora delar av tarmen hos människa och vi har, för första gången, visat att melatonin också finns i tunntarmskarcinoider. Det finns sannolikt en kopp-pling mellan melatonin producerat i tarmen och symptom från mag-tarmkanalen, även om ytterligare studier behövs för att undersöka verkningsmekanismerna bakom detta samband. Patienter med depression och ångest har en ökad förekomst av mag-tarmbesvär jämfört med kontroller och detta bör efterfrågas vid behandling och uppföljning av psykiatriska sjukdomar. Ökad kunskap om kommunikationen mellan mage och tarm är nödvändig för att bättre förstå och kunna styra behandlingen av såväl depression och ångest som IBS. Den här avhandlingen är en pusselbit i arbetet att försöka hitta biologiska markörer som kan användas för att skilja mellan olika subgrupper inom den heterogena gruppen unga med depression och ångest.
Acknowledgements

This work was carried out at the Department of Neuroscience, Psychiatry, Uppsala University. I would like to express my deepest gratitude to everyone involved in this thesis project.

Janet Cunningham, my brilliant, energetic, inspiring supervisor. Thank you for believing in me and understanding that life is complex and multifaceted. Without you, I would never have taken on this challenge. Thank you for always looking after (all of) me.

Annica Rasmusson, my co-supervisor and general fixer. Your ability to identify the gaps and find the solutions to bridge them has been instrumental for this work. Thank you for your practical compassion.

Lisa Ekselius, originally my associate supervisor, for making this work possible and supporting my supervisor so she could support me.

Mikaela Syk, thank you for your brilliant mind, eternal patience and accuracy.

Isak Sundberg, for outstanding collaboration and picking up the slack when sleep was non-existing.

Mia Ramklint, for sound scientific discussions and understanding of psychometric data.

Per Hellström, for bringing your expertise in gastroenterology in general and IBS in particular.

David Just, for exceptional and immediate help with statistics and visualisations.

Zorana Kurbalija Novicic, for patiently teaching me about PCA and cluster analysis and for great scientific discussions.

Hans Arinell, for your patience and sound scepticism and always willing to answer my many statistical questions.
A-C Fält, our university administrator, who knows everything and everyone. Without you, this PhD process would be an administrative nightmare.

Åsa Forsberg, laboratory technician for excellent technical assistance with immunohistochemical staining.

Leslie Shaps, for brilliant and thorough proofreading of the thesis.

Thank you to all my co-authors that include Eva Tiensuu-Janson, Mats Stridsberg, Abir Ali and Rebecka Widerström, for all your hard work, wise comments and excellent collaboration.

Emelie Ahnfelt, for pep talks throughout the process of this thesis, both on scientific and personal matters.

Thanks to all my colleagues in my clinical everyday life at the department of Gynaecology and Obstetrics, for teaching, inspiring and supporting me in my clinical work. You truly do an amazing work!

Tahmineh Badiei, for your compassion, distraction and support.

My Dala family, for very little research but a lot of everything else!

The entire Brink family and especially Maria and Hans for all the love, help and support with our children. Without you, we would never manage.

My dearest “klicken”, Hanna Minna, Amanda and Agnes, “klacken” and all of your lovely children for a lifetime of friendship and support, completely unrelated to research.

My family, my mother Carina and my father Lennart for unconditional love, support and inspiration. Bibi and my brothers John and Linus with families. Thank you all for being a part of my life.

Most of all, thank you John, my friend and partner in life and love for everything that you do and for being the brilliant, fun, anxiety-free person that you are. Also thank you for our wonderful children, the other two overwhelming projects during this highly full-packed period in our lives. Olle and Mikkel, thank you for all the love and distraction.
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