Impact of the upper gut on body fluid regulation and blood pressure

– potential involvement of a locally expressed renin-angiotensin system

Doctoral thesis

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To my children

ABSTRACT

This thesis explores the role of the upper gut in the regulation of diuresis and blood pressure control in relation to the novel finding of a mucosa-located renin-angiotensin system (RAS). RAS is a regulatory super-system vital for body fluid homeostasis and blood pressure control. Recent research demonstrates that RAS is not only an endocrine (blood borne) system, but also in all respects locally expressed influencing tissue growth and differentiation as well as inflammatory responses.

A first aim of the present thesis-project was to explore if RAS was expressed in the mucosa of the stomach and duodenum. Indeed, by use of western blot and immunohistochemistry most components of RAS were found in several compartments of the gastric mucosa of the Mongolian gerbil (model for human *Helicobacter pylori* infection) and also in the human mucosa. It was also observed that a subset of gastric mucosal endocrine cells expressed AT1 receptors suggesting that activity in a local RAS can influence enteroendocrine signalling. RAS components were found also in the mucosa of the human duodenum.

The second aim of the thesis was to examine the potential functionality of the local mucosal RAS described above. The project was focussed on a previously described sodium/volume sensor postulated to be situated in upper gut. Such a sensor is activated by food ingestion/drinking and increases renal diuresis already in the pre-absorptive state. The upper-gut location of this regulatory principle was demonstrated in healthy volunteers by intragastric instillation of 750 ml saline that almost promptly was followed by an increased diuresis, whereas intrajejunal instillation had an additional 60 min lag-time until response. In a second set of experiments, the volunteer were first exposed to gastric instillation of saline (with sham-intubation as time control) and after 30 to 40 min a gastroduodenoscopy with sampling of mucosal biopsies was performed. The tissue specimens were examined for RAS components and the principal finding was that the concentration of the pro-hormone angiotensinogen decreased in the duodenal mucosa, but not in the stomach. The results confirm that a volume sensor is located to the upper gut in man. Furthermore, local mucosal RAS, particularly in the duodenum, may be involved in mediating the diuresis occurring in the pre-absorptive state after drinking and eating.

The third aim of the project was related to the physiological and clinical relevance of the sodium/volume monitor described above. Patients participating in the Swedish Obese Subjects (SOS) study were investigated. Gastric bypass (GBP), meaning that food and drinks are led directly into the jejunum thus bypassing the major part of the stomach and duodenum, was compared to gastric band constructions. The latter type of weight reducing surgery restricts the food intake capacity with the alimentary route intact. Interestingly, after adjustments for weight loss the GBP patients exhibited a larger 24h diuresis and a markedly more reduced systolic and diastolic pressure than the gastric band patients. These changes were prominent also 10 years after surgical intervention and were not related to the reduced body weight. Furthermore, the GBP patients consumed, despite a lowered blood pressure, approximately 1 g dietary salt more per day than patients operated with the restrictive banding techniques. This picture is compatible with that the sodium/volume sensor induces diuresis in an anticipatory fashion in relation to ingestive load and also inhibits salt appetite. Upon removal of this pre-absorptive regulatory mechanisms (as following GBP), more rough post-absorptive regulatory principles dominate that very probably results in an overshooting diuretic effect with depressor action and an increased salt intake.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Hallersund P, Helander HF, Casselbrant A, Edebo A, Fändriks L, Elfvin A. Angiotensin II receptor expression and relation to Helicobacter pylori-infection in the stomach of the Mongolian gerbil. *BMC Gastroenterol*. 2010 Jan 14;10:3

II. Hallersund P, Elfvin A, Helander HF, Fändriks L. The expression of renin-angiotensin system components in the human gastric mucosa. *J Renin Angiotensin Aldosterone Syst.* 2011 Mar 12;54-64. Epub 2010 Aug

III. Hallersund, P, Edebo A, Casselbrant A, Spak E, Fändriks L. The sodium/volume sensor in the upper gut in man – potential involvement of a local renin-angiotensin system. *In manuscript*

IV. Hallersund P, Sjöström L, Olbers T, Lönroth H, Jacobson P, Wallenius V, Näslund I, Carlsson LM, Fändriks L. Long-term effects on blood pressure and dietary salt intake by weight reducing surgery – an analysis of 10-year follow-up data from the Swedish Obese Subjects study. *In manuscript*

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LIST OF ABBREVIATIONS

ACE	angiotensin-converting enzyme
AGT	angiotensinogen
AngII	angiotensin II (1-8)
Ang-(1-7)	angiotensin (1-7)
AT1R	AngII type 1 receptor
AT2R	AngII type 2 receptor
ANP	atrial natriuretic peptide
BNP	B-type natriuretic peptide
CgA	chromogranin A
ECF	extracellular fluid
GBP	gastric bypass
GI	gastrointestinal
Na	sodium
NaCl	salt
NEP	neprilysin
RAS	renin-angiotensin system
SOS study	Swedish Obese Subjects study
Upper gut	stomach and duodenum
VBG/B	gastric banding procedures

BACKGROUND

INTRODUCTION

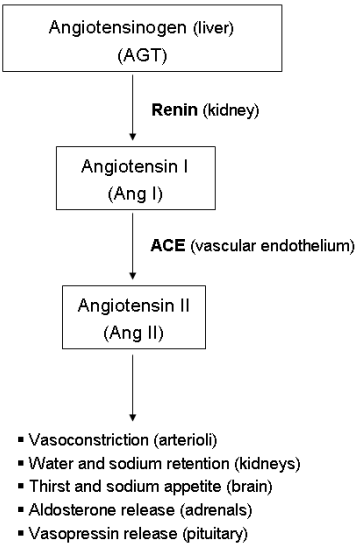
This thesis project explores the role of the upper gut in the regulation of diuresis and blood pressure control in relation to the novel finding of a mucosa-located renin-angiotensin system (RAS) in the stomach and duodenum. The project emanated from an exploration of mechanisms by which the human pathogen *Helicobacter pylori* "manipulated" host defence-dependent cytotoxic radical formation in the human gastric mucosa[1]. The findings were further explored in Mongolian gerbils being regarded as a good model for the human *H. pylori* infection and its related pathology[2]. The rationale for investigating RAS in this animal model can perhaps not be conceived as intuitive and therefore deserves some explanation. Our research team had previously linked *H. pylori* to inhibition of duodenal mucosal bicarbonate secretion. This secretion provides a neutralising zone close to the surface epithelium protecting the mucosa from intraluminal acid disposed by the stomach. Hence, the ulcerogenic property of *H. pylori* could to some degree be explained as due to inhibited mucosal bicarbonate secretion[3]. In a parallel project in our laboratory, RAS was found to regulate such duodenal mucosal bicarbonate transport[4, 5]. In addition, data in the literature show that RAS is involved in inflammation, tissue growth and differentiation, as well as carcinogenesis, all being of great clinical interest for GI pathology. Based on this background we occasionally checked for the presence of angiotensin II (AngII) receptors in the *H. pylori* infected and inflamed gastric mucosa of the abovementioned Mongolian gerbils. The intriguing finding of a widespread presence of AngII receptors in gastric mucosae, also in those devoid of infection/inflammation, became the starting point for this thesis project. The project has since then evolved from mucosal expression of RAS to the role of the upper gut as part of fluid homeostasis and arterial pressure control. Below are the today's paradigms regarding RAS, body fluid homeostasis and gut chemosensing briefly reviewed. Novel findings are then presented and discussed.

THE RENIN-ANGIOTENSIN SYSTEM (RAS)

The classical RAS

Textbooks in physiology still describe RAS as an endocrine system for hemodynamic regulation and body fluid homeostasis (Figure 1). This classical picture relates to a system that is activated when blood circulation is challenged, for example due to hemorrhage or uncompensated profuse sweating. The reduced blood volume will be manifested as a lowered arterial pressure or a sodium deficiency that will initiate the release of the enzyme renin from the juxtaglomerular apparatus of the kidneys. Renin cleaves off the decapeptide angiotensin I (AngI) from the precursor protein angiotensinogen (AGT; 452 amino acids long) released by the liver. AngI is then degraded to the signal mediator octapeptide angiotensin II (AngII) by angiotensin-converting enzyme (ACE) expressed by endothelial cells mainly in pulmonary vessels. Circulating AngII acts vasoconstrictive and induces renal sodium and fluid retention to maintain arterial pressure and to compensate for the reduced blood volume. AngII also mediates the thirst sensation and salt appetite driving the individual to a final fluid compensation by increased oral intake of water and sodium[6, 7] (Figure 1). AngII regulates cardiovascular and body fluid homeostasis both directly on the vascular system, kidney and brain, as well as indirectly via other regulatory factors, for example by liberation of aldosterone from the adrenals, or by facilitation of vasoconstrictive sympathetic nervous activity[8, 9].

Figure 1.
The classical endocrine renin-angiotensin system (RAS)



The novel RAS

It has become evident that various components of the RAS are locally expressed in many organs and tissues[10-12], e.g. brain, pancreas and adipose tissue, and can act by paracrine or autocrine mechanisms[13]. These systems are reported to interact with the blood borne "classical" RAS in several aspects. For instance, adipocytes synthesise the pro-hormone AGT and evidence are accumulating in the literature that the adipose tissue is a source of the AGT circulating in the blood[14].

The updated "novel" RAS differs from the classical RAS also with regard to proteolytic enzymatic pathways. These cause generation of bioactive AGT-fragments other than AngII with specific receptors expressed differentially in tissues (Figure 2). For example, tissue production of AngII, or other AGT-fragments such as Ang-(1-7), may occur following local production of AGT, renin, ACE and NEP, or through alternative pathways including cleavage of circulating AGT by other locally enzymes such as cathepsin G and chymase[13, 15, 16].

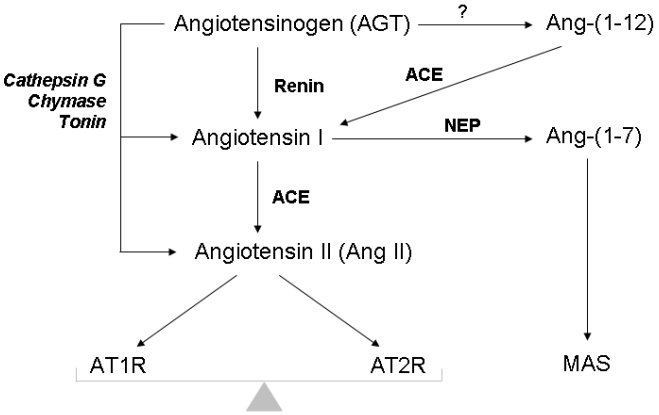


Figure 2. **Some novel aspects of RAS**

AT1R and AT2R, AngII receptor type 1 and 2, respectively

MAS, rceptor for Ang-(1-7)

NEP, neprilysin

Angiotensin II – the principal mediator of RAS

AngII works principally through two receptors (Figure 2) designated AngII type 1 receptor (AT1R) and AngII type 2 receptor (AT2R)[17]. Classical effects of AngII, such as vasoconstriction and aldosterone release, are mediated via AT1R. Less is known about the actions of AT2R. Several studies indicate that activation of the AT2R generally has effects that oppose those mediated by the AT1R, thus modulating the responses to stimulation with AngII[18]. These two receptor subtypes belong to the seven-transmembrane G-protein-coupled receptor superfamily, and binding of AngII to AT1R and AT2T can activate several intracellular second-messenger systems[19-21], resulting in e.g. hormonal release (e.g. AGT, aldosterone and vasopressin) or activation of transcription factors inducing gene expression, such as Activator Protein 1 (AP-1), Signal Transducer and Activator of Transcription (STATs), and Nuclear Factor-kB (NF-kB).

RAS in the gastrointestinal (GI) mucosa

The presence of RAS in the GI mucosa is sparsely reported in the literature, and particularly so with regard to the situation in man[22]. Nevertheless, AngII receptors of both subtypes (AT1R and AT2R) have been reported to be expressed in the esophageal[23], small intestinal[24] and in the colonic mucosa[25], and data suggest that in these parts of the GI tract, RAS is involved in epithelial fluid/electrolyte and glucose transport, as well as in mucosal inflammation[12, 26-29].

Potential roles for the RAS in the gastric and duodenal mucosa are very little explored. Effects on duodenal bicarbonate secretion and gastric blood perfusion in relation to circulatory stress (and reperfusion) have been reported in animal studies[5, 30, 31], and also involvement in the postulated sodium monitor suggested to be situated in the upper part of the gut[32-34]. This latter mechanism is of particular interest for the present thesis project and will be described in detail below. Briefly, it represents the sensor of an entero-renal signalling mechanism demonstrated by the phenomenon that dietary sodium induces a more prompt natriuresis than does the similar amount sodium given intravenously[35, 36]. Filling the gap regarding information on RAS location in the human gastric and duodenal mucosa is one important aim of the present thesis project (see Aims of the thesis).

BODY FLUID HOMEOSTASIS AND THE GUT SODIUM/VOLUME SENSOR

Body fluid homeostasis is a core element in physiology and detailed descriptions are given in most textbooks and many comprehensive reviews[37-39]. A brief summary is given below with some extra attention given to systematic mediators of importance for the experimental project presented later in this thesis.

Of the total water content in the body, the intracellular fluid compartment constitutes 2/3. The remaining 1/3 is extracellular fluid (ECF). 3/4 of the ECF volume surrounds the cells (interstitial fluid) and the rest (1/4) circulates in blood as plasma. Because of its abundance, sodium (Na) is the major determinant of the osmolarity of the ECF. Therefore, the sodium concentration of the ECF constitutes the major osmotic force that moves water in or out of cells. It follows that body fluid homeostasis requires mechanisms that strive to maintain an optimal distribution within the intracellular and extracellular fluid compartments; as well as mechanisms that maintain a precise balance between the intake and excretion of sodium and water of the body.

The input of sodium and water to the ECF is determined by the net absorptive capacity (mucosal absorption minus secretion) of the intestinal mucosa and by the ingested amounts. The latter is in turn dependent on central regulation of ingestive behavior in relation to the sensations of thirst and salt (NaCl) appetite. Sodium and water output is during resting conditions determined mainly by the kidneys, which can control the rates of excretion of water and sodium independently of each other[40]. During exercise one also has to count losses by transpiration and respiration. Stool water contents can vary considerably but is during physiological conditions not regarded of importance for volume output.

Aberrations from normal body fluid conditions are counteracted by regulatory mechanisms on all functional levels of the organism. Local ion concentrations influence directly the state of membrane transporters to protect functions on the cellular level. On the tissue and organ level, specialized sensor structures activate humoral factors and neural activity that forces distant organs to compensatory actions. One such principle is the sensing of blood pressure at specific sites within the cardiovascular system. Blood pressure is by definition dependent

on blood volume which in turn is associated to ECF and its sodium concentration. Pressure sensing takes place in the heart and pulmonary vessels (low-pressure sensing) and in the carotid sinus, aortic arch, and juxtaglumerular apparatus of the kidneys (high-pressure sensing). In addition to integrating pressure information, the organism also senses sodium concentration *per se* in e.g. the juxtaglumerular cells and at certain brain areas. Regulatory signals are mediated via the sympathetic nervous system (partly by renin release), via RAS (AngII and indirectly via the production of aldosterone), and via cardiac natriuretic peptides[41] (ANP and BNP), as well as via vasopressin from the pituitary.

Peripheral markers of body fluid control

From a research perspective, sympathetic neural activity is a difficult variable to assess whereas the humoral mediators (e.g. AngII, aldosterone, BNP, vasopressin) are easy accessible by blood sampling and therefore often are used as good markers on actions related to body fluid control. As mentioned, the circulating blood volume is part of the body fluids and consequently hemodynamic regulation and body fluid homeostasis are integrated. Therefore, mechanisms that regulate blood circulation are also the major determinants of sodium and water balance. It follows that ECF volume partly determines venous and arterial pressure. Blood pressure recordings (particularly in the low pressure parts) can briefly reflect the state of the ECF.

The gut sodium/volume sensor

The above described sensors in the cardiovascular system, brain and kidneys detect changes in plasma volume or sodium concentration. Additionally, experiments have indicated that there also exists a "pre-absorptive" sodium/volume sensor in the GI tract[33, 42-44]. This sensor is activated by salt ingestion and drinking and signals to the kidneys to increase natriuresis before any detectable changes in plasma sodium concentration are observed. A similar mechanism inhibits salt appetite and thirst in an anticipatory fashion (Figure 3).

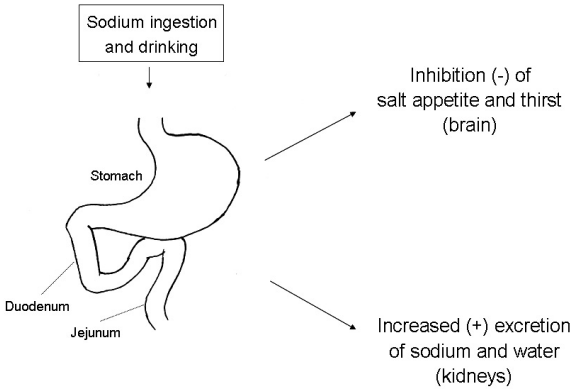


Figure 3. **The proposed sodium/volume sensor in the upper gut**

For example, gastric salt loading inhibits salt appetite in sodium depleted rats before plasma sodium concentration is enhanced by absorption of the salt[43]. Likewise, water intake causes satiety in thirsty humans and animals (initially given hypertonic saline intravenously to induce thirst) before plasma sodium concentration is corrected[44].

The cellular and molecular mechanisms underlying pre-absorptive body fluid regulation are unknown, as well as the exact location of the sensor. Suggested mediators linking the sodium/volume sensor to the central nervous system and the kidneys include vagal afferents[45], enteroendocrine "taste" cells[33] and several humoral factors including AngII[32, 46]. For example, experiments in rats given ACE-inhibitors (decreasing AngII generation) have indicated that an intact renin-angiotensin system is necessary for the interplay between the gastrointestinal tract and kidney[47].

CHEMOSENSING IN THE GUT MUCOSA AND ENTEROENDOCRINE CELLS

The physicochemical properties of the luminal bulk influence markedly the secretion of gastric acid and proteolytic enzymes, the gastric emptying rate and the type of intestinal motility. Sensing of the luminal contents by the GI mucosa is necessary for these adaptive responses that optimize the digestive and absorptive conditions. In addition, the detection of constituents within the GI tract is important also for extra-GI organs and the organism as a whole. Many important physiological processes are initiated or modulated from the GI tract, e.g. immune responses, glycemic control (demonstrated for example by the fact that oral ingestion of glucose triggers more insulin release than glucose delivered intravenously) and food intake[48-51].

Gut chemosensing is usually regarded as a neuro-endocrine process involving hormone releasing cells in the gut mucosa; the *enteroendocrine cells*. When activated, these cells exert endocrine actions (the hormone reach distant targets via the blood stream), or paracrine activation (local release and actions) of, for example, local enteric nerves and/or afferent fibers of the vagal nerve mediating the signal to the central nervous system (Figure 4).

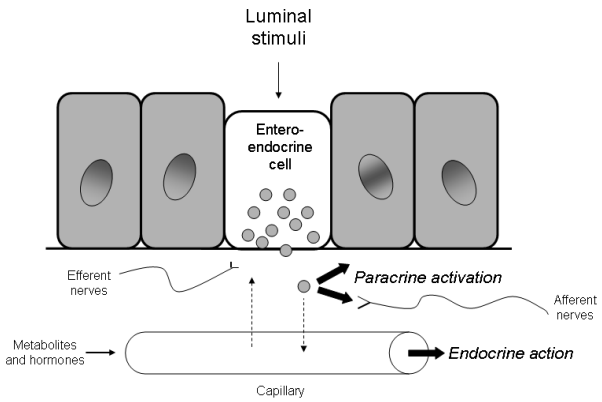


Figure 4. **Endocrine and paracrine signalling by enteroendocrine cells**

The enteroendocrine cells are confined to the epithelial layer of the mucosa and have two principal morphological shapes, the "open type" having contact with the GI lumen, and the "closed type" not reaching the luminal contents. Despite being a numerically small proportion of the total epithelial cells these cells are regarded as the largest endocrine "organ" of the body, both in terms of number of cells and variety of hormones produced. Vagal mucosal fibers do not reach the epithelial surface, but are closely associated with the enteroendocrine cells (Figure 4) and express specific receptors for GI hormones.

A common feature for enteroendocrine cells is the presence of chromogranins[52] which are vesicle storage proteins, reflecting the secretory granules present in endocrine cells. Chromogranin A (CgA) is often visualized in the initial immunohistochemical identification of enteroendocrine cells[53]. The release of hormones from enteroendocrine cells (Figure 4) is partly regulated by agents in the GI lumen, such as nutrients[54, 55] (lipids, proteins and carbohydrates), acidity, and gas tensions[56, 57] (e.g. $C0_2$, NO). Some enteroendocrine cells are also acting secondary to other signalling principles, e.g. neural impulses, blood borne signal substances and nutrients, and gastrointestinal mechanical properties (for example wall tension reflecting the degree of distension due to presence of food and/or muscular activity). On the other hand, chemosensitivive enteroendocrine cells can elicit muscular activity that in turn activate mechanosensors belonging to the extrinsic vagal and spinal afferents that in turn mediates signals to the central nervous system eliciting reflex feedback and/or perceptions.

One example of polymodal enteroendocrine signalling is the mediator glucagon-like peptide 1 (GLP-1)[58]. This peptide is liberated when nutrients reach the enteroendocrine L-cells in the distal small intestine and colon. GLP-1 has multiple effects based on its endocrine mode of action (for example stimulates insulin secretion from the pancreas) but does also activate vagal afferents in turn resulting in rapid reflex effects.

Recently, much interest has been focused on the role of "taste cells" in the GI mucosa. These cells express modality-specialized sensing molecules originally described in the taste receptor cells of the tongue[59, 60]. Interestingly, recent findings suggest that the molecular pathways similar to those mediating oral taste perceptions also operate in the gut mucosa[55].

Taste molecules have been found in enteroendocrine cells and in other morphologically similar cells, called “brush cells”. However, no secretory granules of the type that characterize endocrine cells can be demonstrated in brush cells[61]. Studies indicate that brush cells can release nitric oxide (NO) that may be an important signalling molecule[62], activating vagal afferent nerve fibers or influencing adjacent mucosal cells.

In general, nutrient sensing mechanisms in the gut are not well understood but this is an area of increasing scientific interest, given its importance in the regulation of glucose homeostasis and food intake. It is difficult to study enteroendocrine cells directly within the gut mucosa and particularly their paracrine actions because plasma levels may not be helpful in assessing local roles of a particular hormone or determing the mechanism of its release. Consequently, much of what we know of direct chemosensing by enteroendocrine cells comes from experiments on cell lines[50].

HYPOTHESES

It is well established that the endocrine renin-angiotensin system (RAS) is a powerful signalling system involved in the electrolyte and fluid homeostasis and blood pressure control. It was hypothesised that RAS components expressed locally in the mucosa of the upper gut exert such regulatory impact already in relation to the ingestion of electrolytes and fluid. Based on this hypothesis it was assumed that intervention with the gastrointestinal continuity should affect blood pressure control.

AIMS OF THE THESIS

The general aim of this thesis was to investigate the presence of the renin-angiotensin system (RAS) in the mucosa of the human stomach and duodenum and to position the findings in a physiological and clinically relevant context.

The specific aims of the project were related to the following questions:

1. Is RAS present in the gastric and duodenal mucosa?

2. Is the upper-gut mucosal RAS involved in gut-renal diuretic responses?

3. Does permanent exclusion of the upper-gut sodium/volume sensor influence diuresis, salt appetite and blood pressure?

REVIEW OF RESULTS

1. Is RAS present in the gastric and duodenal mucosa?

The presence and location of representative RAS components in the gastric and duodenal mucosa was investigated by use of Western blot and immunohistochemistry (I, II and III). Gastric mucosal infection with *Helicobacter pylori* is extremely common in the population. Although severe morbidity, e.g. peptic ulcers and gastric carcinomas, can be associated to this infection most individuals remain asymptomatic[63]. Because of its high prevalence it was considered of importance to rule out if and how an *H. pylori* infection influenced the expression of RAS. The mapping of RAS components in the gastric mucosa (I, II), therefore, was related to if *H. pylori* was present or not.

Study setting

A systematic mapping of immunoreactivity to AngII receptors (AT1R and AT2R) was first performed in the stomach of the Mongolian gerbil (commonly used as a model for human *H. pylori* associated gastritis) in presence or absence of experimentally induced *H. pylori* infection (I). These results were subsequently confirmed in endoscopic biopsies from the human mucosa of *H. pylori*-negative *and H. pylori*-positive volunteers, where also immunoreactivity to angiotensin generating enzymes (renin, ACE and NEP) and the prohormone AGT were assessed (II). Mapping of RAS components in the human duodenal mucosa was performed on endoscopic biopsies from healthy volunteers (III).

Presence and location of RAS components in the gastric mucosa

The proteins of the examined RAS components were all identified by Western blotting in samples from the gerbil and human stomach, and immunoreactivity to AT1R and AT2R was found in a variety of cells in the gastric mucosa (I, II). A summary of the immunohistochemical results from various mucosal compartments of the human stomach is given in Table 1.

Table 1. **Location of RAS proteins in the human gastric mucosa (from II)**

Protein	Parietal cells	Endocrine cells	Epithelial cells in glands and in gastric pits	Mesencymal cells in lamina propria	Vascular endothelial cells
AT1R	n.o.	+++ In a subpopulation in antrum	++ Mainly in the basal parts	+++ Some cells in *H. pylori* neg. +++ Inflammatory cells in *H. pylori* pos.	++
AT2R	n.o.	n.o.	+ Mainly in the basal parts	++ Some cells in *H. pylori* neg. ++ Inflammatory cells in *H. pylori* pos.	++
AGT	n.o.	n.o.	n.o.	+++ Some cells in *H. pylori* neg. +++ Some cells in *H. pylori* pos.	++
Renin	n.o.	n.o.	n.o.	+++ Some cells in *H. pylori* neg. +++ Some cells in *H. pylori* pos.	++
ACE	n.o.	n.o.	n.o.	n.o.	+++
NEP	n.o.	n.o.	n.o.	n.o.	+++

n.o.: not observed (or unspecific) immunoreactivity
+: weak immunoreactivity but with distinct location
++: easily recognized immunoreactivity
+++: strong immunoreactivity

Interestingly, strong immunoreactivity to the AT1R protein was found (independent of *H. pylori* infection) in some epithelial cells in the antral mucosa of both the gerbil and human stomach. These cells had the typical appearance of enteroendocrine cells, e.g. in some cases a narrow string of cytoplasm was observed. Co-expression of AT1R and CgA (a marker for endocrine cells[53]) by a subpopulation of gastric enteroendocrine cells was confirmed using double immunostaining (Figure 5). Hence, these results suggest that activity in a local RAS can influence enteroendocrine signalling.

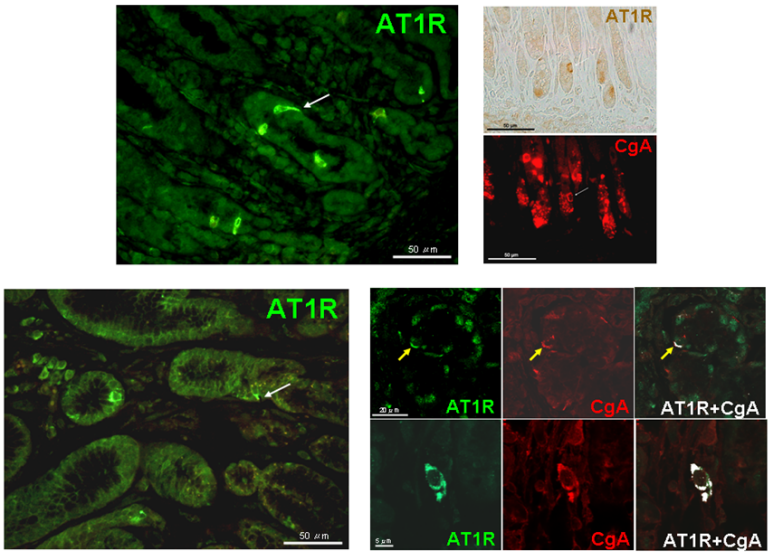


Figure 5. **Enteroendocrine cells in gastric mucosa staining positive for AT1R** A marker for endocrine cells (Cga) was used for confirmation. Stainings from the gerbil (upper sections) and human (lower sections) gastric mucosa are displayed.

RAS expression in relation to Helicobacter pylori infection

In the human gastric mucosa, immunoreactivity to the proteins of AGT, renin, ACE, NEP did not differ quantitatively between *H. pylori*-positive and *H. pylori*-negative subjects. However, AT1R protein expression was significantly more pronounced in the gerbil and human *H. pylori*-positive mucosa compared to *H. pylori*-negative mucosa. Immunohistochemistry also showed an abundance of inflammatory cells (lymphocytes and neutrophils) in the mucosa with immunoreactivity to AT1R (I, II). By quantifying lymphocytes and neutrophils present in the mucosa, we found that the AT1R protein expression correlated with the number of neutrophils, but not with the number of lymphocytes (Figure 6). Thus, these results indicate that *H. pylori* induced gastritis is associated with higher prevalence of AT1R, most probably due to presence of infiltrating neutrophils carrying this receptor.

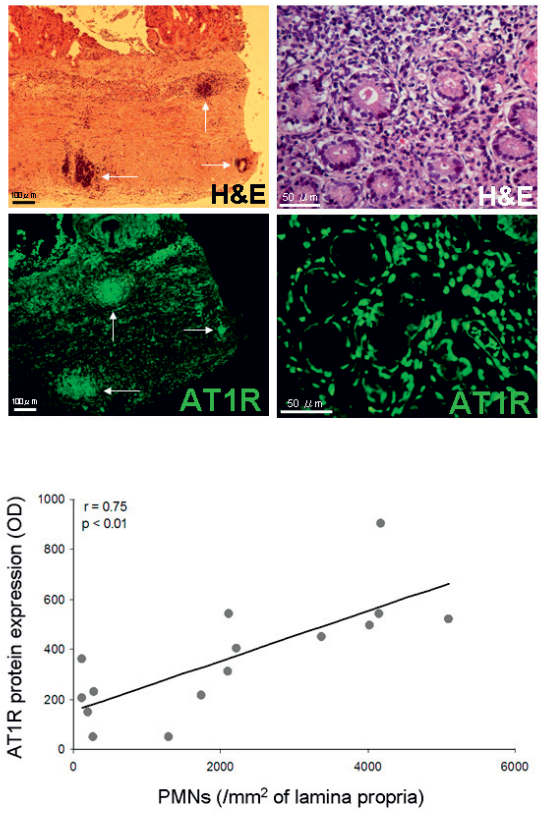


Figure 6. **The *Helicobacter pylori* positive gastric mucosa**

Upper panel: inflammatory cells showing immunoreactivity to AT1R in the gerbil (left sections) and human (right sections) *H. pylori* positive mucosa. H&E, haematoxylin/eosin staining. Lower panel: relationship between AT1R expression and the number of neutrophils (PMNs) in *H. pylori* infected gerbil antral mucosa. OD, optical density.

The observed relationship between AT1R and neutrophils is interesting as epidemiological and experimental studies have indicated that RAS can influence the pathogenesis of *H. pylori* associated gastritis and gastric cancer[64-67]. However, despite of great medical interest this aspect of mucosal RAS was not further investigated in the present thesis project. The investigation was instead focused on the potential to influence enteroendocrine signalling and the possibility that RAS components are involved in a previously described sodium/volume sensor postulated to be situated in the upper gut[32, 34, 43].

RAS components in the duodenal mucosa

Ingested liquid meals are rapidly disposed by the stomach into the duodenum. The gastric emptying rate differs depending on the physicochemical properties of the stomach contents, e.g. energy density, osmolality etc. Water and non-caloric isotonic solutions are almost instantly delivered into the duodenal lumen[68, 69]. Thus, drinks do not only expose the gastric mucosa, but also the duodenal one. The expression of RAS in the human duodenum during basal conditions had not been previously investigated so this was done as part of Paper III. Indeed, the proteins of AT1R, AT2R, renin, ACE, NEP and AGT were all identified by Western blotting in samples of duodenal mucosa from healthy volunteers (III). Immunohistochemistry showed staining for AT1R and AT2R in the basal parts of most epithelial cells. Interestingly, immunoreactivity to AGT was found in the basal parts of solitary epithelial cells in the duodenal mucosa (Figure 7).

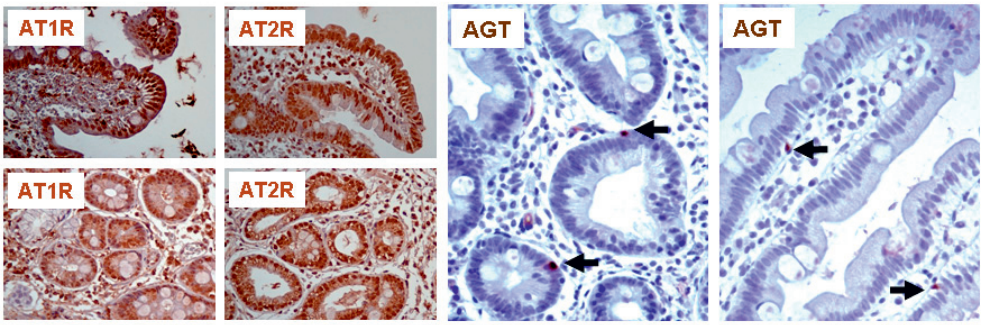


Figure 7. **Immunoreactivity to AT1R, AT2R, and AGT in the human duodenal mucosa**
Left: Immunostainings for AT1R and AT2R. Right: AGT was found in the basal parts of solitary epithelial cells in villi and crypts (arrows) and in blood vessels (not indicated). Original magnification of images: x40

1st conclusion

Prominent components of RAS are present in the human gastric and duodenal mucosa. *H. pylori* induced gastritis is associated with higher prevalence of AT1 receptors, most probably due to presence of infiltrating neutrophils carrying this receptor. Immunoreactivity to AT1R and AGT in solitary epithelial cells suggest that local RAS activity can influence gastro-duodenal enteroendocrine signalling.

2. Is the upper-gut mucosal RAS involved in gut-renal diuretic responses?

The project was then directed towards the potential functionality of RAS in the gastric and duodenal mucosa. The aim was to investigate if the mucosa-located RAS is involved in the GI sodium/volume sensor that upon drinking and eating induces diuresis in an anticipatory fashion. The presence and location of the gastrointestinal sodium/volume sensor was first investigated. Acute signs of mucosal RAS reactions to an intraluminal saline load were then explored.

Study setting

To confirm presence and location of pre-absorptive regulation, 750 ml isotonic NaCl was installed intralluminally via a nasogastro (-jejunal) tube either in the stomach or in the jejunum of healthy male volunteers. The time course of the diuretic response was characterized. Blood borne factors of importance for body fluid homeostasis were also analyzed using radioimmunoassay (RIA) or enzyme immunoassay (EIA). Potential changes in the gastroduodenal mucosal RAS to an intragastric luminal saline load were assessed by Western blot and EIA targeting AGT and AngII levels in the mucosa, respectively. In these experiments, the volunteers were first exposed to instillation of saline via a nasogastric tube (with sham-intubation as time control) and after 30-40 min a gastroduodenoscopy with sampling of mucosal biopsies (usually 45 min after the exposure procedure) was performed. All subjects were instructed to avoid high salt intake 4 days before examinations and each subject participated at two separate study days to be able to serve as its own control.

Diuretic response and plasma hormones after a gastric or jejunal saline load

The latency of onset to a diuretic response was markedly shorter after gastric loading than after jejunal loading of 750 ml isotonic saline (Figure 8). Thus, these results confirm that a diuresis regulating mechanism is activated in the upper gut at a time point where blood volume expansion following absorption is unlikely to have occurred.

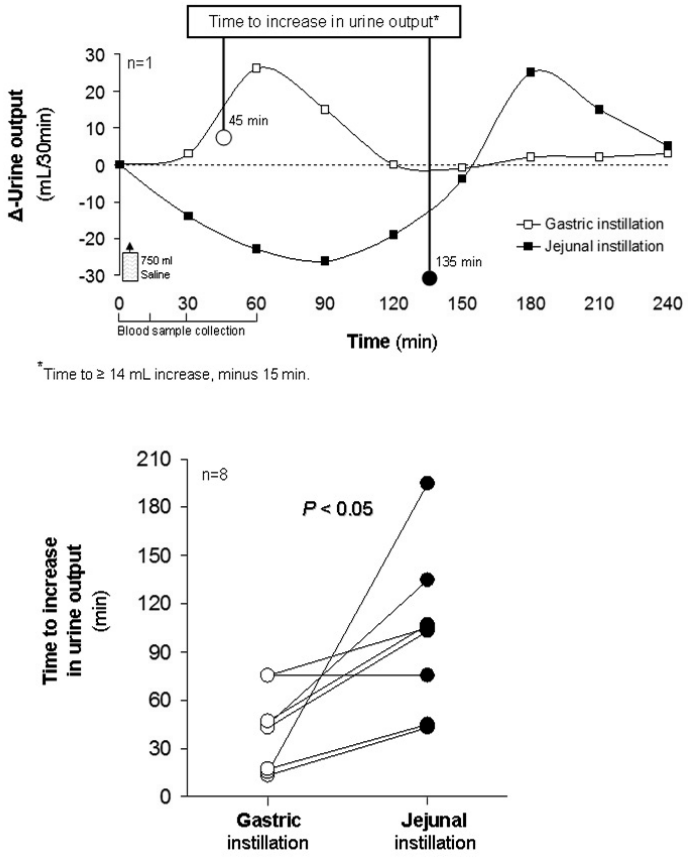


Figure 8. **Diuretic response to a gastric or jejunal instillation of 750 ml isotonic saline** Eight healthy male volunteers were studied on 2 separate study days. Upper panel: Representative responses in urine output in one study subject. Lower panel: Time until increase in urine output after gastric or jejunal instillations, respectively.

Strong support for this interpretation was gained from the finding that hormonal changes typically involved in extracellular volume regulation were not observed following gastric loading, but were apparent following jejunal instillation. Hence, plasma levels of BNP, vasopressin and AngII/aldosterone did not change significantly after the gastric instillation procedure. Intrajejunal saline instillation, on the other hand, was associated with increased plasma BNP concentrations indicating that a systemic volume expansion had preceded the diuretic response[70] (Figure 9). When taken together, the data indicate that the intrajejunal instillation induces diuresis mainly via post-absorptive mechanisms.

Δ AUC (60 min)	**Gastric** instillation mean (SEM)	**Jejunal** instillation mean (SEM)	**Diff.** mean (SEM)	*P*
Ang II (pg/mL)	6216 (9390)	-10931 (7540)	17148 (13973)	*0.31*
Renin activity (µg/L/h)	-13.09 (6.57)	-15.03 (14.36)	1.95 (16.99)	*0.82*
Aldosterone (pmol/L)	-1519 (1153)	-5050 (3217)	3531 (3689)	*0.82*
Vasopressin (pmol/L)	-0.17 (1.28)	0.83 (0.74)	-1.00 (1.47)	*0.59*
BNP (ng/L)	-15 (38)	157 (79)	-172 (84)	*0.04*

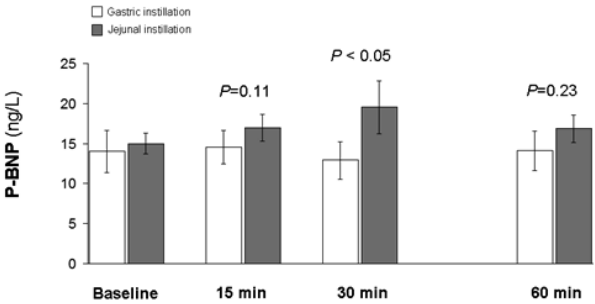


Figure 9. **Plasma hormone responses to intragastric or intrajejunal saline load** Hormone concentrations were measured at baseline, 15, 30 and 60 min after instillation. Upper panel: Area under curve (AUC) calculated from the net changes from baseline (Δ). Lower panel: Plasma BNP concentrations (mean ± SEM).

The time course of natriuresis in response to gastric or jejunal isotonic saline instillations did not show any consistent pattern and the onset time for increased natriuresis did not differ significantly between the intragastric and intrajejunal instillation. It should be noted that we chose instillation of isotonic NaCl in order to avoid effects of transmucosal osmotic and sodium gradients. Still, from a quantitative perspective the net sodium load (6.8 g NaCl) is to be regarded as quite high, being in the order of 50-60% of normal daily salt intake in the Swedish population[71].

AGT and AngII in the gastro-duodenal mucosa subsequent to a gastric saline load

In the next set of experiments, instillation of isotonic saline in the stomach was used to provoke the gastroduodenal mucosa and potentially the local RAS. The volume saline installed was, compared to the previous experimentation, reduced from 750 to 500 ml (4.5 g NaCl) to minimize the risk for aspiration in association to the subsequently performed endoscopy. The endoscopic mucosal biopsy takings were performed approximately 45 min after instillation. According to the previous results, this time point corresponded to onset of the diuretic response following gastric instillation (Figure 9) and, hypothetically, the mucosal reaction inducing gastroduodeno-renal signalling. For practical reasons the tissue-analyses were limited to AngII and the prohormone AGT representing two important mediator factors. Vasopressin, that in addition of being a pituitary hormone also is expressed in the GI mucosa[72], was measured as reference.

Interestingly, the content of AGT in the duodenal mucosa decreased significantly subsequent to the gastric saline instillation and this was not the case in antral specimens (Figure 10). The saline load did not significantly influence AngII or vasopressin, neither in antral nor in duodenal mucosae. These observations suggest that the duodenum might be the primary site for this type of luminal sensing and that the local duodenal RAS reacts upon a luminal saline load with a mobilization of stored AGT.

2nd conclusion

The temporal relationship between increased diuresis induced by an intragastric saline load and the reduced quantity of AGT in duodenum suggest a role for RAS in the duodenal mucosa in the pre-absorptive induction of diuresis occurring after drinking and eating.

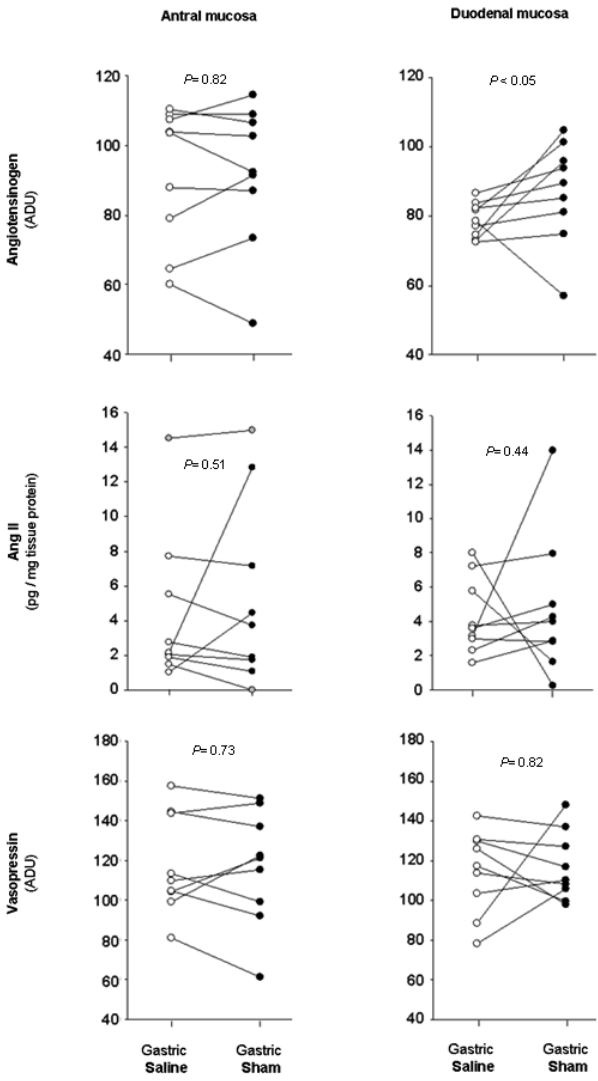


Figure 10. **Tissue levels of angiotensinogen (AGT), AngII and vasopressin in antral and duodenal mucosa subsequent to a gastric saline load.** The assessments were performed 45 min after gastric instillation of isotonic saline (500 ml) or a gastric sham instillation procedure. ADU, arbitrary densitometric units. Gray circles (AngII in antral mucosa) denote levels under the limit of detection or absorbance levels to high to be quantified.

3. Does permanent exclusion of the upper-gut sodium/volume sensor influence diuresis, salt appetite and blood pressure?

To further investigate the upper-gut sodium/volume sensor, as well as its potential physiological and clinical relevance, the next study focused on body fluid regulation following gastric bypass surgery for weight reducing purpose (IV). The background to this study was that after gastric bypass surgery (GBP), food and drinks are led directly into the jejunum thus bypassing the major part of the stomach and duodenum (and the above described upper-gut sodium/volume sensor). This is contrary to weight reducing gastric banding procedures (such as vertical banded gastroplasty or gastric banding) that restrict the food intake capacity with the alimentary route intact (Figure 11).

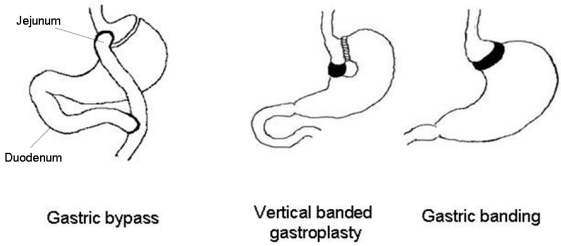


Figure 11. **Two weight reducing surgical principles: gastric bypass (GBP) and gastric banding procedures**

Interestingly, GBP is associated with an improved glucose homeostasis already in the early postoperative phase[73] and the operation cause longstanding changes in appetite and taste preference[74]. One hypothesis for these effects is that exclusion of the upper GI tract from contact with ingested food influences neuro-endocrine signals normally originating from nutrient sensing mechanisms in the stomach, duodenum or proximal jejunum[75] ("the foregut hypothesis"). Another hypothesis is that neuroendocrine signalling following the direct loading of the distal small intestine ("the hindgut hypothesis") improves metabolic aberrations. The latter is true regarding liberation of certain incretins, for example GLP-1,

that stimulate insulin release from the pancreas. However, neither the foregut, nor the hindgut hypothesis can fully explain the early effect on glucose homeostasis by GBP, implicating that unknown mechanisms are operating as well. It has been reported that GBP also reduces blood pressure before significant weight loss has occurred[76]. It was therefore hypothesised that the exclusion of the gastroduodenum and the previously mentioned gut sodium/volume monitor could be a mechanism of action. If so, the GBP-patients should exhibit a diuretic pattern and/or salt ingestive behaviour that differ from patients operated with banding procedures and with their GI continuity intact.

Study setting

Subjects participating in the Swedish Obese Subjects (SOS) study[77, 78] were examined. The prospective large scale SOS study compares obese patients undergoing weight-reducing surgery, with contemporaneously matched, non-operated obese control patients. The subjects who underwent weight-reducing surgery were for the purpose of the present analysis divided into two groups: gastric bypass (GBP) and vertical banded gastroplasty or gastric banding (VBG/B) (Figure 11). Diurnal urine collections and blood pressure levels were investigated at baseline and at 2y and 10y after study-inclusion. Dietary salt intake was assessed by measurement of 24h urinary excretion of sodium, which is considered the gold standard for assessing salt intake[79].

Diurnal urine output, salt intake and blood pressure after gastric bypass surgery

After adjustments for weight loss, the GBP patients exhibited a larger 24h urine output and a larger 24h natriuresis than the gastric band or control patients. The GBP operated individuals also displayed a markedly more reduced systolic and diastolic pressure (Figure 12). These changes were prominent also 10 years after surgical intervention (median follow-up time) and were not related to the reduced body weight. Furthermore, regression analyses demonstrated that changes in diuresis were linearly associated with blood pressure changes only in the GBP cohort, indicating that blood pressure reduction following GBP can be attributed to its diuretic action (Figure 13).

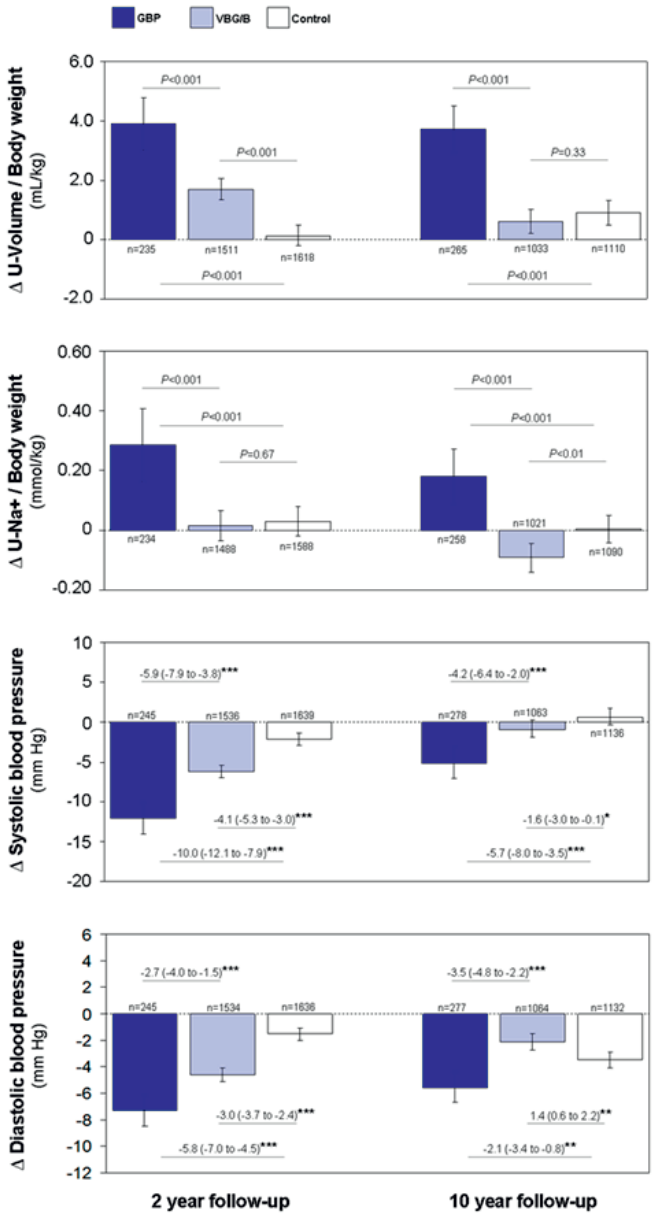


Figure 12. **Changes in diurnal urinary output (U-Volume) and excretion of sodium (U-Na+) in relation to body weight (upper panels), and changes in blood pressure (lower panels) after gastric bypass surgery (GBP), after pure restrictive bariatric surgery (VBG/B) and in non-operated obese controls**. Changes from baseline (Δ) at the 2y and 10y follow-up visits are displayed. Data are mean values adjusted for sex, age, baseline BMI and the baseline level of the respective variables. The bars represent the 95% confidence intervals. Differences between groups are given as mean (95% confidence intervals). **P*<0.05, ***P*<0.01 and ****P*<0.001

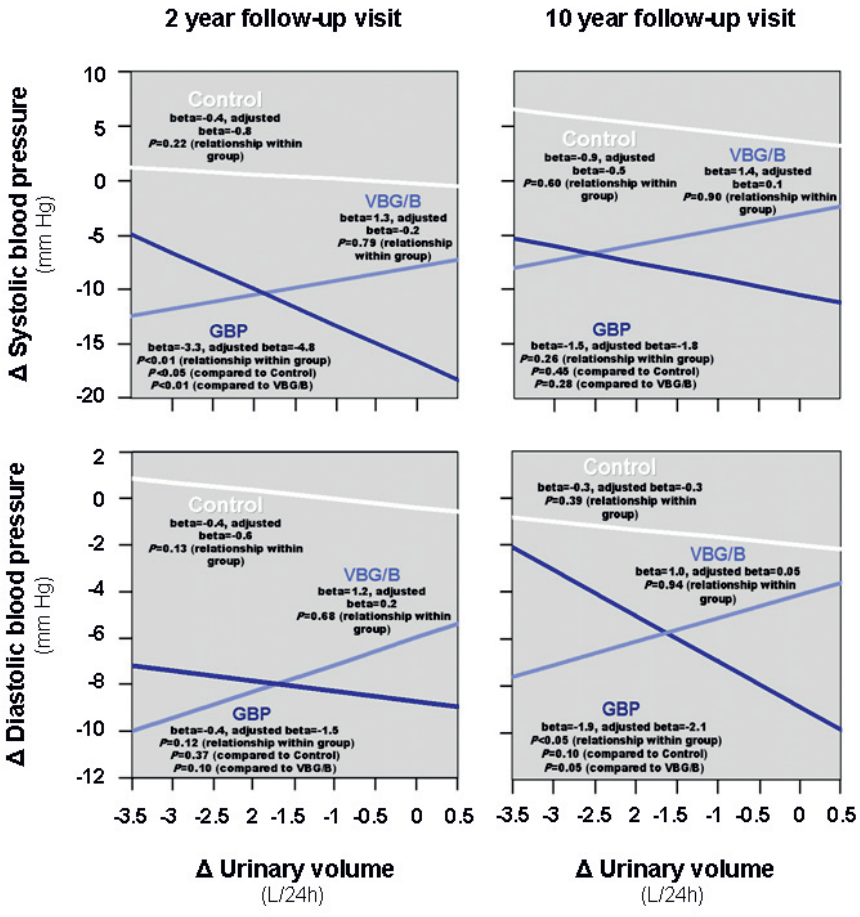


Figure 13. **Linear relationship between blood pressure changes and changes in diurnal urinary output (U-Volume) after gastric bypass surgery (GBP), after pure restrictive bariatric surgery (VBG/B) and in non-operated obese controls at the 2 and 10 year follow-up visits.** Regression lines and beta values (unadjusted) illustrate results of simple linear regression analysis, while adjusted beta values and P-values are results of multiple linear regression analysis adjusted for both BMI change and change in daily salt intake, as well as for sex, age and baseline BMI.

Additionally, the clinical relevance of the observed GBP associated enhancement of diuresis was supported by comparison to effect of use of pharmacological diuretics in the non-operated control arm of the SOS-study: The magnitude of enhanced diuresis after GBP (100-200 ml) was similar to the difference in urinary output observed between users and non-users of diuretics in the non-operated cohort.

Interpretation

If GBP silences out the diuresis promoting monitor of the upper gut, why did these patients excrete more urine than did the weight matched subjects that had their gastrointestinal continuity intact? In order to explain gastrointestino-renal diuretic regulation one has to consider the short-time course of the gastroduodeno-signalling (related to ingestive behaviour) and that after GBP there is probably an additional effect of direct volume-loading into the rapidly absorbing jejunum. Over 24h it may be that diuresis-promoting post-absorptive mechanisms become more pronounced when gastroduodenal short-term coordination is bypassed, resulting in an "overshoot" of fluid excretion by the kidneys. The situation after GBP is actually mimicked by the jejunal infusion in Paper III where plasma BNP increased already within 1h, strongly indicating the induction of post-absorptive diuresis-promoting mechanisms.

However, a primary natriuretic mechanism seems unlikely as the GBP patients were found to have a slightly increased serum sodium concentration. Contributing to this intriguing picture was the finding that GBP patients consumed approximately 1 g dietary salt more per day than the group operated with the restrictive banding techniques. The picture can be compatible with that the upper-gut sodium/volume sensor, in addition to short-term (i.e. <2h) diuretic regulation, normally inhibits salt appetite and that salt intake increases following GBP. Alternative explanations and need for future research will be discussed below in General Discussion.

3^{d} conclusion

Permanent exclusion of the upper GI tract increases salt intake and diuresis suggesting that the upper gut sodium/volume sensor participates in the regulation of salt appetite. Blood pressure reduction following gastric bypass surgery can be attributed to a diuretic action.

CONCLUSIONS

1. Prominent components of RAS are present in the human gastric and duodenal mucosa. *H. pylori* induced gastritis is associated with higher prevalence of AT1 receptors, most probably due to presence of infiltrating neutrophils carrying this receptor. Immunoreactivity to AT1R and AGT in solitary epithelial cells suggest that local RAS activity can influence gastroduodenal enteroendocrine signalling.

2. The temporal relationship between increased diuresis induced by an intragastric saline load and the reduced quantity of AGT in duodenum suggest a role for RAS in the duodenal mucosa in the pre-absorptive induction of diuresis occurring after drinking and eating.

3. Permanent exclusion of the upper GI tract increases salt intake and diuresis suggesting that the upper gut sodium/volume sensor participates in the regulation of salt appetite. Blood pressure reduction following gastric bypass surgery is attributed to a diuretic action.

GENERAL DISCUSSION

Some methodological considerations

This thesis project includes several methodological principles regarding both laboratory analyses and to the design of studies. Papers I, II and III are exploratory studies on carefully standardized small samples of populations, whereas IV is an *ad hoc* analysis of data from a prospective interventional trial involving in total more than 4000 included patients.

The antibody dependent assessments (Western blotting and immunohistochemistry) were performed in accordance to validated standard procedures and included internal controls. The quality of the commercially acquired antibodies can differ markedly and all new batches were therefore tested for specificity. The immunoassay (EIA) used for plasma-AngII analysis was used carefully according to the manufacturer's instructions. Immunoassays used for other plasma peptide analyses, and the analyses of electrolyte concentrations in serum and urine, were outsourced to accredited clinical chemistry laboratories. In general, all the analytical procedures were confirmed as specific and sensitive. In Paper III, however, tissue concentration of AngII was assessed using an EIA not validated for its use in solid tissues. The biological halftime of AngII in tissue is short and it cannot be excluded that, despite addition of enzyme inhibitor during work up, the amount of the octapeptide in the mucosal samples had already been reduced due to endogenous enzymatic activity.

Another potential source of error in the present project is the 24h sampling of urine in the SOS study (IV). As the urine was collected over 24h by the study subjects them selves, sampling errors related to over- or under-collections must be considered. However, there is little reason to believe that any of the surgery groups would be more likely to provide over- or under-collections. Furthermore, the differences in diurnal

urine output and sodium excretion between GBP- and gastric banding subjects remained significant after adjusting these analyses for U-creatinine[79, 80].

RAS in the upper gut mucosa

The presence of AngII receptors is a good indication of the potential impact by RAS on the functional state of a tissue. A few previous studies have indicated expression of AngII receptors in the upper GI tract. Autoradiography of the rat stomach indicated that AT1R and AT2R are present in all layers of the stomach[81]. Matsuo *et al.* used immunohistochemistry to locate AT1R in human antrum and found it in vascular smooth muscle cells, mesenchymal cells and smooth muscle cells in the muscular layers of the mucosa and muscularis propria[82]. Further, Bregonzio *et al*. located the AT1R protein by means of immunohistochemistry in vascular endothelial cells in the rat stomach[81]. Johansson *et al* localised both AT1 and AT2 receptors in the lamina propria in duodenal mucosal villi in the rat[5]. The present study confirms presence of AngII receptors in the human gastroduodenal mucosa and provides novel data on the presence of several other RAS components (I, II, III). Of particular interest is the finding of storage/depletion of the prohormone AGT in the duodenal mucosa (III). These novel aspects of RAS will be further discussed later.

The presence of AngII receptors at specific locations suggests functional importance. Not surprisingly, vascular endothelium expressed both the AT1R and AT2R subtypes in the present study (I, II). The former mediates the classical vasoconstrictive effect of AngII, whereas AT2R has been reported to mediate vasodilatation[12]. However, the superficial vessels of the mucosa studied in the present study were very thin-walled (i.e. without blood flow regulating vascular smooth muscle cells) and consisted mainly of capillaries and venules. AngII receptors in this tissue compartment are probably related to microvascular permeability as has been reported for rat mesenteric venules[83, 84]. To what extent AngII influences mucosal microvascular permeability in the human upper gut remains to be tested. Interestingly, AT1R and AT2R were localised also to cells in the lamina propria and the epithelium suggesting functional impact on epithelial functions.

In the duodenal mucosa, distinct immunoreactivity for AT1R and AT2R was seen primarily in the basal part of most epithelial cells. This differs from the picture in rat duodenum where immunoreactivity was confined to cells in the lamina propria[5]. Speculatively, sodium/water[26] and glucose transport[28, 29] as well as bicarbonate secretion[5] or regulation of cell growth[12] can be targeted by activation of these juxta-epithelial and epithelial receptors, but more research is needed to elucidate physiological significances in man.

The present study also indicated that AT1R is highly expressed by a subpopulation of antral endocrine cells in the stomach. It has been reported that AngII through AT1R can influence gastric acid secretion in rats[85]. In the present study, however, no AT1R was found on the acid producing parietal cells so if AT1R influences gastric acid secretion in man, it is possible that such an effect first involves antral endocrine cells. In the human duodenal mucosa, distinct immunoreactivity for AT1R and AT2R was found principally in all epithelial cells (which will also include the endocrine cells), as mentioned above. Therefore, it's reasonable to assume that also some duodenal enteroendocrine cells express the AT1R protein. The localisation of AT1R to endocrine cells suggests that activity in a local RAS can influence enteroendocrine signalling and represent an interesting field for future research.

A perquisite for AT1R and AT2R activation is the presence of the ligand AngII. This peptide is the result of enzymatic degradation of AGT by renin and ACE and both these enzymes were found in the human gastric and duodenal mucosa. The present study also identified significant levels of AngII in these tissues (III), supporting that the main effector peptide of RAS is also generated in the mucosa of the upper gut. In addition to AngII receptors, angiotensin generating enzymes and AngII, human gastric and duodenal mucosa also showed immunoreactivity to AGT, the prohormone of RAS. AGT was located to solitary epithelial cells in the duodenal mucosa. This observation indicates that AGT is produced and stored at certain cellular locations in the upper gut mucosa. This is a novel finding that certainly demand further investigation. For example, by use of laser capture microscopy it will be possible to assess mRNA expression of the AGT-

immunoreactive epithelial cells without any interference of other celltypes (such as for example the vascular endothelial cells).

Furthermore, data in Paper III suggest that luminal stimulation (in this case isotonic NaCl) influence AGT liberation in the duodenal mucosa that, depending on presence and type of degradation enzymes, may result in formation of bioactive angiotensins with potential to exert endocrine or paracrine effects. Furthermore, mRNA expression of AGT by intestinal "taste cells" in mice has been reported by others[86], supporting that this protein has a relation to mucosal sensing mechanisms. Future double immunostaining studies, using specific markers to taste cells, endocrine cells and brush cells, are needed to further characterise the AGT-positive solitary epithelial cells found in the human duodenal mucosa. Still, this observation supports the possibility that paracrine RAS activity might influence also duodenal enteroendocrine signalling.

Is the gastroduodenal RAS involved in sensing of luminal contents?

We considered that the previously postulated salt/volume monitor in the upper gut[32, 34] could to be of particular interest as a physiological context for the mucosal RAS in the stomach and duodenum. Therefore, levels of AGT and AngII in the human gastro-duodenal mucosa subsequent to a gastric saline load were measured *in-situ*, at a time point corresponding to onset of the diuretic response following gastric instillation of saline (determined in our previous experiments). Indeed, the content of AGT in the duodenal mucosa decreased significantly subsequent to the gastric saline instillation and that was not the case in antral specimens. This observation thus suggests that the duodenum rather than the stomach is the primary site for luminal sensing of a volume load.

Enzymatic degradation of the pro-hormone AGT can result in several bioactive "angiotensins" and the one of particular interest is of course the octapeptide AngII, being the principal mediator in RAS. However, the present investigation did not show

increased AngII concentration in the duodenal mucosa upon intragastric saline loading, suggesting that this mediator is of less importance for the diuretic response. Yet, the absence of changes in local mucosal AngII levels does not completely rule out a role for this mediator. The paracrine mode of action of AngII on, for example, enteroendocrine cells in turn eliciting distant hormonal or autonomic neural effects, may demand local liberation of rather small amounts that are subthreshold to the presently used assay. Paracrine arrangements are difficult to study in human's *in-situ*. A feasible way for further investigation of the role of RAS in duodenal sensing of luminal content may be to isolate the mucosa in an Ussing chamber and use different luminal stimuli (e.g. hypertonic saline and nutrients) with pharmacological inhibitors to for example renin, ACE etc. Also other bioactive AGT-fragments with diuretic effects have been described[87], such as Ang-(1-7). These peptides can be generated locally in the GI-mucosa, depending on type of proteolytic enzymes present in the tissues, and remain to be investigated in the future.

The gut sodium/volume sensor?

The pre-absorptive sodium/volume sensor in the GI tract is postulated to be situated in the upper gut but the exact location of this sensor has not been determined. In the present study, we approached this mechanism by studying the diuretic response to instillation of saline at two sites along the gut lumen (III). These results indeed support that a pre-absorptive diuretic signal is elicited when the gastroduodenal region is subjected to a sodium-water load. The latency of onset to increased diuresis was markedly shorter after gastric loading than after jejunal loading. Furthermore, the diuretic response was not associated with hormonal changes that typically are involved in relation to extracellular volume regulation. The diuretic response to intrajejunal saline instillation, on the other hand, had a much slower onset and was associated with increased plasma BNP concentrations suggesting that a systemic volume expansion had preceded the diuretic response[70]. Hence, the data indicate that the intrajejunal instillation induces diuresis mainly via post-absorptive mechanisms. The interpretation of these experiments is that a pre-absorptive fluid sensor is situated in the upper gut.

Because the rate of gastric emptying of non-energetic, isotonic liquids is high with halftimes in the order of 8-10 min[68, 69], the duodenum almost instantly will be targeted by liquid disposed in the stomach. Consequently, it seems reasonable to assume a more precise location of the pre-absorptive sensor being the stomach and/or the duodenum. The natriuretic response in the present study did not show any consistent temporal patterns in relation to infusion site. Still, total sodium outputs were similar over the observation time. Therefore, these results do not support that the upper gut senses sodium specifically. However, we chose instillation of isotonic NaCl (6.8 g NaCl) in order to avoid effects of osmotic gradients. It follows that isotonic saline mainly is a volume stimulus. A transmucosal sodium gradient might be needed to stimulate the sodium sensor mechanism. Such experiments, using higher luminal salt concentrations, are in progress.

Role of the gut in body fluid homeostasis and arterial pressure control

To further investigate the upper-gut sodium/volume sensor, as well as its potential impact on arterial blood pressure control, we analysed measurements of salt intake, diuresis and arterial blood pressure following two weight reducing surgical principles: gastric banding and gastric bypass (GBP). The GBP operated individuals consumed approximately 1 g dietary salt more per day and had a slightly increased serum sodium concentration, compared to the weight matched subjects that had undergone a restrictive procedure with the gastrointestinal continuity intact. The increased salt consumption together with a small hypernatremia is consistent with an increased preference for salty foods[88]. Actually, Tichansky *et al.* reported that GBP patients experience an increased salt appetite[89]. However, a diet-induced hypernatremia is controversial in relation to the view that increased salt intake should contribute to increased blood pressure[90-92], not the opposite as was observed in the present study. Furthermore, the GBP patients exhibited a larger 24h diuresis being linearly associated with blood pressure reduction.

Apparently, the GBP procedure functions as a diuretic agent, very probably explaining the long term lowered blood pressure. The increased diuresis, however, cannot simply be explained as a compensation for increased salt intake as hypernatremia normally is associated with reduced diuresis. Low vasopressin concentrations can be associated with hypernatremia, but does not explain the increased salt intake. It is likely that the GBP procedure elicits a mixed action, for example increased salt appetite/intake and increased natriuresis due to increased BNP-levels. The nature of the increased diurnal diuresis following GBP should be reasonably easy to sort out by assessing plasma and urine osmolality as well as the humoral mediators of interest for fluid homeostasis.

The relation between "pre-absorptive" and "post-absorptive" mechanisms

In Paper III, a diuresis promoting pre-absorptive mechanism sensitive to gastroduodenal filling was described. Permanent exclusion of this mechanism due to GBP was followed by an even more pronounced diuretic action (IV). How to put this together? Well, it is a very common phenomenon in physiology that anticipatory mechanisms override the more basal adaptation to an existing aberration. In this case, volume and sodium loading of the upper gut (III) probably activates anticipatory mechanisms via the circulation or the central nervous system that counteract the oncoming absorptive phase and plasma volume expansion by changing appetite, ingestive behaviour and urinary output. This "pre-absorptive" (or foregut) fluid regulation normally avoids activation of "post-absorptive" mechanisms. For example, the liberation of BNP is not needed because the organism has already taken the relevant measures so that an expansion of ECF has been prevented. In the case of GBP, the anticipatory foregut regulatory mechanisms are removed and the organism has to count on its post-absorptive regulatory capacity (or perhaps so far unknown hindgut factors).

Physiological and clinical relevance

From the perspective depicted above, one cannot say that GBP normalise blood pressure control. The procedure rather manipulates the physiology so that post-absorptive "overshooting" makes the blood pressure decrease. In that sense, the foregut sodium/volume sensor is of physiological significance by being one principal mechanism behind why GBP surgery has beneficial effect on obesity-associated blood pressure elevation. Furthermore, Paper IV also shows that GBP reduced blood pressure with a magnitude of clinical relevance[93] over long time (median follow-up time: 10 years). This contrasted to gastric banding techniques that exerted a short-lasting and weight-related depressor effect.

The clinical relevance of excluding the foregut and the volume/sodium monitor is obvious. Several findings suggest that a local mucosal RAS is involved in the pre-absorptive volume and sodium sensor of the upper gut, but conclusive data are still lacking and represents an area for urgent future research.

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