“Det man i”
Artur Sjöberg

Se acknowledgements
**ABSTRACT**

**BACKGROUND**

Acute pancreatitis is a disease with potentially lethal consequences. The hormone angiotensin II inhibits the protective mucosa alkaline secretion in the duodenum via the angiotensin II type 1 receptor. A local pancreatic renin-angiotensin system affects enzyme secretion and inflammatory response in the pancreas. Animal experiments have shown that blocking the angiotensin II type 1 receptor could prevent acute pancreatitis. Endoscopic retrograde cholangiopancreatography (ERCP) is followed by an increased risk of elevated pancreatic enzymes (hyperenzymemia) and acute pancreatitis, and no pharmacological prevention exists.

**METHODS**

Paper I: A laboratory study of the response to angiotensin II infusion by measuring the ratio of angiotensin II type 1 and type 2 receptors and the duodenal mucosal alkaline secretion in rats.

Paper II and IV: The effect of angiotensin II receptor blockers (ARB) and relative risk of acute pancreatitis was estimated in two case-control studies: a) A matched case-control study among patients with hypertension in 1996-2005, nested within the Health Improvement Network in the United Kingdom (II), and b) A nationwide population-based case-control study during 2006 in Sweden (IV).

Paper III: A randomized triple-blind placebo controlled clinical trial at two Swedish hospitals was performed in 2005-2008 to evaluate whether 50 mg of the ARB losartan, given one hour before ERCP, prevents hyperenzymemia 24 hours after ERCP.

**RESULTS**

Paper I: Angiotensin II did not elicit any response in duodenal mucosal alkaline secretion. The expression of angiotensin II type 2 receptor was decreased compared to previous studies. Administration of angiotensin II increased the relative expression of angiotensin II type 2 receptor compared to the type 1 receptor.

Paper II: During follow-up of more than 600,00 person-years at risk, 265 cases of acute pancreatitis and 2,000 control people were compared. The risk of acute pancreatitis was statistically non-significantly decreased in ARB users (odds ratio 0.63, 95% confidence interval 0.38-1.03) compared to non-users.

Paper III: Among 38 losartan treated patients, 9 developed hyperenzymemia, compared to 7 out of 38 placebo treated individuals. Losartan conferred an OR of 1.6 (95% CI 0.3-7.8) with regard to hyperenzymemia.

Paper IV: 1,961 case patients with acute pancreatitis and 20,000 control individuals from the general population were compared. The risk of acute pancreatitis in users of ARB was statistically significantly decreased (odds ratio 0.81, 95% confidence interval 0.69-0.97) after adjustment for age, sex, education, number of distinct drugs and cardiovascular disease.

**CONCLUSIONS**

Paper I: The response in duodenal mucosal alkaline secretion depends on the level of angiotensin II type 2 receptor expression, which in turn is affected by angiotensin II infusion.

Paper II and IV: The use of ARB might decrease the risk of acute pancreatitis.

Paper III: An oral dose of 50 mg of the ARB losartan may not prevent post-ERCP hyperenzymemia.
LIST OF PUBLICATIONS


II  Use of angiotensin II receptor blockers and the risk of acute pancreatitis: a nested case-control study. Accepted for publication in Pancreatology.

III Angiotensin II receptor blocker losartan in the prevention of hyperenzymemia after endoscopic retrograde cholangiopancreatography: a randomized clinical trial. Submitted manuscript.

IV Angiotensin II receptor blockers and risk of acute pancreatitis: a population based case-control study using the Prescribed Drug Register in Sweden. In manuscript.
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LIST OF ABBREVIATIONS

ARB  Angiotensin II receptor blockers
AT1  Angiotensin II type 1
AT2  Angiotensin II type 2
BMI  Body mass index
DMAS Duodenal mucosal alkaline secretion
ERCP Endoscopic retrograde cholangiopancreatography
CI   Confidence interval
OR   Odds ratio
Q-PCR Real-time quantitative polymerase chain reaction
THIN The Health Improvement Network
INTRODUCTION

Acute pancreatitis is a disease with rising incidence causing a large amount of morbidity and in its most severe form high mortality. \(^2\)-\(^5\) There are many unanswered questions regarding the prevention, etiology, pathophysiology, and treatment of acute pancreatitis. New and improved approaches of these issues are desirable. The emphasis of the present thesis is on a potential pharmacological prevention of acute pancreatitis.

The renin-angiotensin system, traditionally known as a hormonal system regulating blood pressure and electrolyte homeostasis, is being addressed in new areas of application. Angiotensin II receptor blockers (ARB) are commonly used in the treatment of patients with hypertension and heart failure to prevent cardiovascular complications like stroke and myocardial infarction. \(^6\) The renin-angiotensin system is physiologically activated, for instance, in response to blood loss and dehydration. \(^7,\) \(^8\) Under these conditions, fluid can be reabsorbed from the gastrointestinal tract, which contributes to the integrity of the systemic circulation. This response is partially mediated by stimulation of angiotensin II signaling in the gastrointestinal tract as shown already in 1980’s. \(^7\)-\(^9\) Angiotensin II signaling also inhibits the protective mucosa duodenal alkaline secretion, an effect that can be reversed by ARB. \(^10\)

The local renin-angiotensin system in the pancreas has recently been suggested to play interesting roles in many aspects of the functions of the pancreas. \(^11\) Angiotensin II stimulation in the pancreas affects endocrine \(^12\) and exocrine \(^13\) secretion as well as its microcirculation. \(^11\) Interestingly, conditions like hypoxia, \(^14,\) \(^15\) and pancreatitis \(^16\) affect expression of angiotensin II receptors. In pancreatic inflammation and fibrosis, angiotensin II facilitates production of inflammatory mediators and recruitment of inflammatory cells. \(^17,\) \(^18\)

Thus, there is a potential benefit from pharmacologically blocking angiotensin II signaling in the pancreas to reduce or inhibit the inflammatory response. The present thesis investigates this hypothesis. In this thesis, experiments were used to investigate how the angiotensin II receptor expression is regulated, and the following studies investigated the potential protective effect of ARB on pancreatic inflammation.
BACKGROUND

RENIN-ANGIOTENSIN SYSTEM AND THE GASTROINTESTINAL TRACT

Renin-angiotensin system and angiotensin II receptor blockers
The systemic effects of the renin-angiotensin system are well characterized elsewhere and will not be described in detail here. Briefly, the kidney produces renin, which facilitates hepatic angiotensinogen conversion to angiotensin I. Pulmonary angiotensin-converting enzyme (ACE) generates angiotensin II from angiotensin I. The highly active octapeptide angiotensin II constricts blood vessels, mediates sodium re-absorption from the kidney, and increases thirst and sodium appetite by stimuli in the central nervous system.

Different opportunities for pharmacological interventions of the renin-angiotensin system exist. ACE inhibitors, and ARB act directly on the renin-angiotensin system in different ways. ARB antagonize angiotensin II binding to the AT1 receptor, but do not decrease circulating angiotensin II levels, which is a mechanism of ACE inhibitors. These two drug types are therefore used simultaneously in some instances, because of their different pharmacological mechanisms. The main indications for ACE inhibitors and ARB are hypertension and heart failure. In general, ARB are recommended as a secondary choice after testing ACE inhibitors for these conditions.

Approximately 10–20% of patients experience side effects from ACE inhibitors, i.e. coughing and prescribed ARB instead. Regarding side effects, ARB are generally well tolerated apart from electrolyte disturbances, hypotension and very rarely angioedema. The first ARB losartan was released 1994, and is used in paper III. Losartan has a 10x lower affinity to AT1 receptor than angiotensin II and is 3000x more selective for AT1 than AT2 receptors. Losartan is metabolized in the liver to its more active metabolite EXP3174, which accounts for most of the known pharmacological actions of the losartan drug. Compared to other ARB, losartan has a relatively low affinity for AT1 receptors but still sufficient to reach an efficient blockade in therapeutic dosage. Losartan peaks in plasma in one hour, whereas EXP3174 reach its maximum level after 3-4 hours. Other ARB has similar effects, although some have even higher affinity to the AT1 receptor and in some cases longer half-life, e.g telmisartan. The side-effect profile is similar between the different ARB. As a rule, ARB is considered to have similar effects and is regarded as a pharmacological class with similar properties and effects. Recently, tissue-specific renin-angiotensin systems have been identified, which is introduced below.

Role of the renin-angiotensin system in the gastrointestinal tract
In the jejunum, angiotensin II has been shown to act in concert with sympathoadrenergic system to increase water and sodium transport in situations like hypovolemia and dehydration. This reaction is in line with the body’s short-term survival reaction to loss of extra-vascular and blood volume. Subsequent
studies showed a role for angiotensin II receptors in jejunal fluid transport. The angiotensin II type 1 (AT1) receptor is commonly expressed in the body and mediates most of the angiotensin II actions. In the gastrointestinal tract, it influences vasoconstriction, smooth muscle contraction e.g. of the lower esophageal sphincter, and inhibits jejunal electrolyte and fluid transport. The latter effect is counteracted by the angiotensin II type 2 (AT2) receptor. In general, the AT2 receptor has a dynamic expression pattern, which is the object of study in paper I.

Regulation of duodenal mucosal alkaline secretion

The regulation of the protective mucosa alkaline bicarbonate secretion is complex, and some important aspects will be presented here. In general, the mucosal alkaline secretion creates a pH-gradient by which gastric acid is neutralized. As a result, the underlying epithelium is protected from injury. This bicarbonate secretion is influenced by neural, hormonal, and other factors, e.g. prostaglandins. This is exemplified by the vagal nerve stimulation of alkaline secretions. This protective action acts in concert with its stimulation of gastric acidity. The alkaline secretion is also stimulated by somatostatin and vasoactive intestinal peptide. In contrast, increased sympathetic activity, as seen in hypovolemia, inhibits the alkaline secretion. Interestingly, the renin-angiotensin system prolongs the sympathetic inhibition of duodenal mucosal alkaline secretion, mediated by angiotensin II and AT1 receptor. The AT2 receptor is initially shown to stimulate the duodenal alkaline secretion. This stimulatory effect of duodenal AT2 receptor was not reproduced in another line of Sprague-Dawley rats. This led to the investigation of the dynamics of the AT2 receptor expression in paper I. Some known functions of the AT2 receptor are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Summary: Some known functions of AT2 receptors in the gastrointestinal tract:</th>
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<tr>
<td>• Influences jejunal fluid and electrolyte absorption.</td>
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<tr>
<td>• Increases duodenal bicarbonate secretion.</td>
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<tr>
<td>• Affects smooth muscle contraction along the gastrointestinal tract.</td>
</tr>
<tr>
<td>• Counteracts the AT1 receptor in these functions</td>
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Function and localization of angiotensin II receptors in the pancreas

The function of the renin-angiotensin system in the human pancreas is not completely known. Angiotensin II receptors are responsive in situations like hypoxia, hyperglycemia, and cancer. The exact role of angiotensin II receptors in these conditions, however, is not known. Exocrine and insulin secretion are influenced by angiotensin II signaling. Stimulation of angiotensin II in the pancreas also mediates inflammatory response via e.g. production of nicotinamide adenine dinucleotide phosphate oxidase (NADPH) and nuclear factor κB. AT1 receptors have been mapped in the pancreatic acinar, beta cells, islet, ductal, as well as in blood vessels. Expression of AT2 receptors, has been
shown, in the acinar cells and ductal cells, while the function has not been completely characterized in the pancreas.

Pancreatitis and the renin-angiotensin system

Acute pancreatitis activates a local pancreatic renin-angiotensin system as well as the circulating renin-angiotensin system. 44, 45 For example, angiotensinogen and AT1 receptor are up-regulated in inflamed pancreatic tissue. 13 In addition, ACE inhibitors and ARB affect chronic pancreatic inflammation. 18, 46 In one study decreased signs of histological inflammation and increased AT2 receptor expression after pretreatment with an ARB. 18 Another paper reported that angiotensin II infusion elicited amylase and lipase secretion, 13 an effect that was reversible by use of an ARB. 13 Moreover, other studies, 16, 17, 47 have shown attenuated acute pancreatic inflammatory response in ARB treated rats after experimentally induced pancreatitis, using cerulein 16 and taurechlaceate, 47 and after bile duct obstruction. 17 These data motivated the investigation of ARB in the development of hyperenzymemia after ERCP in paper III and acute pancreatitis in paper II-IV.

NATURE OF ACUTE PANCREATITIS

Physiology and patophysiology

The exocrine part of the pancreas constitutes approximately 85% of its volume, the supportive tissue 10%, and the endocrine part 5%. 48 Physiologically, the pancreas produces and stores many potent digestive enzymes. These are stored as inactive pro-enzymes or zymogens (except amylase) to avoid premature activation followed by pancreatic tissue damage. In the exocrine pancreas, the acinar cells store zymogens in separate granules in its apical part, draining to the ducts and further transported to the duodenum, where activation normally occurs. Under physiological conditions trypsin inhibitors, trypsin degrading proteases, and adequate pH (normally the pancreatic tissue is mildly alkaline) all protect the integrity of the pancreatic tissue. This delicate balance could be disturbed by a number of events, 49 some of which are:

- Mechanical obstruction of the pancreatic duct, inducing an increased hydrostatic pressure, e.g. as seen in gallstone pancreatitis, ERCP and in case of malignant bile duct obstruction.
- Repeated high alcohol exposure.
- Genotype of decreased trypsin inhibitor activity. 50
- Exposure to acute acid load.
- Arterial ischemia inducing vasospasm. 51
- Pathological regulation of cytosolic calcium levels, resulting in inappropriate activation of trypsinogen.

Pathogenesis of acute pancreatitis

The inflammatory reaction following initiation of pancreatitis will briefly be presented here. Many different events could trigger the initiation of pancreatitis, some of which are mentioned above. Independent of the etiology, a key event in the pathogenesis of pancreatitis is inappropriate activation of
trypsin from trypsinogen. Initially, acinar cells are damaged, and cytokines are produced. This leads to recruitment of inflammatory cells, e.g. neutrophils and macrophages, which propagates the inflammation. In pancreatitis, inappropriate secretion of the acinar cell to the interstitial space could also lead to premature activation of digestive enzymes. In general, the danger is that the inflammatory reaction escalates to the extent of a systemic inflammatory response and possibly multi organ failure and eventually death. Characterization of the pathogenesis of acute pancreatitis is not completely known, and as a result, effective pharmacological treatment is lacking. 49

**Diagnostic Criteria of Acute Pancreatitis**

Acute pancreatitis is a clinical diagnosis. Acute onset of upper abdominal pain, often radiating to upper part of the back, and vomiting are symptoms in favor of the diagnosis of acute pancreatitis. These in combination with elevation of pancreatic amylase or lipase (often arbitrarily set to >3 times above the upper reference value) are sensitive markers for acute pancreatitis. Pancreatic enzymes are released into the circulation during an acute attack. Levels peak early and decline over 3–4 days. The British Society of Gastroenterology has published guidelines for management of acute pancreatitis. 52 It is there stated that: “An important concept derives from this: the diagnosis of acute pancreatitis should not rely on arbitrary limits of values 3 or 4 times greater than normal, but values should be interpreted in light of the time since the onset of abdominal pain” Alternatively, clinical imaging such as ultrasound may show pancreatic swelling, but the pancreas is visualized in only 25–50% of patients with acute pancreatitis. Contrast enhanced computed tomography often provides good evidence of acute pancreatitis. In some cases, autopsy findings are used to set the diagnosis. The specific criteria for acute pancreatitis used in paper II-IV are presented in later sections.

**Clinical Course of Events of Acute Pancreatitis**

The clinical course of an attack of acute pancreatitis varies from mild, (comprising < 1 week of hospitalization with supportive care), and severe i.e. prolonged hospitalization with intensive care, and presence of organ failure or local complications, e.g. necrosis or cysts. The ratio of severe acute pancreatitis is between 14% 4 and 28%, 2 according to Swedish studies defined by the Atlanta classification 53 and the Bradley definition, 54 respectively. The mortality is reported to be approximately 3-11% in most Western studies 2-5, 55 and seems to be falling. 2, 3, 5 The population-based mortality in acute pancreatitis appears to be stable with time. 2, 3, 56 The course of recurrent episodes of alcohol-related acute pancreatitis is usually milder.

**Management of Acute Pancreatitis**

Determination of the etiology is important for guiding immediate management and preventing recurrence. 52 The management of patients with pancreatitis depends further on the severity and of patient characteristics. Management of gallstone-related pancreatitis is described in a later section. Generally, the main treatment of other types of acute pancreatitis is symptomatic and conservative
with e.g., fluid resuscitation, pain relief, and antibiotic treatment. The aim is to avoid clinical deterioration and appearance of severe complications, i.e. multi organ failure, sepsis, and death. Surgery or endoscopic treatment other than ERCP for acute pancreatitis occurs only in selected cases, e.g. for some cases of severe necrotizing pancreatitis or pseudocysts.

**INCIDENCE OF FIRST-TIME ACUTE PANCREATITIS**

**Sweden**

The incidence of first-time acute pancreatitis in Sweden seems to be increasing during the last decades according to some observational reports, but not in all. The latter report observed an annual incidence of 30 per 100,000 person-years, which was stable during the period 1975-1996. However, this study included recurrences of the disease. The reported trend in another study between 1985 to 1999 was an annual increase in incidence of 3.9%, approximately from 18 to 32 per 100,000 person-years. A nationwide survey in 1988 to 2003 described a rising incidence from 21 to 32 per 100,000 person-years during the study period.

**Western world**

Overall, acute pancreatitis seems to be increasingly common. The increased use of analyses of amylase and lipase in emergency departments could partly explain this (detection bias) as well as the aging population in Western countries. The geographical variations in the incidence rates are likely due to disparities of the prevalence of risk factors and to different genetic susceptibility. In the Netherlands, the incidence has risen from 12 to 16 per 100,000 person-years from 1985 to 1995, and in Ireland from 18 to 24 per 100,000 person-years in 1997 to 2004. The incidence in the UK has been reported to be lower than in other European countries, contrary to one study based on hospital admissions observing a similarly rising incidence from 16 to 21 during 1989 to 2000. In the United States, and specifically California, the incidence had risen from 33 to 42 per 100,000 person-years during 1994 to 2001.

**Gallstone-related pancreatitis**

The rising overall incidence of acute pancreatitis could partly be attributed to an increase in gallstone-related pancreatitis. This etiology accounts for about a third (27-42%) of cases in Swedish studies and up to half of the cases in a some studies in a recent review. The prevalence of gallstones, a precondition for gallstone-related pancreatitis, has been thoroughly explored. For example, a population-based cohort study reported that 53% of women and 32% of men above 35 years old had ultrasound verified gallstones. The incidence rate, however, has been less investigated. Using the same study base as mentioned above, incident cases of asymptomatic and symptomatic gallstones were investigated. Individuals without gallstones at baseline were followed and re-examined after at least 5 years. Eight percent, or 42 out of 402, had developed gallstones. As a comparison, a recent population-based Italian
study reported an annual incidence rate of 0.66% in men, and 0.88% in women.\textsuperscript{63}

**Alcohol-related acute pancreatitis**

In contrast to gallstone-related pancreatitis, the incidence of alcohol-related pancreatitis has decreased in Sweden, in parallel with a decrease of \textit{delirium tremens}, an acute disorder usually caused by alcohol withdrawal in alcoholics.\textsuperscript{4} Alcohol use accounts for between 5 and 50\% of all cases of acute pancreatitis.\textsuperscript{56} This large variation could due to geographical differences in the prevalence of risk factors. Moreover, the accuracy in measuring alcohol exposure is often low. Consequently, the contribution of alcohol to the burden of acute pancreatitis is often more or less under-estimated.

**Etiology of acute pancreatitis**

**Gallstone disease and acute pancreatitis**

In the Western world, gallstones mainly contain cholesterol in approximately 80\% of cases. The rest consists of mainly pigmented stones. Main components of the bile include bile acids, bilirubin, phospholipids, and cholesterol. Supersaturation of cholesterol-containing vesicles may lead to crystallization and formation of gallstones. Gallstone formation is facilitated by e.g. old age, female sex, obesity, estrogen therapy, and sudden weight loss.\textsuperscript{48} Impacted gallstones in the common bile duct that obstruct \textit{papilla Vateri} is one mechanism by which gallstones may cause pancreatitis, which was proposed already 1901 by Opie.\textsuperscript{65} Another explanation is that bile reflux to the pancreatic duct results in an inflammatory response.\textsuperscript{66} According to a longitudinal study, 3\% of patients with gallstones experience symptoms like biliary colic.\textsuperscript{67} Among these symptomatic patients, about 3-5\% annually have complications, i.e. gallstone pancreatitis, cholecystitis, choledocholithiasis, or cholangitis.\textsuperscript{67}

**Alcohol and acute pancreatitis**

Repeated high alcohol exposure may cause pancreatitis through a number of proposed mechanisms, which are not completely elucidated.\textsuperscript{49} The effect of alcohol differs largely between individuals, indicating modifying genetic and environmental factors that influence the result. There is no doubt, however, that ethanol and its metabolites predispose the pancreas to inflammation. Ethanol may decrease perfusion to the exocrine pancreas, which cause pathologically microcirculatory changes.\textsuperscript{68} Ethanol metabolizes in the pancreas via two pathways. One is the non oxidative way to fatty alcohol esters causing calcium release.\textsuperscript{70} The other oxidative pathway is present in e.g. the pancreatic stellate cells.\textsuperscript{71} This breakdown causes oxidative stress, which might activate an inflammatory state in pancreatic stellate cell.\textsuperscript{71} This cell type, named after its star-like appearance, resembles the Kupffer cell of the liver and regulates fibrosis development in that organ.\textsuperscript{72}

Alcohol pancreatitis is more common among males than females and decreases with higher age, which is likely due to sex and age differences in the habits of
alcohol consumption. Following a high-risk population, 2-4% developed acute pancreatitis according to prospective cohorts after decades of heavy drinking. Alcohol-related acute pancreatitis is often recurrent and could progress into chronic pancreatitis.

**Tobacco smoking**

Tobacco smoking has in several studies been reported to be independently associated with acute pancreatitis. A prospective cohort study investigated the effect of smoking on the risk of acute pancreatitis in Southern Sweden, reporting a more than doubled risk among current smokers after adjustment for age, sex, BMI, and alcohol consumption (by both survey and biochemical assay of γ-glutamyl transferase which increased with alcohol over consumption). No adjustment for gallstone disease was made, however. There was a dose-dependent relation and the increased risk was present also among non-alcoholics. Similarly, a Danish cohort study found a time-dependant and dose-response relation between smoking and acute pancreatitis, after controlling for alcohol and gallstone disease. Despite adjustment for alcohol, residual confounding could nevertheless exist in these studies due to unmeasured alcohol exposure.

**Obesity and risk of acute pancreatitis**

There is no consistent evidence that obesity per se is a risk factor for acute pancreatitis. A Swedish case-control study reported a weak positive correlation, whereas another study found no association after adjustment for other known risk factors. Obese people have, however, a higher risk of more severe acute pancreatitis than non-obese. A potential biological explanation is the low-grade sub-clinically inflammatory state that characterizes obesity. Also, the prevalence of hypertriglyceridemia, a risk factor for pancreatitis, increases in obese patients.

**Drugs**

In general, many medical drugs e.g. tetracycline, valproate, and metronidazole are suspected of causing acute pancreatitis. Yet, drug related disease account for no more than approximately 2% of the cases. In a paper on drug-induced pancreatitis, it was concluded that warnings of drugs causing acute pancreatitis are seldom confirmed on a population-based level. A recent review proposed a systematic way of classifying drugs suspected of causing acute pancreatitis according to four levels. This classification is based on strength of evidence, i.e. class I: positive re-challenge, class II: consistent latency, class III: at least two cases without re-challenge or consistent latency, and class IV: presence of at least one case report. This section will focus on anti-hypertensive drugs and the risk of acute pancreatitis.

**Angiotensin II receptor blockers**

Two reviews on drug-induced pancreatitis classified ARB as drugs that probably cause acute pancreatitis. The first released ARB, losartan, has been implicated in causing acute pancreatitis in a number of case reports.
On the population-based level, however, ARB users have not been found to be at an increased risk of acute pancreatitis compared to non users according to a European case-control study. 89

Angiotensin-converting enzyme inhibitors

Evidence of the risk of acute pancreatitis linked to ACE inhibitors is equivocal. Two reviews on drug-induced pancreatitis supported the notion of a higher risk of acute pancreatitis. 83, 84 In the former, 83 the evidence concerned mainly enalapril. A large European case-control study reported a positive association between intake of ACE inhibitors and the risk of acute pancreatitis. 89 In contrast, a Canadian study of an elderly population did not show any such association. 90 Additionally, a study from the General Practitioner’s research Database in the United Kingdom found a weakly increased risk estimate for captopril, but not for enalapril. 85 The authors suspected that underlying diseases could have confounded the association between ACE inhibitors and acute pancreatitis.

Hypothetically, different types of ACE inhibitors may have different risk effects. The class effect of ACE inhibitors has been questioned in other conditions. 91, 92 For example, among patients with heart failure enalapril and captopril users had a higher mortality than did other ACE inhibitor users. 91

Other antihypertensive drugs

Loop and thiazide diuretics have been linked with an increased risk of acute pancreatitis in some case reports, 83, 84 but not in a population-based study. 89 In the same study, no increased risk was found with regard to beta blockers. Calcium channel blockers, however, conferred an OR of 1.5 (95% CI 1.1-2.1). In general, beta-blockers and calcium channel blockers are not suspected to increase risk of acute pancreatitis. 83, 84

Hypertriglyceridemia

Hypertriglyceridemia (usually above 1,000 mg/dL) is an established cause of acute pancreatitis. 93 Appropriate treatment of the triglyceridemia aids in resolution of the acute illness and prevents future episodes of pancreatitis. 94 Although the biological mechanism is not completely understood, high serum levels of triglycerides could be converted to toxic fatty acids in the presence of lipase leaking from acinar cells. Accumulation of fatty acids in the pancreatic microcirculation could induce endothelial injury and activation of trypsinogen to trypsin, leading to pancreatic inflammation. Hypertriglyceridemia is an uncommon cause of pancreatitis, occurring mainly in patients with familial hypercholesterolemia. 49

Endoscopic retrograde cholangiopancreatography

Endoscopic retrograde cholangiopancreatography (ERCP) is a common invasive investigation where the distal bile and pancreatic ducts are reached by a catheter put through a flexible endoscope with a side-viewing camera positioned in the duodenum. The ducts are cannulated via the papilla Vateri
through the endoscope by a thin catheter and visualized after radio opaque contrast injection under dynamic radiographic imaging. ERCP allows opportunity for endoscopic diagnostic and therapeutic surgical procedures, such as cutting the sphincter Oddi, sphincterotomy, specimen collection, extraction of stones, and bridging of duct strictures by insertion of a stent. Common indications for ERCP are icterus, cholangitis, common bile duct stones, and suspected malignancy in the vicinity of the biliary or pancreatic ducts. After the development of magnetic resonance cholangiopancreatography, a non-invasive investigation of bile and pancreatic ducts, ERCP have become less common for diagnostic indications. ERCP is associated with some potentially severe complications, mainly acute pancreatitis, bleeding, and perforation. Post-ERCP pancreatitis is encountered in about 2-7% of cases. 95-97 Frequently, the instrumental manipulation and injection of contrast causes elevated serum levels of pancreatic exocrine enzymes, i.e. pancreatic amylase and lipase (> 3x above the upper reference value defined as hyperenzymemia). Hyperenzymemia is a prerequisite for developing acute pancreatitis, which occurs after 25-40% of the ERCP procedures. 95, 98-100 The wide range of reported incidences is probably due to differences in patient populations, indications, endoscopic experience, type of procedure, and definitions of the outcome. 101 Known risk factors for post-ERCP pancreatitis include female sex, sphincter Oddi dysfunction, previous pancreatitis, and procedure-related factors, such as experience of endoscopist, extent of pancreatic duct injection, cannulation difficulties, and use of sphincterotomy. 96, 97, 102 ERCP is responsible for a limited part (5-7%) of all cases of acute pancreatitis. Nevertheless, ERCP provides a good opportunity for testing of pharmacological agents to prevent acute pancreatitis.

Other rare causes
Hypercalcemia, scorpion venom, trauma, and anatomical aberrations, i.e. pancreas divisum, are rare conditions that have been recognized as risk factors of acute pancreatitis. 49 Hereditary susceptibility, e.g. due to inherited alterations of genes regulating trypsinogen degradation i.e. serine protease inhibitor Kazal type 1 (SPINK1), raises the risk of acute pancreatitis in some families. 50

Idiopathic pancreatitis
Many aspects of the etiology of acute pancreatitis are still not characterized, underlining the complexity of the disease. Many environmental and hereditary factors interact to generate a state of pancreatic inflammation. Generally, the share of idiopathic pancreatitis is over-estimated from register-based data due to incomplete information on all risk factors. Excluding other reasons, idiopathic pancreatitis currently accounts for no more than 25% of all cases. 52

**PREVENTION OF ACUTE PANCREATITIS**
In general, treatment of risk factors and avoidance of risk behavior prevent development of acute pancreatitis. Primary prevention aims to avoid first-time
pancreatitis, whereas secondary prevention refers to recurrence of the disease. Some of the most effective tools for prevention are treatment of alcohol abuse and gallstones. In the case of gallstone disease, appropriate clinical action would be to remove the stones, e.g. by cholecystectomy or ERCP to facilitate the uncomplicated passage of stones through the papilla Vateri by sphincterotomy. Many pharmacological agents have been tested in the prevention of acute pancreatitis, of which some of the principal are described below.

**Prevention of gallstone pancreatitis**

To prevent new episodes of gallstone-related pancreatitis, cholecystectomy is recommended for patients suitable for surgery for removal of any common bile duct stones. However, the timing of surgery is not clear-cut. In general, the recommendation is an early intervention if the pancreatitis is not too severe and the patient is fit enough. In surgically unsuitable patients, endoscopic sphincterotomy by ERCP could replace surgery. In most cases of gallstone-related acute pancreatitis, gallstones have already passed through the papilla, but in case of persistent common bile duct stones, ERCP could be used to allow clearance. Cholecystectomy is recommended during or in conjunction with the same hospital stay to prevent recurrence of acute pancreatitis. If gallstones are left untreated, the risk of recurrence is high, i.e. in about a third of patients. Interestingly, in a Swedish sample, cholecystectomy was conducted on average 107 days from the first attack of gallstone pancreatitis. Trials have found that treatment with ursodeoxycholic acid in patients loosing weight because of diet or surgery could counteract the development of gallstone disease.

**Prevention of post-ERCP pancreatitis**

Several pharmacological strategies have been evaluated in the drug-related prevention of post-ERCP pancreatitis as described below. For example, relaxation of sphincter Oddi using calcium channel blockers, lidocaine or nitroglycerine, has been evaluated without convincing results. Antibiotics like cephtazidime have indicated positive, but not replicated, results. Studies of anti-inflammatory drugs, i.e. corticosteroids, allopurinol, plasma activating factors (PAF) inhibitors, interleukin 10, heparin and low molecular weight heparin, have not provided convincing results.

Another approach is to influence the pancreatic enzyme secretion by glucagone (also by relaxing sphincter Oddi), somatostatin, gabexate or somatostatin analogues, such as long-acting octreotide. According to a comprehensive review on post-ERCP pancreatitis: “In summary, somatostatin is possibly efficacious in the prevention of post-ERCP pancreatitis. “ Regarding octreotide, this has not been successful in preventing post-ERCP pancreatitis. In the review mentioned earlier, the author conclude that: “Octreotide increases basal sphincter pressure and might contribute to pancreatic outflow obstruction and, hence, pancreatitis”. Among protease inhibitors, aprotinin has not shown positive results. Similarly, gabexate cannot be recommended
for pharmacological prevention of post-ERCP pancreatitis.  

Ulinastatin has shown some promising results, but not been proven to be cost-effective in the prevention of post-ERCP pancreatitis.  

Pending successful pharmacological prevention of post-ERCP, careful patient selection should be employed to avoid border-line indicated ERCP, particularly in high-risk patients. In these patients, one has to consider alternative imaging techniques, i.e. endoscopic ultrasound and MRCP. Moreover, minimizing pancreatic injections and placement of pancreatic stents could be considered for high-risk groups.
**Aims**

In paper I, the overall aim was to better understand the dynamics of the angiotensin II receptor expression in the duodenum with regard to duodenal mucosal alkaline secretion.

The following specific questions were addressed in rats:

- Does the angiotensin II mediated response in increased DMAS depend on the expression status of AT1 and AT2 receptor?
- Can the expression of angiotensin II receptors and the response in DMAS be modulated by exogenous angiotensin II infusion in rats?

In paper II-IV the overall aim was to clarify if use of ARB decreases the risk of pancreatic hyperenzymemia (paper III) or acute pancreatitis (paper II and IV). The following specific questions were addressed in humans:

- Do users of ARB have a decreased risk of first-time acute pancreatitis than other patients with hypertension?
- Does oral administration of the angiotensin II receptor blocker, losartan decrease the incidence of pancreatic hyperenzymemia after ERCP?
- Are users of angiotensin II receptor blocker at a decreased risk of acute pancreatitis, compared to community controls?
MATERIAL AND METHODS

Figure 1. Overview of studies in the thesis.
The laboratory experiments were performed during two periods of time:

- During 2000-2002 (referred to in the text as 'previous line' delivered by Møllegard A/S Denmark).
- In 2003 (referred to as 'new line' bred by B&K Universal in Sweden).

In these experiments, analyses on the AT1a and AT1b receptor were conducted. Of note, most of the actions in rats are mediated through the AT1a receptor. 126, 127 Duodenal secretion and mean arterial pressure were monitored according to a standard protocol. 34

Anesthesia and surgical procedure on rats

Animals were housed in thermostatically controlled humidified rooms with a daylight-darkness cycle of 12 h and fed with standard rat chow and water as needed. All experiments were performed on non-fasted similarly weighing male Sprague-Dawley rats. The anesthesia was induced by methohexital, which was maintained by α-chloralose or by pentobarbitalum, until adequate anesthetic condition was confirmed by lack of response to the interdigital reflex. A thermostatically controlled heating pad and a lamp kept the body temperature at a physiological level (38°C). Tracheal intubation ensured free airways. The right femoral vein and artery were catheterized for drug infusions and measurements of arterial blood pressure, respectively. To gain access to the intraperitoneal space, a midline laparotomy was performed. The common bile duct was catheterized 5 mm proximal to the papilla Vateri to avoid contamination of the duodenal perfusate from bile and pancreatic juice.

Recording of duodenal mucosal alkaline secretion

A duodenal segment with the proximal end 0.5 cm distal to the pylorus and a length of 1.5 cm was isolated between two glass tubes connected to a reservoir enclosed by a water jacket for maintenance of 38°C. Saline solution (150 mM NaCl) was perfused and re-circulated through the reservoir and duodenal segment by means of a gas lift (room air) The duodenal mucosal alkaline secretion was titrated to pH 7.40 using well-calibrated pH-stat equipment in the luminal perfusate. 128

Western blot and measurement of angiotensin II receptor protein

Full wall thickness specimens from the duodenum were excised after the abdominal incision snap frozen in liquid nitrogen and stored at −70°C. Specimens were later thawed and homogenized on ice in buffer A. 129 Centrifugation was performed at 30,000 g for 30 min at 4°C. The pellet was re-suspended in buffer B (buffer A and a detergent) and subsequently stirred and centrifugated. Protein concentration of the resulting supernatant was then determined by Bradford’s method 130 and deep frozen until additional analysis. The electrophoresis was performed on a 10% Bis-Tris gel. One lane of each gel was loaded with pre-stained molecular weight standards, and two lanes on each gel were loaded with a positive control for AT1 receptors from
Pheochromocytoma cell line (PC-12 whole cell lysate) and for AT2 receptors from ras-transformed rat kidney cells (KNRK), respectively. Positive controls were used for intergel standardization when necessary. The proteins were subsequently transferred to a polyvinylidifluoride membrane and incubated with polyclonal specific antibodies of rabbit origin directed to the AT1 receptor and AT2 receptor, respectively. An alkaline phosphatase conjugated goat anti-rabbit IgG antibody and CDP-Star as a substrate were utilized to identify immunoreactive proteins by chemiluminescence. Images were captured by charge couple device camera, and semi-quantification was performed based on optical density per microgram of protein, using the software Gauge 3.3.

**Real-time PCR and measurement of angiotensin II receptor RNA**

For the quantification of mRNA expression, quantitative PCR was performed with SYBR Green I as a marker. The software supplied by Roche Diagnostics was used to perform the quantification. To reduce background and optimize signal intensity, a 4 mM concentration of MgCl2 was used. Sample concentration was determined from a standard curve for each pair of primers. Details on primer sequences, primer concentrations, PCR conditions, and references are shown in paper I. To standardize the expression of angiotensin II receptors a house keeping gene glyceraldehyde-3-phosphate dehydrogenase was used.

**Experimental protocol**

The experimental protocols are shown in detail in paper I. Some aspects are described below.

**Comparison of results from previous and new Sprague-Dawley line**

After 60 minutes of recovery from surgery, baseline recordings were made over a 30 min period after which drugs were administered, CGP42112A (AT2 receptor agonist) or saline vehicle infused intravenously during 45 min, or prostaglandin E2 (PGE2) applied intraluminally. Subsequently, net induced response in DMAS to the drug or placebo, expressed as percent of the individual baseline, was recorded. In some animals, antemesenteric duodenal specimens of full wall thickness were collected immediately after the abdominal incision. Finally, the animals were sacrificed.

**Angiotensin II infusion and response in DMAS**

The following experiments were divided into two parts. Firstly, dose-finding experiments were conducted to determine what concentration of angiotensin II that influenced the mRNA and the protein level of angiotensin II receptor. Secondly, a longer pre-treatment with high dose of angiotensin II was tested with analysis of angiotensin II receptor expression and the functional response in DMAS. See details in paper I.
Outcomes

The DMAS was evaluated with regard to differences between groups. Net change in DMAS was defined as the difference between an average of the last 15 minute-period of drug administration and basal conditions. The mRNA level of AT1a, AT1b, and AT2 receptors and individual ratio of AT1a to AT2 receptor were calculated. Comparisons were then made between the groups. Similarly regarding protein expression, levels of AT1 receptor and AT2 receptor was analyzed, and the individual ratios of AT1 to AT2 receptor were compared between the different groups.

Statistical analysis

Differences in DMAS and hemodynamic parameters between groups were analyzed by analysis of variance (ANOVA) with Bonferroni post-hoc test and unpaired Student’s t-test, respectively. Kruskal-Wallis or Mann-Whitney U-test evaluated receptor expression differences between groups. Data are presented, in general, as means (+/- standard error of the mean) where appropriate. For significance testing, a p-value of <0.05 was considered statistically significant.

PAPER II AND IV

Design of paper II

A matched case-control study was nested within the Health Improvement Network (THIN) research database in the United Kingdom. The main exposure prescriptions of ARB, the outcome of acute pancreatitis and covariate information were received from this database.

Design of paper IV

This was a Swedish population-based matched case-control study conducted during 2006. The main exposure was dispensed prescriptions of ARB. Information on such prescriptions was collected from the Prescribed Drug Register in Sweden (described below). The outcome was acute pancreatitis and this data was received from the Patient Register. The Swedish personal identity number assigned to all Swedish residents was used to link the data between different registers for each study participant.

Case identification II and IV

Cases were defined as persons in the source population having a first time acute pancreatitis, i.e. code K85 (ICD-10) as a discharge diagnosis in the Patient Register during year 2006 (IV). In paper II, a manual review to confirm the diagnosis acute pancreatitis was made of all charts belonging to patients with: 1) The diagnosis acute pancreatitis mentioned in a discharge letter from the hospital or 2) presence of elevated pancreatic enzymes i.e. amylase or lipase >2x above reference value in combination with clinical symptoms or confirmatory investigations, i.e. ultrasound, computed tomography, laparotomy, or autopsy.
Case validation II and IV

To ensure the validity of the data on acute pancreatitis diagnosis registered in THIN, one researcher (TS) manually reviewed all the medical charts of patients with a potential acute pancreatitis. The researcher was blinded to exposure status. In a subset of 50 cases, we requested the general practitioners to confirm the cases of acute pancreatitis. This yielded a good positive predictive value (94%) in predicting acute pancreatitis based on the THIN data. Therefore, no further efforts to confirm the diagnosis were conducted.

Regarding paper IV, a validation study of the acute pancreatitis diagnosis in the Patient Register, performed by our group, found that the diagnosis in the Patient Register yielded a high (96%) positive predictive value (unpublished data). This validation study was performed by manually reviewing all charts from suspected acute pancreatitis cases.

Control selection II and IV

Controls were randomly selected using the principle of density-based sampling. Controls were frequency matched on sex, age (within the same year) in paper II and calendar year (same year) in paper IV. Individuals eligible as controls were all members of the cohorts with assigned random dates included in that person’s follow-up time. This date was set to be the index date. From the eligible persons, 2,000 control subjects were randomly selected in paper II and 20,000 in paper IV.

Drug exposure II and IV

Drug exposure was classified based on the presence of a lasting prescription in relation to the index date. These prescriptions were regarded as current if the prescription lasted until index date or ended <14 days before index date, past if the prescription ended 15-365 days before index date, or non use if the prescription ended >365 days from index date. If no prescription was administered, that person was regarded as non-users. Duration was dichotomized into <6 months or ≥6 months of drug exposure, summing all consecutive prescriptions. Dosage was classified as high or low depending on the drug type as shown in Appendix 2 (II).

In paper IV, exposure was classified in three ways: 1) 114 days for a lasting prescription in relation to index date was defined as current use; 115-180 days since the last prescription was defined as past use; and > 180 days or no recorded prescription defined non-use. 2) We re-defined the cut-off for current exposure to 90 days in relation to index date. Past use was defined as 91-180 days, and non-use as before. 3) A manual validation scanned all prescriptions for ARB and calculated actual prescription length based on each dose and amount of medication. Anatomical, Therapeutic, and Chemical (ATC) codes were used to identify drugs in the Prescribed Drug Register, as shown in paper IV, appendix 2.
Outcome definition

Paper II

The main outcome was occurrence of acute pancreatitis as defined in earlier section.

Paper IV

We used the discharge diagnosis code K85 from the Patient Register to define acute pancreatitis. A sub-classification of this diagnosis was made by etiology into gallstone-related, alcohol-related, and other etiologies.

Statistical analysis in paper II and IV

To estimate the relative risk for acute pancreatitis, unconditional logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (CI), including adjustment for potential confounders in multivariable analysis. In paper II, the adjustment for confounding was made in three different models: 1) ‘Crude’ model including adjustment for matched variables, i.e. age (in increments of 10 years per category), sex, BMI (in five categories) and calendar year; 2) Basic model, i.e., ‘crude’ model adding alcohol consumption (in five categories of weekly consumption) and tobacco smoking (current, past, never and missing categories); and 3) Full model included a number of potentially important covariates added one-by-one to the basic model. Each variable affecting the OR of acute pancreatitis for ARB >10% was included in the final full model. After testing several covariates, these covariates age, sex, calendar year, alcohol consumption, tobacco smoking, number of visits to the general practitioner during the previous year, and use of angiotensin-converting enzyme inhibitors, adrenergic beta-blockers, calcium channel blockers, other hypertensive drugs and diuretics remained in the full model.

The same principle as above in selecting covariates for multivariable modelling was applied for paper IV. The crude model included age and sex. The full model consisted after testing several drugs and diseases age, gender, education (four categories), cardiovascular disease (any of the following conditions combined to one variable: hypertension, heart failure, ischemic disease, and cerebrovascular disease), and number of distinct medications (in steps of five unique prescriptions per category during the last six months from index date specified in Appendix 1, paper IV).

The health improvement network research database (II)

THIN is a database established among general practitioners in the United Kingdom for research purposes. Data are collected from routine clinical care for approximately 500,000 patients from more than 300 general practitioners’ practices. Data contains diagnosis (based on the Read Clinical System, comparable with the International Classification of Diseases, see paper IV), laboratory tests, and prescriptions on an individual basis. Moreover, correspondence between the general practitioner and the hospital is recorded in a free text section.
The Patient Register (IV)
Since 1987 the Patient Register provides nationwide coverage on all inhospitalized patients in Sweden, and since 2001 also on all specialized outpatient care. The register contains codes for surgical procedures and diagnoses, according to ICD-9 during 1987-96 and ICD-10 from 1997 and onwards. Duration of stay, main diagnosis, surgical procedures, and up to 8 concomitant diagnoses at discharge were recorded. Surgical procedure codes were used for the etiological classification of acute pancreatitis as shown in paper IV.

Prescribed Drug Register in Sweden (IV)
Since July 1 2005, the Prescribed Drug Register \(^{137}\) comprises prescriptions dispensed in Sweden, i.e. about 100 million annually, and the corresponding personal identity number. During the study period all pharmacies in Sweden were run by the government. The register contains details on all dispensed prescriptions, including date of filling, amount, and drug substance recorded according to the specific ATC classification code, as exemplified in Appendix 2, paper IV. \(^{138}\) Each prescription contains a free text area with dosage detail.

PAPER III

Design
A triple-blind randomized clinical trial on patients undergoing ERCP at two Swedish hospitals, Karolinska University Hospital and Kalmar County Hospital from 2005 to 2008.

Patients
Patients eligible for the study were at least 18 years of age, able to give informed consent, and scheduled for ERCP. An overview of some characteristic could be found in Figure 1.

The endoscopist recruited possible study patients at the time for ERCP procedure. Upon inclusion, patients received either a placebo capsule or losartan capsule (50 mg) given orally one hour before the ERCP.

Randomization and blinding
The participants, endoscopists, and evaluators were all kept unaware of the treatment status until after the analysis of the study. The study coordinator held the key to the study code and allocated active or placebo drug to the participants using computer-generated numbers. The randomization was made in blocks of 10, and active and placebo drugs were equally distributed between the study centers.

ERCP procedure
During the ERCP procedure, the patients received midazolam or diazepam for sedation and ketobemidone for analgesia. Glucagon or butylscopolamine was
given to reduce intestinal motility if needed. Omnipaque (140-240 mg I/ml) was used as contrast medium to visualize the biliary and pancreatic ducts. The endoscopist documented indications, procedural characteristics and findings directly after the ERCP.

**Statistical analysis**

Sample size estimation

We made a sample size calculation based on the following assumptions: significance level of alpha=0.05, power of 80 % (beta=0.20), and a reduction of the outcome (hyperenzymemia) from 40% in the placebo group to 10% in the losartan group (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Outcome, proportion of hyperenzymemia</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>40%</td>
<td>38</td>
</tr>
<tr>
<td>Losartan</td>
<td>10%</td>
<td>38</td>
</tr>
</tbody>
</table>

We followed the analytical rule of intention to treat, i.e. analyzing the patients according to the randomization status independent of received treatment. To lessen the impact of missing data on the outcome, we utilized the method of last observation carried forward.

Analysis

Significance testing for difference between groups was made for continuous variables by use of Student’s t-test. For categorical variables, $\chi^2$-test and the Fischer exact test were utilized. The median test was used for comparisons between groups, comparing continuous non-parametric variables like amylase and lipase.

We used multivariable logistic regression to calculate OR with 95% confidence interval of hyperenzymemia. This also facilitated the adjustment for any imbalance in the distribution of potential confounders, occurring in spite of randomization. The final multivariable model included the following covariates: sex, age (grouped into < or $\geq$ 65 years), BMI (categorized as <20, 20-25, or >25), history of pancreatitis (yes or no), study center (Karolinska University Hospital or Kalmar County Hospital), and ERCP duration (in minutes).

**Outcome definition**

Post-ERCP hyperenzymemia and pancreatitis

Hyperenzymemia was used as a surrogate market for post-ERCP pancreatitis, and defined as at least 3-fold elevated levels of the upper reference value for serum pancreatic amylase or lipase as measured 24 hours from the ERCP.
Acute pancreatitis, which was a secondary outcome, was defined as presence of upper abdominal pain in combination with hyperenzymemia 24 hours after ERCP.
RESULTS

PAPER I

DYNAMIC EXPRESSION OF THE ANGIOTENSIN II TYPE2 RECEPTOR AND DUODENAL MUCOSAL ALKALINE SECRETION IN THE SPRAGUE–DAWLEY RAT

The duodenal mucosal alkaline secretion response in previous and new line

In the previous line of rats the AT2 receptor agonist, CGP42112A, administered intravenously (0.1 µg kg\(^{-1}\) min\(^{-1}\)) elicited a significant 45% (+/-8%) net increase in DMAS. In the new line, however, such response was not evoked. Administration of the prostaglandin E2 (10\(^{-5}\) M) elicited a response in DMAS in both the previous and the new line. Thus the secretion capacity of the mucosa per se was intact and could not explain the differences found in DMAS between the lines. (Figure 3a)

![Figure 3a. Comparison of the net response in duodenal mucosal bicarbonate secretion (y-axis) to intravenous CGP42112A, intravenous saline or intraluminal prostaglandin E2 (PGE2) in the previous and new line, respectively (x-axis). * p-value<0.05. Data shown are mean values and standard error of the mean.](image)

Expression levels of angiotensin II receptors

The level of AT2 receptor mRNA expression was significantly higher in the previous line compared to the new line. In concurrence with this, the ratio of individual AT1a receptor and AT2 receptor mRNA expression was lower in the previous line compared to the new line. The level of AT2 receptor protein expression was elevated in the previous line compared to the new line.
Additionally, the individual ratios of AT1 to AT2 receptor protein were lower in the previous line compared to the new line.

**Dose-finding experiments with two doses of angiotensin II administration**

In the angiotensin II treated (10 μg kg\(^{-1}\) h\(^{-1}\)) group, the ratio of AT1a receptor to AT2 receptor mRNA was lower compared to that of saline treated group. However, the level of angiotensin II receptor protein was similar in the angiotensin II treated and saline groups.

**Modulation of angiotensin II receptor expression and functional response after angiotensin II infusion**

The protein level of AT2 receptor was higher in the group treated with angiotensin II than in the saline group. Moreover, in the angiotensin II treated, the AT2 receptor agonist elicited a 42% (11%) response in DMAS, which was higher compared to the saline treated group (Figure 3b). Furthermore, both the saline treated and the angiotensin II treated reacted with DMAS to prostaglandin E2 applied intraluminally.

![Figure 3b](image-url)

**Figure 3b.** Net response in duodenal mucosal alkaline secretion (y-axis) to application of CGP42112A (left) and PGE2 (right) in angiotensin II (10 μg kg\(^{-1}\) h\(^{-1}\)) and saline vehicle treated groups, respectively (x-axis). * p-value <0.05. Abbreviation used: PGE2, prostaglandin E2; Ang II, angiotensin II
PAPER II

USE OF ANGIOTENSIN II RECEPTOR BLOCKERS AND RISK OF ACUTE PANCREATITIS: A NESTED CASE-CONTROL STUDY

Descriptive results of the study population

In about 167,000 individuals registered with hypertension, 281 cases of acute pancreatitis were registered, rendering a crude incidence rate of 41 per 100,000 person-years. From the manual review, 16 cases could not be confirmed as cases of first-time acute pancreatitis, and were, therefore, excluded. Some characteristics of study patients are found in Table 2a. Notably, there was an overrepresentation of obesity (BMI >30 kg/m²) in the case group (39%) compared to the control group (30%). Current tobacco smoking was more common among cases (29%) than controls (23%). High alcohol consumption (>34 units per week) was not more common in case groups than controls.

Table 2a. Distribution of some characteristics among cases of acute pancreatitis and controls

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Total</td>
<td>2,000 (100)</td>
<td>265 (100)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>957 (47.9)</td>
<td>126 (47.6)</td>
</tr>
<tr>
<td>Female</td>
<td>1,043 (52.1)</td>
<td>139 (52.4)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-59</td>
<td>673 (33.7)</td>
<td>83 (31.3)</td>
</tr>
<tr>
<td>60-69</td>
<td>656 (32.8)</td>
<td>87 (32.8)</td>
</tr>
<tr>
<td>70-79</td>
<td>671 (33.5)</td>
<td>95 (35.9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown BMI</td>
<td>216 (10.7)</td>
<td>24 (9.0)</td>
</tr>
<tr>
<td>Non smoker</td>
<td>1,090 (54.5)</td>
<td>129 (48.7)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>374 (18.7)</td>
<td>53 (20.0)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>456 (22.8)</td>
<td>76 (28.7)</td>
</tr>
<tr>
<td>Unknown status</td>
<td>80 (4.0)</td>
<td>7 (2.6)</td>
</tr>
<tr>
<td>Use of alcohol (units/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>1,027 (51.4)</td>
<td>162 (61.1)</td>
</tr>
<tr>
<td>3-15</td>
<td>506 (25.3)</td>
<td>56 (21.1)</td>
</tr>
<tr>
<td>16-34</td>
<td>193 (9.7)</td>
<td>20 (7.6)</td>
</tr>
<tr>
<td>&gt;34</td>
<td>73 (3.6)</td>
<td>8 (3.0)</td>
</tr>
<tr>
<td>Unknown use</td>
<td>201 (10.0)</td>
<td>19 (7.2)</td>
</tr>
</tbody>
</table>

Exposure to angiotensin II receptor blockers and risk of acute pancreatitis

Among cases, 8% were classified as current users of ARB compared to 11% of the controls. Current use of ARB rendered a statistically non-significant decreased OR of 0.63 (95% CI 0.38-1.02) of acute pancreatitis, compared to
non-users in the full multivariable adjustment. Correspondingly, past users of ARB had a statistically non-significantly raised OR of 2.58 (95% CI 0.93-7.17) compared to non users. See Table 2b.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Controls</th>
<th>Cases</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2000 (100)</td>
<td>265 (100)</td>
<td>Reference (1.00)</td>
</tr>
<tr>
<td>ARB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1,773 (89)</td>
<td>237 (89)</td>
<td>Reference (1.00)</td>
</tr>
<tr>
<td>Past use</td>
<td>14 (1)</td>
<td>6 (2)</td>
<td>2.58 (0.93-7.17)*</td>
</tr>
<tr>
<td>Current use</td>
<td>213 (11)</td>
<td>22 (8)</td>
<td>0.63 (0.38-1.02) *</td>
</tr>
</tbody>
</table>

* OR adjusted for sex, age, calendar year, smoking, alcohol consumption, body mass index, use of ACE-inhibitors, CCB, beta-blockers, diuretics, other antihypertensive drugs, and general practitioner's visits the year before. Percentages are rounded, therefore the sum could be >100.

### Angiotensin-converting enzyme inhibitors and risk of acute pancreatitis

No significant association was seen in current users of ACE inhibitors and acute pancreatitis (OR 1.03, 95% CI 0.77-1.39). Past use did not render a statistically significant association (OR 1.43, 95% CI 0.86-2.38).

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Controls</th>
<th>Cases</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>Never</td>
<td>1,295 (65)</td>
<td>155 (59)</td>
</tr>
<tr>
<td></td>
<td>Past use</td>
<td>115 (6)</td>
<td>24 (9)</td>
</tr>
<tr>
<td></td>
<td>Current use</td>
<td>590 (30)</td>
<td>86 (33)</td>
</tr>
</tbody>
</table>

* OR adjusted for sex, age, calendar year, smoking, alcohol consumption, body mass index, use of CCB, beta-blockers, diuretics, ARB, other antihypertensive drugs, and general practitioner's visits the year before. Percentages are rounded, therefore the sum could be >100.
PAPER III

ANGIOTENSIN II RECEPTOR BLOCKER LOSARTAN IN THE PREVENTION OF HYPERENZYMEMIA AFTER ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATEOGRAPHY (ERCP): A RANDOMIZED CLINICAL TRIAL

Study population and procedure characteristics

In total, 291 patients were considered for inclusion in the study, and 215 patients were excluded for different reasons shown in the flowchart in Figure 3. Recent ERCP was the most common explanation for exclusion (n=142).

![Figure 2](image)

**Figure 2.** Flowchart of selection of study participants in paper III. Abbreviations used: ERCP, endoscopic retrograde cholangiopancreatography; ARB, angiotensin II receptor blockers; ACE, angiotensin-converting enzyme; DIC, disseminated intravascular coagulation.

There were 38 patients randomized to each of the losartan and placebo group. There was an even distribution of treatment status between the two study centers. Some characteristics of the study participants are presented in Table 3a. Some slightly unequal distribution of variables deserves highlighting: there were fewer females and patients with jaundice and cholangitis in the losartan group. Biliary sphincterotomy was performed more often in the losartan group (n=27), than in the placebo group (n=24). The blood pressure was lower in the losartan group compared to the placebo group (93 versus 98 mm Hg, p<0.05) after 24 hours, while the baseline-recorded blood pressure was equal (100 mm Hg).
Table 3a. Characteristics of the 76 participating patients and their indications for endoscopic retrograde cholangiopancreatography (ERCP)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Losartan group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Total</td>
<td>38 (100)</td>
<td>38 (100)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (58)</td>
<td>16 (42)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (42)</td>
<td>22 (58)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥65</td>
<td>13 (34)</td>
<td>14 (37)</td>
</tr>
<tr>
<td>&lt;65</td>
<td>25 (66)</td>
<td>24 (63)</td>
</tr>
<tr>
<td>Previous pancreatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34 (89)</td>
<td>35 (92)</td>
</tr>
<tr>
<td>Yes</td>
<td>4 (11)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Indication for ERCP*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspected tumor in pancreas or bile ducts</td>
<td>20 (53)</td>
<td>21 (55)</td>
</tr>
<tr>
<td>Suspected benign disease</td>
<td>7 (18)</td>
<td>9 (24)</td>
</tr>
<tr>
<td>Jaundice without cholangitis</td>
<td>10 (26)</td>
<td>13 (34)</td>
</tr>
<tr>
<td>Jaundice with cholangitis</td>
<td>20 (53)</td>
<td>16 (42)</td>
</tr>
</tbody>
</table>

*Since each procedure could have several indications, the sum of percentages could be >100.

Risk of hyperenzymemia and acute pancreatitis

The incidence rates of hyperenzymemia and acute pancreatitis among all 76 participating patients were 21% and 12%, respectively. Overall, losartan treated did not have lower a lower OR of post-ERCP hyperenzymemia, compared to placebo treated individual (OR 1.6, 95% CI 0.3-7.8), after adjustment for sex, age, study center, and length of ERCP. In total, 9 patients in the losartan group and 7 patients in the placebo group showed hyperenzymemia 24 hours after ERCP (p=0.51, Table 3b).

Serum amylase levels in the losartan and placebo group

No significant differences were detected either at baseline in terms of median serum amylase values (0.44 in the losartan group and 0.46 in the placebo group, p=0.64), or after 24 hours (0.62 in the losartan group and 0.82 in the placebo group, p=0.33) after the ERCP (Table 3b).

Serum lipase levels in the losartan and placebo group

The median serum lipase value at baseline was similar among the groups, i.e. 0.53 and 0.48 in the losartan and placebo groups, respectively (p=0.47). Moreover the median serum lipase was similar in the study groups 24 hours post-ERCP (0.77 and 1.07 in the losartan and placebo groups, respectively (p=0.62), Table 3b).
Table 3b. Serum pancreatic enzyme levels, abdominal pain, and pancreatitis after endoscopic retrograde cholangiopancreatography (ERCP) among 76 participating patients*

<table>
<thead>
<tr>
<th>Pancreatic enzyme level in serum</th>
<th>Losartan group</th>
<th>Placebo group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amylase (microkat/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>Median, (interquartile range)</td>
<td>0.44 (0.3)</td>
<td>0.46 (0.4)</td>
</tr>
<tr>
<td>24 hours after ERCP</td>
<td>0.62 (2.3)</td>
<td>0.82 (1.0)</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>Lipase (microkat/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>Median, (interquartile range)</td>
<td>0.53 (0.3)</td>
<td>0.48 (0.5)</td>
</tr>
<tr>
<td>24 hours after ERCP</td>
<td>0.77 (1.1)</td>
<td>1.07 (1.5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Hyperenzymemia‡ 24 hours after ERCP, number (%)</td>
<td>9 (24)</td>
<td>7 (18)</td>
<td>0.51</td>
</tr>
<tr>
<td>Missing data</td>
<td>4 (11)</td>
<td>3 (8)</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain 24 hours after ERCP, number (%)</td>
<td>8 (23)</td>
<td>9 (26)</td>
<td>0.93</td>
</tr>
<tr>
<td>Missing data</td>
<td>3 (8)</td>
<td>3 (8)</td>
<td></td>
</tr>
<tr>
<td>Acute pancreatitis (hyperenzymemia and abdominal pain after 24 hours), number (%)</td>
<td>5 (13)</td>
<td>4 (11)</td>
<td>0.57</td>
</tr>
<tr>
<td>Missing data</td>
<td>7 (18)</td>
<td>4 (11)</td>
<td></td>
</tr>
</tbody>
</table>

*In all analyses missing values were included as a separate category; p-values refer to overall differences between groups.

‡Occurrence of hyperamylasemia or hyperlipasemia.
PAPER IV

ANGIOTENSIN II RECEPTOR BLOCKERS AND RISK OF ACUTE PANCREATITIS: A POPULATION-BASED CASE-CONTROL STUDY USING THE PRESCRIBED DRUG REGISTER IN SWEDEN

Basic characteristics of cases and control persons

We identified 1,961 cases of first-time acute pancreatitis during 2006. Study participants are described in Table 4a. Men were slightly overrepresented among the cases (55% versus 45% women). The education level was lower among cases than controls (34% with elementary school category versus 29% in controls). Gallstone disease (39%) was the most common reason for pancreatitis, followed by alcohol use (10%). Many cases were categorized as of unknown or idiopathic etiology. Cases had a higher median number of distinct medications (6 compared to 4 for controls, data not shown).

Table 4a. Basic characteristics among case patients with acute pancreatitis and matched control persons

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>20,000 (100)</td>
<td>1,961 (100)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11,125 (56)</td>
<td>1,088 (55)</td>
</tr>
<tr>
<td>Female</td>
<td>8,875 (44)</td>
<td>873 (45)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>3,280 (16)</td>
<td>323 (16)</td>
</tr>
<tr>
<td>50-59</td>
<td>4,418 (22)</td>
<td>430 (22)</td>
</tr>
<tr>
<td>60-69</td>
<td>5,364 (27)</td>
<td>525 (27)</td>
</tr>
<tr>
<td>70-79</td>
<td>4,547 (23)</td>
<td>446 (23)</td>
</tr>
<tr>
<td>80-84</td>
<td>2,391 (12)</td>
<td>237 (12)</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>5,876 (29)</td>
<td>659 (34)</td>
</tr>
<tr>
<td>Secondary school</td>
<td>7,298 (36)</td>
<td>733 (37)</td>
</tr>
<tr>
<td>University studies</td>
<td>4,419 (22)</td>
<td>324 (17)</td>
</tr>
<tr>
<td>Missing data</td>
<td>2,407 (12)</td>
<td>245 (12)</td>
</tr>
<tr>
<td>Number of distinct medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No prescription</td>
<td>5,664 (28)</td>
<td>267 (14)</td>
</tr>
<tr>
<td>1-4</td>
<td>7,920 (40)</td>
<td>696 (35)</td>
</tr>
<tr>
<td>5-9</td>
<td>4,467 (22)</td>
<td>616 (31)</td>
</tr>
<tr>
<td>10-14</td>
<td>1,416 (7)</td>
<td>250 (13)</td>
</tr>
<tr>
<td>15-35</td>
<td>533 (3)</td>
<td>132 (7)</td>
</tr>
</tbody>
</table>

*The percentages were rounded, why the sum could be more or less than 100.
Angiotensin II receptor blockers and risk of acute pancreatitis

The results from the multivariable regression analysis are presented in Table 4b. Among cases, 8% were currently exposed to ARB, compared to 7% of the controls. The OR of acute pancreatitis among current users was 1.28 (95% CI 1.08-1.52), controlling for sex and age. To evaluate the effect of socioeconomic status, education was added to the model without substantial effect; the OR remained 1.28 (95% CI 1.08-1.52). The OR shifted to 0.81 (95% CI 0.69-0.97) after adjustment in the full model.

Past use, rendered a statistically non-significant 59% higher OR of acute pancreatitis (OR 1.59, 95% CI 0.90-2.80) controlling for age, sex, and education, compared to non-use. In contrast, no association remained after controlling for these factors in the full model (OR 1.08, 95% CI 0.61-1.93) (Table 4b).

Table 4b. Exposure to angiotensin II receptor blockers (ARB) and relative risk for acute pancreatitis estimated by odds ratio (OR) with 95% confidence intervals (CI)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Controls</th>
<th>Cases</th>
<th>OR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20,000 (100)</td>
<td>1,961 (100)</td>
<td></td>
</tr>
<tr>
<td>ARB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never use</td>
<td>18,560 (93)</td>
<td>1,782 (91)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Current use</td>
<td>1,348 (7)</td>
<td>165 (8)</td>
<td>1.28 (1.08-1.52)</td>
</tr>
<tr>
<td>Past use</td>
<td>92 (0)</td>
<td>14 (1)</td>
<td>1.59 (0.90-2.80)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.08 (0.61-1.93)</td>
</tr>
</tbody>
</table>

1) Adjusting for sex and age.
2) Adjusting for the full model (sex, age, education, alcohol-related disease, cardiovascular disorders and number of distinct medications).

The effect of dosage of ARB was also evaluated. No large difference was detected with regard to high dose (OR 0.90, 95% CI 0.68-1.20) and low dose (OR 0.82, 95% CI 0.65-1.04) compared to non users. The OR were similar in gallstone-related pancreatitis (OR 0.76, 95% CI 0.57-1.01), alcohol-related (OR 0.67, 95% CI 0.30-1.51), and other pancreatitis (OR 0.86, 95% CI 0.69-1.08).

Angiotensin-converting enzyme inhibitors and risk of acute pancreatitis

For comparison reasons, the association between current exposure to ACE inhibitors and risk of acute pancreatitis was estimated. The OR of angiotensin-converting enzyme inhibitors was 1.05 (95% CI, 0.91-1.22) with regard to acute pancreatitis.

Stratified analysis on distinct medications and risk of acute pancreatitis

A stratified analysis with regard to number of distinct medications on the effect of ARB in development of acute pancreatitis was also conducted. In the category of 1-4 distinct medications, ARB had an OR of 0.69, (95% CI 0.46-
1.03). In the strata of 15-35 distinct medications ARB usage rendered an OR of 0.68 (95 % CI 0.40-1.15), as compared to non-use of ARB.

Number of distinct medications and risk of acute pancreatitis

The effect of number of distinct medications on acute pancreatitis was measured. As a covariate in the regression analysis, the use of 1-4 distinct medications compared to no drugs conferred an OR of 2.04 (95% CI 1.77-2.37), adjusting for sex and age. In comparison, having 15-35 distinct medications generated an adjusted OR of 6.76 (95% CI 5.33-8.56).
DISCUSSION: METHODOLOGICAL ASPECTS

To evaluate our hypotheses, we have utilized three fundamentally different study types. First, an experimental study in animals was conducted to investigate a biological response to exogenous angiotensin II in the duodenum. Second, two case-control studies in different settings were performed. Third, a randomized clinical trial evaluated the effect of ARB on the risk of developing post-ERCP hyperenzymemia. Some aspects on these study designs are discussed below.

INTERNAL VALIDITY

Internal validity can be improved by well-designed studies, by reducing systematic errors. Selection bias occurs if the selection of study participants is influenced by factors related to the exposure and outcome. Information bias represents misclassification of the exposure or outcome, which may be a major problem in observational research. Confounding may occur if factors related to both the exposure and outcome influence the findings, and not properly handled. These aspects are described more in later sections.

EXPERIMENTAL RESEARCH

Paper I provides an example of the, at times, problematic use of in vivo models in experimental research. In general, the internal validity of this type of research is stated to be high. As a rule, experimental animals should have experienced the same environmental conditions and the exposure to factors like sex, weight, and nutrients should be controlled. Even genetic factors could be modified as seen in knockout mouse models. However, the experimental equality and standardized conditions for all animals could be questioned. The fact that our results were not replicated in a new line of Sprague-Dawley rats bred by another farm raises concerns. The dynamic expression pattern of angiotensin II receptors also underlines the importance of critically scrutinizing models used in this type of research. In our study (I), the influence of environmental factors on the expression of AT2 receptors could not be ruled out. The impact of previous living conditions for the rats, i.e. amount of stress, infections, and salt content of nutrition, are factors that could have contributed to the differential expression of receptors in the two lines of rats. Intriguingly, the farm of the previous line reported that the animals had undergone several viral infections. This could have contributed to the initial finding of high AT2 receptor content in the duodenum. Due to the retrospective nature of the investigation, other tissue samples could not be analyzed to confirm this hypothesis. In general, to ensure the validity of the findings confounding factors needs to be considered also in experimental studies. Specifically, AT2 receptor expression could be highly variable depending on the individual's or animal's previous experiences and exposures to known and
unknown confounding factors. Another methodological issue is if the AT2 receptor agonist acts selectively on AT2 receptors at the dose given in paper I. This has been proved with regard to stimulation of duodenal bicarbonate secretion in other studies, 24, 25, 34

**CLINICAL EPIDEMIOLOGY**

Clinical epidemiology, or the study of patterns of health or disease in humans and the factors that influence these patterns, is a cornerstone of modern evidence-based medicine. A deep understanding of the strengths and weaknesses of these methodologies is paramount for sensible clinical decision-making. Study design is a major determinant of quality in clinical research. Careful planning and execution of the studies directly influences the validity and potential for interpretation of the findings. To address our research question, we have employed two different study designs: case-control studies and a randomized clinical trial.

**COHORT STUDY**

The cohort study type, although not fully used in this thesis, serves as a template for observational study types in humans. The basic structure is a comparison of incidence rates of a disease among exposed individuals versus unexposed individuals. A strength is the ability to follow how the incidence develops over time, making it possible to establish temporal and causal relations. Large cohort studies have, however, often the drawback of being time and resource consuming. Therefore, the need for faster, more efficient study design was a driving force behind the development of the case-control study design. In theory, cohort studies could have replaced the case-control study in paper II and IV. The reason for not using a cohort design in paper II and IV was our ambition for comprehensive data on potential confounders, which was more readily available from a subset of cases and controls rather than on a large cohort including all antihypertensive users in a large database (II) or a major part of the Swedish population (IV).

**CASE-CONTROL STUDY**

Adequately designed and stringently analyzed, case-control studies, employed in paper II and IV, may play an important role in evaluating associations between exposures and clinical outcomes. Disease and exposure have already occurred when the participants enter the study, and the researcher is retrospectively looking back into relevant exposures contributing to the disease of interest. Incidence rate and true relative risk are not possible to calculate in contrast to cohort studies. OR is regarded as a good estimate of the relative risk, especially when the outcome is rare. In common outcomes the OR could over-estimate the true relative risk. In the present thesis, addressing a fairly uncommon outcome, OR should be a good proxy for the relative risk. Recall bias is a frequent problem in case-control studies, since cases tend to recall extent and type of exposures differently as compared to healthy subjects.
However, use of objectively and prospectively recorded exposure data, i.e. before the development of the disease mimics the cohort study design and minimizes this bias as in paper II, the data was obtained from THIN, and in paper IV from validated health data registers.

Control selection
The selection of controls is a key feature of case-control studies. Controls should ideally be chosen from the same source population that generates the cases. The probability of being selected for participation should not, in an uncontrolled manner, be influenced by exposure status, e.g. gender, age or socioeconomy. In both our case-control studies (II and IV), the risk for selection bias should be small due to population-based design and, in paper IV, the use of close to complete nationwide health data registers. The controls in paper II and IV were selected using density-based sampling. In this method, the probability of being selected as a control is in proportion to the time at risk.

**RANDOMIZED CONTROLLED TRIAL**

"One must attend in medical practice not primarily to plausible theories but to experience combined with reason. In other words, a treatment plan should be reasonable in theory but should also be tested experimentally"

This was stated already by Hippocrates. Randomized clinical trial or the clinical experiment is the only study type where the intervention is de facto assigned in a truly random manner. This is attractive because, in clinical practice, a pharmacological treatment decision is never randomly done, i.e., it is at the preference of the individual physician to decide and could be affected by patient characteristics and stage and type of the disease. Moreover, irrational factors like socioeconomy and gender might influence the treatment decision, which could introduce bias. The randomization ideally deals with this by distributing all known and unknown risk factors equally between the groups; while in observational studies there is a need for careful control for such potential confounding. Large, well-designed, and thoroughly executed randomized controlled trials carry the highest level of evidence.

Sample size
Inadequate power is a common problem in randomized clinical trials. This could generate problems with regard to acceptance of the null-hypothesis and risk of type 2 errors, or false negative results. In paper III, a larger sample size would have been desirable, but the study sample size estimation was deliberately carried out with the purpose of detecting a strong decrease in hyperenzymemia only. A strong decrease would indicate a need for a larger study, addressing acute pancreatitis rather than hyperenzymemia only as the outcome.

In general, to improve power and minimize type 2 errors, larger studies or selection of a patient-group with a higher risk of the outcome are needed. In paper III, for instance women or patients with previous pancreatitis could have
been targeted, but this would probably have resulted in an even more prolonged study period.

Another problem with a small sample size is the risk of imbalance regarding known or unknown confounding factors between the comparison groups. To adjust for such error, we analyzed the data using logistic regression to at least adjust for known or suspected confounding variables.

**Missing values**

Many trials have problems with adequate handling of missing outcome data, even those published in high-impact journals. One way of compensating for this is to perform a sensitivity analysis, using for example worst-case scenario of the missing outcomes, or replacing the outcome with earlier values (last observation carried forward). No optimal method exists, but one needs to specify and consider this early when designing and planning a trial. In our trial (III), the proportion of missing values of the main outcome, hyperenzymemia, was around 11%, which cannot be overlooked. Therefore, we used the method of last observation carried forward. This meant that the amylase or lipase values after 4 hours were used to replace the missing 24-hour endpoint value. As a comparison in the paper by Wood, 13 of 71 investigated trials had >20% missing outcome data, and not all clearly reported this, nor if sensitivity analysis had been performed.

**EXPOSURE MISCLASSIFICATION**

Generally, non-differential misclassification dilutes the association towards a null-finding, whereas differential misclassification could wrongly affect the association either way. Non-differential means that the degree of measurement error of the exposure is equally distributed in cases and controls. On the other hand, differential misclassification means that exposure measurement is different in cases and controls, causing systematic errors. In both paper II and IV, the true exposure to ARB differs from our estimated exposure using lasting dispensed prescriptions. The difference between the true and estimated exposure depends e.g. on the compliance to medication and the accuracy in the exposure data. Some current users could be misclassified as non users and past users or vice versa. For example, the exposure to ARB could be over-estimated in a group of alcoholics, whom generally have an increased risk of pancreatitis. This over-estimation could influence the estimated relative risk. In this example, the OR of acute pancreatitis in users of ARB would be over-estimated, compared to the true use due to the confounding effect of differential misclassification of exposure. In our studies, however, we have no reason to suspect large differential misclassification in the exposure of ARB. The risk estimates of ARB were similar in stratified analyses by different age and education groups, as well as in different etiologies of pancreatitis. Taken together, the major part of any misclassification of ARB in our studies should be non-differential, and thus only dilute the risk estimates.
Paper II
The main exposure use of angiotensin II receptors blocker was defined as presence of lasting prescription in relation to the index date. This data was collected from the general practitioners’ computer based charts and is prospectively collected before knowledge of the case status. The validity of the THIN database is high and established indirectly from data regarding a similar research database. 141, 142

Paper III
In study III the performing endoscopists prospectively collected the exposure information, which should reduce misclassification. Incomplete exposure information did not disturb our analysis.

Paper IV

Figure 4. Misclassification example in study IV. In this case, an individual received her last prescription 95 days prior to index date. In the figure the effect on exposure status is shown. Definition 2 and 3 correctly classed the individual as a past user, whereas misclassification occurs assuming 114 days of prescription length.
In study IV, we used data from dispensed prescriptions from the Prescribed Drug Register. An important detail was how to define current exposure to ARB. We approached this in three different ways: 1) The main study definition was that we assumed that a prescription normally included 100 days of drug usage, and added an arbitrarily chosen margin of 14 days. In all, prescriptions were assumed to last for maximum 114 days. Thereafter, drug exposure was defined as current if the drug had been dispensed from zero to 114 days in relation to the index date. 2) We re-defined current exposure to ARB by changing the assumption of prescription length from 114 to 90 days. 3) All prescriptions were manually reviewed of all prescriptions with ARB and actual prescription length calculated. Reassuringly, these three approaches rendered similar risk estimates of ARB and risk of acute pancreatitis.

Misclassification of the outcome
The assessment of outcome needs to be sensitive, i.e. all existing true cases of acute pancreatitis should be identified and specific and true non-cases should be correctly classed as healthy. In study II and IV, this is handled in similar ways. A validation study of a subset of the cases was performed in study II, resulting in positive predictive value of 94%. Thus, 6% of the cases were false positives and not true cases of acute pancreatitis. Similarly, an unpublished validation study for paper IV has tried to single out false positive cases, rendering a positive predictive value of 96%. As for specificity, there exists a possibility in both studies that some cases could have been missed and not classified as cases. The coding Patient Register is not 100% correct. Both the number of false positive and the number of false negative cases of acute pancreatitis ought to be evenly distributed with regard to exposure to ARB. Thus, this would only cause nondifferential misclassification of the outcome, diluting the association towards a null finding, i.e. the OR towards one.

CONFOUNDERS

"Thinking about confounder effects will keep you awake at night"
This was stated already in 2005 by professor Jesper Lagergren. Several possibilities are at hand to reduce any potential confounding of a study. In designing a study, one can use matching of controls to mimic the distribution of selected variables among cases (II and IV), randomization (III, see above), or restriction, e.g. exclusion of a certain risk group to increase validity. When analyzing the data, controlling for potential confounding factors can be done by stratification or by multivariable adjustment in logistic regression analysis, methods we used in the present thesis.
In paper II and IV control individuals were frequency matched, i.e. chosen to reflect the proportion of different exposures among cases with regard to sex, age (II and IV) and calendar year (II). Successful matching should reduce any confounding on matched variables. Frequency matching is much preferred as this permits you to avoid conditional regression analysis (a less efficient
technique than unconditional: the one we performed). It is only possible to perform frequency matched sampling with access to the complete roster or membership of the source population from which the cases have arisen. This is difficult or impossible unless using a complete database or valid registers. The numbers of controls were chosen as round numbers, and being close to 10 controls per case, a ratio that is considered to bring close to as much information as any greater number. Hence, any problems with statistical power were not due to the number of control subjects, but rather a limited number of case subjects.

**Alcohol over-consumption**

When investigating risk factors for acute pancreatitis, one has to take into account potential confounding by alcohol exposure. Many studies 76, 77 attempt to control for alcohol, but unmeasured exposure remains. People who consume alcohol in excess have a raised risk for acute pancreatitis as compared to non-consumers, and the main question is if they also differ in their exposure of ARB. Alcohol exposure is in turn also correlated to smoking and socioeconomic status, which are separately discussed below. In paper IV, we control for alcohol exposure recorded by general practitioners. The fact that alcohol exposure was not a risk factor *per se* for acute pancreatitis indicates residual unmeasured exposure to alcohol. In paper IV, however, indirect proxies of alcohol exposure are used, i.e. recordings of alcohol-related disorders, exposure to anxiolytics, and anti-depressive medication. The adjustments for these factors did not largely affect the association between ARB and risk of acute pancreatitis. Additionally, the risk estimate of ARB remained constant in alcohol-related, gallstone-related, and other acute pancreatitis. This adds strength to the notion that alcohol exposure does not act as a strong confounder in paper II and IV.

**Socioeconomic status**

In the middle of the 90’s the pharmacological class of ARB was introduced. Initially, two patterns of prescription may be expected. Bias may be introduced if new drugs are more often prescribed to patients with a high socioeconomic status. On the other hand, hospital specialists may prescribe the drug in cases with severe cardiovascular disease, which could in turn be associated with a low socioeconomic status. Currently, this possible bias should have lessened in importance because these drugs have been on the market since 1995 (losartan). However, socioeconomic status needs to be evaluated carefully, especially in paper II and IV. In the latter paper (IV), we adjusted for socioeconomic using education level as a proxy. Interestingly, education level did not influence the risk estimate of ARB with regard to acute pancreatitis. Moreover, we evaluated marital status and country of birth to capture other dimensions of socioeconomic, but this did not materially affect the risk estimates of ARB (data not shown). Alcohol over-consumption can be associated with a low socioeconomic status. In paper II and IV, we control for this variable without markedly changing the risk estimates of ARB. In summary, we could not find
that socioeconomic status was a significant confounding factor with regard to the association between ARB and acute pancreatitis.

Comorbidity

Comorbidity is often a confounding factor in epidemiological research, which needs to be properly handled. In paper II, the number of visits to the general practitioner the previous year was a surrogate marker for comorbidity. Based on the literature and the use of potentially healthier controls in relation to cases, we decided, a priori, to create a comorbidity score in paper IV. We summed all unique drugs (based on ATC codes) dispensed in the Prescribed Drug Register to each individual during the six months preceding index date. This sum was used to create a comorbidity score in different categories in steps of five distinct medications. The scientific literature lends some support of creating such a variable. Number of distinct medications is a good predictor of coming morbidity and mortality in comparison to other comorbidity scores. 143

The association between use of ACE inhibitors and the risk of developing acute pancreatitis was estimated in paper II and IV to evaluate the potential effect of confounding by indication. Confounding by indication could appear if the indication (or contra indication) for use of ARB, e.g. hypertension or heart failure, also is associated with the outcome, i.e. acute pancreatitis. ACE inhibitors have similar indications and contra indications as ARB and ACE inhibitors represent is a class of drugs for which we did not hypothesize an association with acute pancreatitis. 85, 90 The absence of an association with ACE inhibitors shown in paper II and IV strengthens our main finding of a specific association between ARB and acute pancreatitis.

Tobacco smoking

Smoking tobacco is recognized as a risk factor for acute pancreatitis in some studies. 76-78 Data on tobacco smoking emanated from self-reported data in paper II, which was continuously updated by the general practitioners. Adding smoking as a covariate did not affect the association between ARB and acute pancreatitis, indicating lack of confounding by smoking. In paper IV, however, exposure data on smoking was not available, but instead an indirect measure was used, i.e. the inclusion of chronic obstructive pulmonary disease. This adjustment did not affect the risk estimates of ARB. Therefore, the effect of smoking as a confounding factor seems to be small or absent in paper II and IV.

Precision and Random Errors

Precision relates to the existence of random errors, which could be reduced by increasing the sample size. The wideness of the confidence intervals is a measure of precision. In contrast, the magnitude of the association is estimated by the size of the OR and is not related to the precision. In randomized clinical trials (paper III), a common problem is false negative findings as discussed earlier. In paper II, the confidence intervals were wide and crossing one. Thus, our findings were statistically non-significant. This could be due to a lack of true association or a lack of precision, possibly due to limited number of
patients. This was the main reason for initiating study IV, where a larger number of cases with acute pancreatitis could be identified. Thereby, the precision improved and the risk of random errors decreased.

**Statistical versus clinical significance**

The importance of having predefined study questions cannot be overstated. Research should be based on pre-defined hypotheses and not on the existence of data sources. In keeping with this, when constructing studies the underlying biological knowledge of disease mechanism should not be replaced by skillful or statistical manipulation of data. It can be argued that a single significant finding without biological plausible mechanism or clinical importance is less interesting than a reasonable *a priori* hypothesis rendering a border-line significant result, as seen in paper II.
FINDINGS AND INTERPRETATIONS

ROLE AND FUNCTION OF THE ANGIOTENSIN II TYPE 2 RECEPTOR IN THE DUODENUM

The function of the AT2 receptor in the gastrointestinal tract is not completely elucidated. Data suggest involvement of AT2 receptor in formation of nitric oxide \(^{144}\) secretion of protective mucosa alkaline secretion counteracted by the AT1 receptor. \(^{34}\) The AT2 receptor agonist exerts its pro-stimulatory effect via bradykinin receptors located in the crypts of the mucosa. \(^{145}\) The localization of the AT2 receptor is suggested to be in the lamina propria of the duodenal villus. \(^{34}\)

The physiological concept of having two receptors counteracting or modulating each other is not unique for angiotensin II receptors. This has also been suggested for serotonin receptors, \(^{146}\) and the purpose could be to create a feedback inhibition of inappropriate activation of a potentially harmful system. In other words, AT2 receptors are induced and counteract AT1 receptors to protect the local tissue from an over-activation of angiotensin II signalling via the AT1 receptors, a feedback inhibition that could promote short-term survival. We interpret our findings in paper I as follows:

- Four hour infusion of high-dose angiotensin II markedly raises systemic mean arterial blood pressure (>40 mm Hg) at the expense of the splanchnic circulation.
- In the duodenal villi, angiotensin II redirects blood flow to the deeper layers of the mucosa. \(^{147}\)
- Normally the villus is on the brink of ischemia and the vasoconstriction leads to hypoxia and endothelial injury.
- Angiotensin II via stimulation of AT1 receptor or indirectly via hypoxia \(^{148}\) induce expression of AT2 receptors.
- AT2 receptors are expressed to resist the injurious condition and increase the protective mucosa alkaline secretion probably via bradykinin mediation or nitric oxide.

This response from AT2 receptors does not balance the massive stimulation of angiotensin II on AT1 receptors, which implies a role for pharmacological AT1 receptor blockade. This has been tested in severe hypovolemia, another condition causing local ischemia in the gastrointestinal tract. Long-standing hypoperfusion could cause endothelial injury damage to the intestinal barrier, which increases the risk of sepsis. In an animal model, pigs pre-treated with candesartan showed increased survival compared to control animals. \(^{149}\)

INDUCTION OF ANGIOTENSIN II RECEPTORS

The experiments in paper I attempted to induce AT2 receptors by infusing the natural ligand angiotensin II. This has been successful in other organ systems.
According to the latter study by Bonnet et al., a chronic treatment with angiotensin II induced AT2 receptor expression. Simultaneous ARB treatment attenuated this effect suggesting a role for the AT1 receptor pathway.

In the initial experiments of two hours infusion of angiotensin II, changes in gene transcripts of angiotensin II receptors were seen, but not in protein levels. However, after 240 min of infusion, AT2 receptor expression was induced and apparently functional because a secretion response to the AT2 receptor agonist was noted. This was rather surprising, given the relative short infusion, and was previously unproven in the duodenum. A remaining issue is to determine the reason for the AT2 receptor induction. The reason could be the surge in blood pressure in itself and not specific AT1 receptor stimulation of angiotensin II. Moreover, to extrapolate this finding to other species, and within rats, more experimental trials are needed.

In summary, paper I supports the idea of a dynamic expression pattern of AT2 receptors. Moreover, pre-treatment of rats with angiotensin II elicit expression AT2 receptors. AT2 receptor presence could not be taken for granted and is highly inducible to various patophysiological conditions.

**Angiotensin II receptor blockers effects on pancreatic hyperenzymemia and acute pancreatitis**

Several conditions, i.e. blood loss and dehydration, result in activation of the renin-angiotensin system. Some data support this in acute pancreatitis in a circulating renin angiotensin system and local renin-angiotensin system. This activation includes an increase of circulating angiotensin II, of which most effects are mediated by the AT1 receptor. Increased AT1 stimulation can predispose for development of acute pancreatitis via a number of proposed mechanisms (Table 5). A common feature of these mechanisms is the involvement of AT1 receptor signaling on the cellular level. With a potential harmful effect of AT1 receptors, the existence of a counteracting AT2 receptor (discussed in earlier sections) seems logical. In line with this, there is potentially a benefit from specific AT1 receptor blockade. This reasoning is the basis for the underlying hypothesis evaluated in this thesis.
Table 5. AT1 receptor mediated actions potentially involved in the pathogenesis of pancreatitis

<table>
<thead>
<tr>
<th>Component</th>
<th>Cell type</th>
<th>Action of AT1 receptor</th>
<th>Pathological effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exocrine</td>
<td>Acinar cell</td>
<td>Amylase/lipase secretion</td>
<td>Hypersecretion, calcium influx153</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Beta cell</td>
<td>Impaired insulin secretion</td>
<td>Hyperglycemia promotes inflammation and RAS activation</td>
</tr>
<tr>
<td>Blood vessel</td>
<td>Endothelial</td>
<td>Vasoconstriction</td>
<td>Hypoxia, acidity and RAS activation</td>
</tr>
<tr>
<td>Supportive tissue</td>
<td>Stellate cells</td>
<td>Activation to inflammatory state</td>
<td>Increase in NADPH, NF-κB, and ROS</td>
</tr>
<tr>
<td></td>
<td>Ductal cells</td>
<td>Bicarbonate secretion</td>
<td>Acidosis</td>
</tr>
</tbody>
</table>

Abbreviations used: ROS, reactive oxygen species; RAS, renin-angiotensin system NADPH, Nicotinamide adenine dinucleotide phosphate; NF-κB: Nuclear factor κB

In paper III, we evaluated the hypothesis that losartan, an ARB, could prevent pancreatic inappropriate hypersecretion (hyperenzymemia) after ERCP and in keeping with this, post-ERCP pancreatitis. This notion involved several assumptions: 1) Losartan de facto protects against hyperenzymemia in humans at the given dose and administration. The evidence for this is lacking in humans, in contrast to supporting experimental evidence. 16, 17, 47 2) There is a positive correlation between hyperenzymemia and pancreatitis. Previous studies have made a similar assumption 95 and hyperenzymemia is a prerequisite for development of acute pancreatitis. No tendency towards a protective effect of losartan on either hyperenzymemia or pancreatitis was found, however. Therefore, we did not proceed to investigate the effect of losartan on pancreatitis in a larger trial.

Apart from a true absence of an effect, other potential explanations for the lack of identifying a protective effect include: a) Too short period between oral drug administration and ERCP procedure, considering the pharmacodynamics of losartan. b) Too low dosage. A 50 mg dose was potentially not enough to penetrate to the pancreatic tissue. c) Too small sample size. The sample size used limited the possibility to detect small effects. The study was, however, designed only to detect a strong reduction in hyperenzymemia. d) Confounding. The presence of confounding has been discussed in an earlier section.

Strengths of the study include the triple-blind design, the use of identical placebo and drug capsules, the objective outcome measurement, and the short follow-up time between exposure and outcome (24 hours).

To further investigate the hypothesis, which has not been thoroughly explored, 89 we performed two observational studies (paper II and IV).

In study II, we found a statistically non-significantly decreased risk of acute pancreatitis among ARB users. No association was found for other anti-hypertensive medications, e.g. ACE inhibitors. This interesting finding urged us to continue to evaluate the hypothesis in a larger setting (paper IV). The results of paper IV indicate that after controlling for a number of potential confounding factors, current users of ARB have a lower risk of acute pancreatitis. 153
pancreatitis as compared to non-users. Thus, the observed inverse association between use of ARB and risk of acute pancreatitis found in our two observational studies (paper II and IV) was contradicted by the lack of a protective effect in our randomized controlled trial (paper III).

In conclusion, paper II-IV provides support for the notions that:
- Conditions related to AT1 receptor activation in the pancreas can be entailed by an increased risk of acute pancreatitis.
- The potential benefit from pharmacologically blocking AT1 receptor signalling with regard to the development of acute pancreatitis needs to be further explored before any firm conclusions can be made.

**GENERALIZATION OF THE FINDINGS**

Our findings do not establish a protective relation between ARB and acute pancreatitis. The decreased risk estimates found in our observational studies need to be confirmed in other settings before any implications can be drawn. If a risk reducing effect of ARB could receive further observational support, additional trials could be conducted in high risk groups, either as primary prevention trials, e.g. post-ERCP pancreatitis, or secondary prevention trials in patients with recurrent pancreatitis, e.g. in cases of alcohol-related or hereditary pancreatitis.
FUNCTIONAL AND PATOPHYSIOLOGICAL CONSIDERATIONS AND SPECULATIONS

ANGIOTENSIN II AND STRESS

What are the long-term implications of stress on health and disease? Many people experience stress in today’s society despite the improving living conditions throughout the world. Stress is defined in different ways, psychologically and physiologically. The latter definition usually includes events triggering adrenocorticotropic hormone release, which in turn stimulates the adrenal gland to produce diverse hormones including glucocorticoids, the body’s principal stress hormone. Angiotensin II is similarly regarded as a stress hormone. Elevated levels of glucocorticoids bring about increased risk of disease. Glucocorticoids are anti-insulinergic and increase insulin resistance. They also exert a mineralocorticoid effect by raising blood pressure. Interestingly, glucocorticoids affect the expression of angiotensin receptors in the pancreas and down-regulate the AT2 receptor favoring the AT1 receptor. This mechanism could have implications in cardiovascular, renal and pancreatic disease. Given that renin-angiotensin system plays a role in the patophysiology of acute pancreatitis, factors that influence the balance of angiotensin receptors are of interest. Hypothetically, stress via down-regulation of angiotensin receptors could increase risk of acute pancreatitis, predisposing inflammation and the AT1 receptor pathway. In contrast, chronic ARB treatment would up-regulate AT2 receptors and resistance to inflammation, hypertension and diabetes mellitus.

To elucidate the function of the AT2 receptor, knockout mice have shown changes in behavior and resistance to cerebral ischemia depending on sex. Male mice, lacking the AT2 receptor gene, showed worsened resistance to cerebral ischemia compared to females. In line with this, it has been speculated that the delayed incidence of increase in cardiovascular disease in women depends on interaction between estrogen and angiotensin receptors. Recently, the effect of estrogen on intestinal inflammation was investigated. Estrogen down-regulated AT1 receptors, and increased resistance to haemorrhage induced inflammation.

ANGIOTENSIN II AS A GASTROINTESTINAL HORMONE

Traditionally, angiotensin II is not regarded as a hormone with its principal action in the gastrointestinal tract. However, angiotensin II influences important functions here. Angiotensin influences the esophagus, duodenal mucosal alkaline secretion and gastrointestinal fluid transport. Probably, angiotensin II has more unravelled functions. Already it is implicated in response to increased gastric acid; DMAS increases to prepare the duodenal
mucosa for increased strain from gut acidity. Angiotensin II also acts on the pancreas affecting bicarbonate secretion as well as exocrine and endocrine secretion. To understand this in a more physiological way, angiotensin II is regarded as a part of the body’s *fight and flight* reaction. Accordingly, the homeostasis of the organism is at stake and the body’s defense mechanisms activate. Thus all unnecessary functions are inhibited. For example, the vagal mediated gastrointestinal functions, which are connected to the *rest and digest* response, are inhibited. Speculatively, angiotensin II and the AT1 receptor could act analogous to the sympatho-adrenergic system, and might have patophysiological implications if inappropriately stimulated, which is exemplified in Table 6.

<table>
<thead>
<tr>
<th>Function</th>
<th>Angiotensin II, AT1 receptor</th>
<th>Patophysiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower esophageal sphincter</td>
<td>Increase tonus</td>
<td>Reflux</td>
</tr>
<tr>
<td>Motility in the GI tract</td>
<td>Increase 1</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>Gastric acid secretion</td>
<td>Inhibit (?)</td>
<td>Peptic ulcer disease</td>
</tr>
<tr>
<td>Gastric blood flow</td>
<td>Inhibit</td>
<td>Peptic ulcer disease</td>
</tr>
<tr>
<td>Duodenal alkaline secretion</td>
<td>Inhibit</td>
<td>Duodenal ulcer</td>
</tr>
<tr>
<td>Insulin secretion</td>
<td>Inhibit and impair 1</td>
<td>Diabetes mellitus</td>
</tr>
</tbody>
</table>

**Table 6. Potential pathophysiological role for angiotensin II in the gastrointestinal tract**

**INCREASED INCIDENCE OF ACUTE PANCREATITIS**

The incidence of acute pancreatitis has been linked to the prevalence of risk factors. In Sweden at the end of the 1960’s, there was a rise in alcohol-related pancreatitis in parallel with increased availability to beer. Thereafter, this subtype of pancreatitis has decreased in favor of gallstone-related pancreatitis, a type related to high age, obesity and factors regarding cholesterol metabolism. Interestingly, the incidence of acute pancreatitis has risen from 40-70 per 100,000 person-years in 1988-2003 in the United States. Many risk factors, i.e. obesity and gallstones, have correspondingly increased in prevalence. These risk factors could be put in a larger context as part of the metabolic syndrome in which an important component is hypertension. In our cohort of hypertensive patients, the crude incidence rate was higher compared to the general population. Of course antihypertensive drug users generally have more diseases, but one could speculate that hypertension in itself is a risk factor for acute pancreatitis. In other studies hypertension has been shown to be a risk factor.

In summary, it can be argued that the increased incidence of acute pancreatitis depends on mechanisms that are not yet understood. One proposed way to further understand the underlying mechanisms is to consider other risk factors; i.e. obesity, stress and hypertension; in a systematic way. This should be the aim of future studies.
CONCLUSIONS

- Treatment with angiotensin II can induce AT2 receptor expression in duodenum of Sprague–Dawley rats.
- Angiotensin II infusion may lead to increased protective mucosa duodenal secretion.
- Losartan given as a single-dose may not protect against development of hyperenzymemia.
- Users of ARB might have a lower risk of acute pancreatitis.
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Ett jämtländsk uttryck som min farfar, Artur Sjöberg, brukade använda. Han jobbade långa och kalla dagar i skogen som skogshuggare. Innan han begav sig hemåt högg han ned några träd till för att ha en män till morgondagen eller för att mana sig, att arbetet skulle flyta på lättare. Detta har inspirerat mig inom forskningen. Endast genom att anstränga sig lite extra blir slutresultatet riktigt bra. Det finns även andra saker och framför allt personer som jag vill uppmärksamma och tacka:

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SAMMANFATTNING


I nästa studie (III) har vi undersökt en specifik ARB, losartan, jämfört med placebo i en klinisk prövning. Vi har undersökt patienter som har genomgått en endoskopisk undersökning av gallvägarna via magtarmkanalen, till exempel på grund av gallsten. I denna undersökning som benämnas ERCP kan den näraliggande bukspottkörteln retas och reagera med att utsöndra enzym i blodet. I vissa fall leder det också till bukspottkörtelinflammation. Före undersökningen fick patienterna en dos losartan 50 mg eller placebo. Våra resultat visade ingen fördel av att behandlas med losartan jämfört med placebo med avseende på stegrade enzymnivåer.

I den sista studien (IV) har vi återigen undersökt om långtidsanvändare av ARB har en lägre risk att utveckla bukspottkörtelinflammation. Denna gång har vi utfört studien i Sverige under 2006. Vi har jämfört andelen av fallen som står på ARB med andelen hos ett representativt urval från den allmänna befolkningen. Våra resultat tyder på att ARB-användare kan ha en lägre risk för sjukdomen akut bukspottkörtelinflammation.

Sammantaget visar denna avhandling att angiotensin II påverkar bikarbonatsekretionen i experimentella försök. En dos av losartan skyddar inte
mot stegade enzymnivåer efter ERCP. Våra resultat visar att ARB-användare kan ha en lägre risk för bukspottkörtelinflammation, men detta behöver bekräftas i andra studier.
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