Diplomarbeit

Influence of Galle-Donau® on the peristalsis of the guinea-pig small intestine

eingereicht von

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unter der Anleitung von

Univ.- Prof. Dr. Peter Holzer

Univ.- Prof. Dr. Ulrike Holzer

Graz, am 27.1.2010……

Eidesstattliche Erklärung

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Graz, am ……

Acknowledgements

Ein Studium geht seinem Ende zu. Es waren lange Jahre, es waren teils harte Jahre.

Zu Beginn möchte ich meinen Eltern und meiner Schwester für ihre intensive Unterstützung danken! Meine Freunde und Kollegen, wo immer sie auf der Welt gerade verstreut sind, waren mir ein Licht am Ende des Tunnels. Danke an alle, die an mich geglaubt und mich unterstützt haben.

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Kurzfassung

Galle-Donau®, eine Kombination aus α–Naphthylessigsäure (NAA) und p-Tolylmethylcarbinolnikotinsäureester (pTMC-N), ist ein Medikament erster Wahl bei funktionellen Produktions- oder Sekretionsstörungen von Galle. Gallsäuren haben eine potente abführende Wirkung. Ziel unserer Studie ist es, einen möglichen Einfluss von Galle-Donau® auf die Peristaltik des Dünndarms beim Meerschweinchen zu festzustellen und eine mögliche neue Therapie von pathologischen, intestinalen Motilitätsstörungen aufzuzeigen.

Für unser Experiment wurden weibliche Meerschweinchen (TRIK – Stamm) mit einem Gewicht zwischen 630 und 890g verwendet. 10 cm lange Dünndarmsegmente wurden intraluminal mit Tyrode perfundiert, was zu Auslösung der Peristaltik führte. Die Software „Peristal 1.0“ zeichnete hierbei gewonnenen Daten auf. Vier Parameter wurden evaluiert: peristaltische Druckschwelle, Maximaldruck, Basisdruck und maximale Beschleunigung.

Die Resultate zeigten, dass Galle-Donau® und pTMC-N einen inhibitorischen Effekt auf die peristaltische Bewegung im Meerschweinchendünndarm, in unserem in vitro Experiment, hatten. NAA und Nikotinsäure zeigten nur marginale Auswirkung auf intestinale Bewegungsabläufe. Kein Effekt konnte bei Zugabe von p-Tolylmethylcarbinol (pTMC), deionisiertem Wasser oder Dimethylsulfoxid (DMSO) gefunden werden. Unsere Daten zeigten keinen praktischen Unterschied zwischen oralen und aboralen Segmenten.

Unsere Studie konnte zeigen, dass Galle-Donau® und pTMC-N die intestinale Peristaltik blockieren. Kein Unterschied zwischen oralen und aboralen Segmenten konnte gefunden werden. Somit ist es uns gelungen, Galle-Donau® als eine mögliche, neue Therapie für intestinale Motilitätsstörungen aufzuzeigen.

Abstract

Galle-Donau®, a combination of α-naphthylacetic acid (NAA) and p-Tolylmethylcarbinol-nicotinic acid ester (pTMC-N), has been a first line drug in the therapy of functional disorders of bile acid production or secretion.
Bile acids are potent laxatives. As a choleretic agent Galle-Donau® could have an effect on the peristalsis of the intestine and thus may play a part in the future therapy of intestinal motility diseases.

Without exception, all guinea pigs used were female (TRIK strain). They weighed between 630 and 890g**.** 10 cm long oral and aboral segments of the small intestine were intraluminally perfused by Tyrode, eliciting peristalsis. Recording and evaluation was performed by Peristal 1.0 software. Four parameters were evaluated for every segment: the peristaltic pressure threshold, the maximal pressure, the base pressure, and the maximal acceleration.

Results showed that Galle-Donau® and pTMC-N had a rather strong inhibitory effect on the intestinal motility in our in vitro experiment. NAA and nicotinic acid had mild effects on peristalsis. Our data suggested that pTMC, deionised water and DMSO had no relevant influence on intestinal motility. This study pointed out that peristaltic motor parameters were comparable between oral and aboral segments.

The work succeeded in evaluating the influence of Galle-Donau® and its compounds on the peristalsis of the guinea pig's small intestine. We concluded that Galle-Donau® and pTMC-N caused an inhibition of peristaltic motility. According to our data, no relevant difference was found between oral and aboral segments of the small intestine. Thus, Galle-Donau® has potential to play an increasingly important role in the therapy of intestinal motility disorders in the future.

Inhaltsverzeichnis

Abbreviations

ACh: Acetylcholine

ATP: Adenosine triphosphate

BP: Base pressure

C4: 7α-Hydroxy-4-cholesten-3-one

CBD: Common bile duct

CCK: Cholecystokinin

CETP: Cholesterol ester transfer protein

CI: Confidence interval

CNS: Central nervous system

DMSO: Dimethyl sulfoxide

DYN: Dynorphin

ENK: Enkephalin

ENS: Enteric nervous system

GABA: γ-Aminobutyric acid

GAL: Galanin

GOT: Glutamic-oxaloacetic transaminase

GPT: Glutamate Pyruvate Transaminase

GRP: Gastrin releasing peptide

HDL: High density lipoprotein

5-HT: 5-Hydroxytryptamine (serotonin)

IBS: Irritable bowel syndrome

IBS-C: Irritable bowel syndrome with predominant constipation

IBS-D: Irritable bowel syndrome with predominant diarrhea

ICC: Interstitial cells of Cajal

LDL: Low density lipoprotein

MMC: Migrating motor complexes

MP: Maximal pressure

NAA: α-Naphthylacetic acid

NPY: Neuropeptide Y

NO: Nitric oxide

OATT: Oral anal transit time

P2X: Purine receptor 2X

P2Y: Purine receptor 2Y

pTMC: p-Tolylmethylcarbinol

pTMC-N: p-Tolylmethylcarbinol-nicotinic acid ester

PBC: Primary biliary cirrhosis

PPT: Peristaltic pressure threshold

SP: Substance P

TG: Triglycerides

VIP: Vasoactive intestinal peptide

VLDL: Very low density lipoprotein

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1 Introduction

1.1 Background

1.1.1 Macroscopic aspects of the small intestine

The length of the small intestine measures about 5-6m in dead people and 2m in live humans (1). This organ is divided into 3 parts: the duodenum, jejunum, and ileum. Jejunum and ileum are situated in the intraperitoneal cavity, while the duodenum is located in the retroperitoneal cavity. (1)

The uppermost part, the duodenum, has a length of approximately 30 cm (12 inches) and is formed like the letter c. (1) It commences at the pylorus, the end of the stomach, and reaches the flexura duodenojejunalis, where it continues as the jejunum. The duodenum can be divided into a superior part, a pars descendens, the part where the sphincter of Oddi as well as the major and minor duodenal papilla are located, and a pars inferior. Then the pars ascendens and the flexura duodenojejunalis follow. (2)

It is very hard to distinguish between jejunum and ileum by just looking at it from the outside. (1) Overall, the jejunum is rather located in the left part of the peritoneal cavity, the ileum in the right. (1)

The human small intestine absorbs nutrients, vitamins, and electrolytes. With the help of enzymes from the pancreas and the wall of the intestine, food is cleaved into absorbable molecules. (1) These are transported through the intestine's wall. Lipids are taken up by lymph, all other nutrients are distributed by blood. Thus, the small intestine plays a major role in the process of digesting. (1)

1.1.2 Layers of the small bowel

From a histological point of view this organ has an outermost layer, the tunica serosa. (3) The tunica serosa covers layers of longitudinal and then circular muscle fibres. Then the submucosa is followed by the mucosa. (4)

Auerbach's plexus, also called myenteric plexus, is situated in the muscular layer of the gut wall, between the longitudinal and the circular muscle layer. (3) It is made up of a network of neurons, which are interconnected. Its main task is to control and influence the movements of the bowel. When stimulation occurs, mostly muscular tone and frequency of contractions rise. (5)

Further luminal, the tela submucosa can be found. Meissner's plexus, also referred to as submucosal plexus, is found in the submucosal layer. (1) From there, it is acquiring information from chemo- and mechanoreceptors, mostly from the epithelium, and focuses on controlling intestinal secretion, as well as absorption, and local blood flow. The number of neurons is lower than in Auerbach plexus. Around 1000- 5000 neurons per cm^2 have been counted. (6)

1.1.3 Innervation

The gastrointestinal tract has its own, autonomous, nervous system, the enteric nervous system (ENS). It is located in the wall of the gut and reaches from the esophagus to the anus. (7) Similar to the spinal cord, the ENS consists of around 100 million neurons. (6, 7) Its function is to control GI movements and secretion. (7)

The myenteric (or Auerbach's) plexus, located between the circular and the longitudinal muscle, and the submucosal (or Meissner's) plexus situated in the submucosa make up this nervous system. (7)

Local blood flow and secretion is modulated by the submucosal plexus, the GI movements are mainly controlled by the myenteric plexus. (7)

The myenteric plexus consists mostly of a mesh of many interconnecting neurons that extends the entire length of the gastrointestinal tract. It enhances muscle tone of the gut wall, amplitude and frequency of peristaltic waves and conduction of excitatory waves. (6) It also functions in an inhibitory way via nitric oxide (NO)/ vasoactive intestinal peptide (VIP) neurons, which mediate the descending inhibitory reflex. In addition, the myenteric plexus may relax intestinal sphincters, such as the pyloric sphincter or the sphincter of the ileocecal valve. (7)

The submucosal plexus receives sensory input from the nerves in the intestinal epithelium and controls absorption, secretion and contraction of the submucosal muscles. (7,8)

The ENS is influenced by the autonomic nervous system. The parasympathetic system has an excitatory effect, enhancing bowel functions, while the sympathetic system reduces motility and secretion. Sensory input comes from nerve endings in the gut wall, which transmit information to the ENS, the spinal cord, through the vagus to the brain stem, and to the prevertebral ganglia of the sympathetic nervous system. (7)

The parasympathetic supply is composed of cranial and sacral divisions. The cranial nerves, with a few exceptions, travel almost entirely in the vagus and innervate the esophagus, the stomach, the pancreas, and the intestines to the first half of the colon. The sacral fibres pass through the pelvic nerves and supply innervations to the sigmoidal, rectal and anal regions. Postganglionic neurons are located in the two plexuses. (7)

Sympathetic fibres originate from T5 to L2. (1,7) The vast majority of preganglionic fibres joins a sympathetic chain lateral to the spinal cord and continues to several ganglia like the mesenteric or the celiac ganglion. The fibres then spread to all parts of the gut. Mostly norepinephrine, but in some cases also epinephrine, is secreted from the sympathetic nerve endings. (7)

Afferent sensory nerve fibres from the gut have their cell bodies located either in the ENS or the dorsal root ganglia. Signals can have excitatory or inhibitory effects on movements and secretion of the intestine. (7)

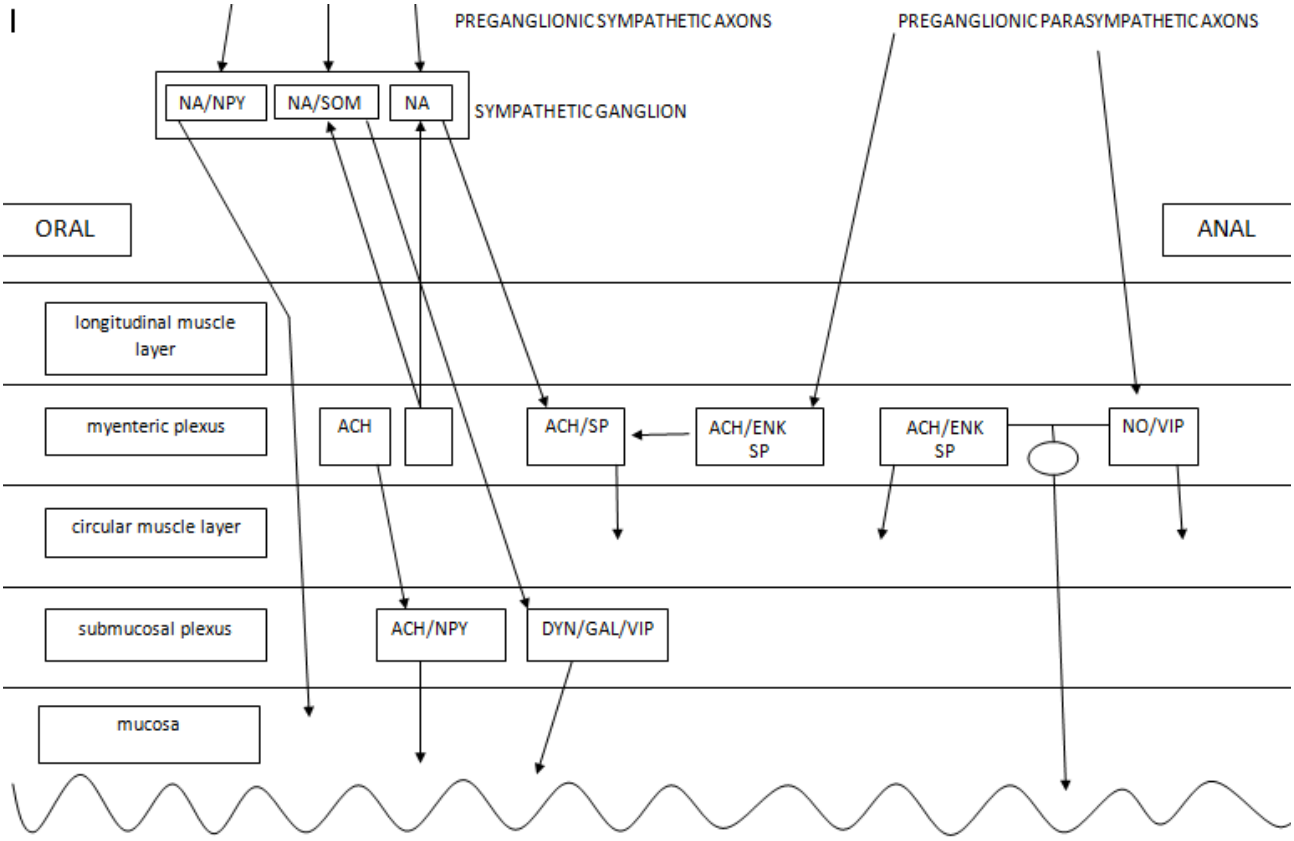

Figure 1: Intrinsic and extrinsic innervation of the enteric nervous system.

From: Furness JB. Types of neurons in the enteric nervous system. J. Auton. Nerv. Syst. 2000; 81(1-3):87–96 and Kunze WA, Furness JB. The enteric nervous system and regulation of intestinal motility. Annu. Rev. Physiol. 1999; 61:117–42.

1.1.3.1 Transmitters

Prevalent transmitters are acetylcholine (ACh), substance P (SP), NO, and VIP. In the myenteric plexus one can find ACh/SP- and Ach/ enkephalin (ENK)/ SP-neurons. ACh and SP have an excitatory effect on the circular smooth muscle layer. Their axons travel in the plexus to the muscle a couple of millimetres orally. On the other hand, smooth muscle decreases in tone when NO and VIP, from

NO/VIP- neurons in Auerbach's plexus, become active. Their axons travel in the plexus a couple of millimetres in the anal direction before they reach the muscular layer. (8)

From ACh/NPY- neurons and VIP from Dynorphin (DYN)/ Galanin (GAL)/VIP-neurons enhance secretion. In the submucosal plexus ACh and NPY have an excitatory effect on secretion, DYN, GAL and VIP an inhibitory effect. (8) Excitatory as well as inhibitory neurons are innervated by preganglionic fibres of the parasympathetic system. Preganglionic parasympathetic axons reach neurons with ACh, enkephalin, and SP, as well as neurons with NO and VIP, all located in the myenteric plexus. *"Excitatory motor neurons and inhibitory motor neurons both release transmitters that act on interstitial cells of Cajal (ICC)."*[1] Inhibitory motor neurons release NO which interacts with a soluble guanylyl cyclase in the cytoplasm. They act inhibitory on both smooth muscle cells and ICCs. Excitatory motor neurons release ACh and tachykinins. ACh interacts with muscarinic receptors on the smooth muscle and epithelium. (5)

The sympathetic nervous system has three major effects on the enteric nervous system. NA and NPY cause vasoconstriction, their axons reach the mucosa. Through inhibition of DYN/GAL/VIP neurons, noradrenaline and somatostatin decrease secretion. Their axons connect the sympathetic ganglia with the submucosal plexus. Sympathetic nerves also inhibit smooth muscles, through the action of noradrenaline, which projects to the myenteric plexus. (8)

Not only transmitters, but also hormones have an effect on intestinal motility. (7) Cholecystokinin (CCK), secretin, glucose dependent insulinotropic peptide (GIP), motilin, somatostatin and histamine have their individual influence on bowel motility. (7, 9)

1.1.4 Motor patterns of the small intestine

[1] Furness JB. The enteric nervous system. Malden Mass.: Blackwell Pub.; 2006.

Slow waves, which occur about eight to twelve times a minute, are responsible for the basic rhythm. Their intensity varies between five to fifteen millivolts. (7,9,10) The rhythm of the slow waves is determined by a mesh-like network of the interstitial cells of Cajal (ICC), the so-called pacemaker cells. The ICCs are characterised by a fusiform nucleus and several processes going into each direction. (6) On top of that, action potentials, also called spike bursts, take place. If the membrane potential reaches a threshold of -40mV several action potentials are elicited. The threshold can be modified by neural, paracrine or endocrine influences. A spike burst is forwarded to smooth muscle myocytes via gap junctions and muscle contractions are triggered. (7,9,10)

The length of the bowel decreases when the longitudinal muscle shortens. Vice versa, it increases when the longitudinal muscle relaxes. This special movement is referred to as pendular movement. (7,9)

When the circular muscle layer contracts and relaxes, this motion is called segmentation contraction. Its purpose is to mix the intestinal contents. When the circular muscle layer contracts the diameter of the lumen decreases. Chyme is partly pushed in oral, partly in anal direction. Relaxation of the muscle leads the contents to move back. (7,9,10) *"After all, segmentation movements mix the chyme, but don't cause any net forward movement."*[2]

Migrating motor complexes (MMC) are interdigestive and with the exception of the distal ileum, low amplitude waves. Those contractions occur during fasting and have the purpose to move nondigestible residue to the colon. The MMC starts often around one hour of fasting and lasts for about 4 minutes. (11)

The peristaltic reflex can only occur when several neurons act in accordance with each other. NO/VIP-, ACh/ENK/SP-, as well as sensory neurons make this complex movement possible. When a bolus distends the wall, the circular layer relaxes anal to the bolus, by release of NO and VIP from NO/VIP neurons. (7) This mechanism is referred to as descending inhibitory reflex, while in the ascending

[2] Linda Costanzo. BRS Physiology. 4 edition. Philadelphia: Lippincott Williams & Wilkins; 2007.

excitatory reflex, ACh and SP from ACh/ENK/SP-neurons lead to an oral contraction. (8) Through this mechanism, the bolus is transported towards the rectum.

1.1.5 Secretion and function of bile

Bile is a fluid produced continuously by the liver. After being drained down the hepatic ducts, it is stored in the gallbladder and finally transported to the duodenum. (7,9) It contains bile salts, phospholipids, cholesterol and bile pigments (bilirubin), electrolytes, steroid hormones, and medications. (7,8,9)

Bile salts are amphipathic molecules. The liver produces 6 grams each day. (7,8) Bile salts have an important role during the process of digestion. First produced by the liver as chenodeoxycholate and cholate, synthesised from cholesterol, they become transformed into secondary bile salts (deoxycholate and lithocholate). (7,8,9) After conjugation with glycine or taurine those bile salts get secreted into the ducts. The gallbladder then is used as storage and secrets bile when triggered i.e. by CCK or ACh. (7,9)

Conjugated bile salts are secreted through the sphincter of Oddi into the small intestine, while the unconjugated ones are reabsorbed by the bile ducts- a process called cholehepatic circulation. The conjugated bile salts make their way to the terminal ileum. (7,10) In the small intestine, bile salts have two important tasks. Firstly, they emulsify lipids by decreasing the surface tension of the fat particles and thus making the breakdown of fats possible. Their second task is to solubilise lipids for easier absorption. (9,10) This is done by forming micelles. Having been used for fat digestion, they are reabsorbed and are transported back to the liver. This is referred to as enterohepatic circulation. (7,10)
During digestion, the amount of bile salts in the portal vein is increased. This inhibits, through the mechanism of negative feedback, further production of primary bile salts by the liver and raises the secretion of bile salts into the biliary canaliculi. (7,9)

Bile acids have an antiresorptive and secretagogue laxative effect. Sodium and thus water is not reabsorbed. (9,10) Additionally, there is secretion of Na^+, K^+, Cl^-, and Ca^{2+}. Thereby feces are softened and, through distention of the colon wall by the retained water, defecation is enhanced. (7,9,10)

1.1.6 Irritable bowel syndrome

Irritable bowel syndrome (IBS) is referred to as a functional GI disorder. Patients present with abdominal discomfort or even pain and a change in frequency and consistency of stool. Diagnosis is established by exclusion. Generally, women are more likely to suffer from IBS. (12)

The Rome III criteria (2006) state that a patient must present with *"recurrent abdominal pain or discomfort at least 3 days per month during the previous 3 months that is associated with 2 or more of the following: relieved by defecation, onset associated with a change in stool frequency, [and] onset associated with a change in stool form or appearance."*[3]
Supporting symptoms are "altered stool frequency, altered stool form, altered stool passage (straining and/or urgency), mucorrhea, abdominal bloating or subjective distention"[4]

One has to differentiate between 4 subtypes. Patients with IBS can present with predominant diarrhea, constipation, a mixed type and an alternating type. Subtypes are likely to change with time. (12)

Symptoms including constipation, diarrhea, or postprandial urgency are common. Abdominal pain occurs. Many patients report abdominal bloating and having to pass gas more frequently. Non GI symptoms are dyspepsia, nausea, heartburn,

[3] Lehrer, Jenifer; Lichtenstein, Gary. Irritable Bowel Syndrome; 9 Aug 2009. Available from: URL:http://emedicine.medscape.com/article/180389-overview.

[4] Lehrer, Jenifer; Lichtenstein, Gary. Irritable Bowel Syndrome; 9 Aug 2009. Available from: URL:http://emedicine.medscape.com/article/180389-overview.

vomiting, fibromyalgia and others. In many patients they worsen during perimenstrual period. (12)

At present, therapy focuses on the symptoms. 40-70% of patients improve after the administration of placebo. Patients presenting with obstipation can be given osmotic laxatives such as lactulose. Loperamide is effective to treat IBS patients with diarrhea. For abdominal pain spasmolytics, such as mebeverine or butylscopolaminebromide, should be the treatment of choice. (8)

1.2 Aims of study

Galle-Donau®, a combination of α-naphthylacetic acid (NAA) and p-Tolylmethylcarbinol-nicotinic acid ester (pTMC-N), has been a first line drug in the therapy of functional disorders of bile acid production or secretion. (13)

Bile acids are potent laxatives. As a choleretic agent Galle-Donau® could have an effect on the peristalsis of the intestine and thus may play a part in the future therapy of irritable bowel syndrome (IBS).

It is assumed that the symptoms of IBS are caused by GI motility disorders and increased visceral pain perception. (8) The colonic motor response to stress, as well as food intake and pain seems to be different in patients with IBS compared to control groups. Constipation-predominant IBS patients have, compared to diarrhea-predominant IBS patients, a delayed intestinal transit time. Contractions in the small intestine showed to be more frequent in IBS patients than in healthy people. (11,12,14)

In our experiment we wanted to focus on the effect of Galle-Donau® on motility changes of the small intestine. In Galle-Donau® dragees pTMC-N and NAA are combined. In case of a visible effect, the causative substance should be identified. Esters are cleaved very quickly in the intestine, hence it was important to test the components of the ester, pTMC-N separately. Therefore, we examined pTMC-N, but also nicotinic acid and pTMC alone. Additionally, NAA, Galle-Donau® itself,

dimethyl sulfoxide (DMSO) and deionised water as their vehicle were used. In previous experiements with clonidine the quantitative effects of the drug differed between oral and aboral segments (P. Holzer personal communication). Therefore, we focused on a possible difference between oral and aboral segments.

2 Methods

2.1 Animals & tissue preparation

Without exception all guinea pigs used were female, weighed between 630 and 890g (TRIK strain)**.** (15,16) They were obtained from the breeding station of the Slovak Academy of Sciences, Dobra Voda (SK). After a month of resting and accommodating in the institute's animal house, the experiment started.

The animals were humanely sacrificed in accordance with national guidelines. (15) Jejunum and ileum were carefully isolated. 5 cm oral to the ileocecal junction the small bowel was cut and one thread with a diameter of about 1 milllimeter was used to mark the aboral end. Then the mesentery was carefully cut off from the small bowel.

After about 40 centimeters oral to the ileocecal junction, the gut was cut and thus divided into an oral and an aboral part. The aboral part was moved into an oxygenated Tyrode solution, kept at room temperature, for up to 5 hours. (15) The Tyrode was composed of: 136.9mM NaCl, 2.7mM KCl, 1.8mM CaCl2, 1.0mM MgCl2, 11.9mM NaHCO3, 0.4mM NaH2PO4 and 5.6mM glucose. It was oxygenated with 95% O2 and 5% CO2. (16) The oral part of the small intestine was marked with two threads. The mesentery was separated from the bowel. The oral part was also placed in Tyrode solution for storage.

A 10 ml syringe filled with Tyrode solution was used to clean the lumen of the intestine by flushing the segments from the oral end. Very little pressure was applied to avoid overstretching the enteric musculature. The syringe was always introduced from the oral end, resembling the flow of liquids and chyme through the intestine. (15)

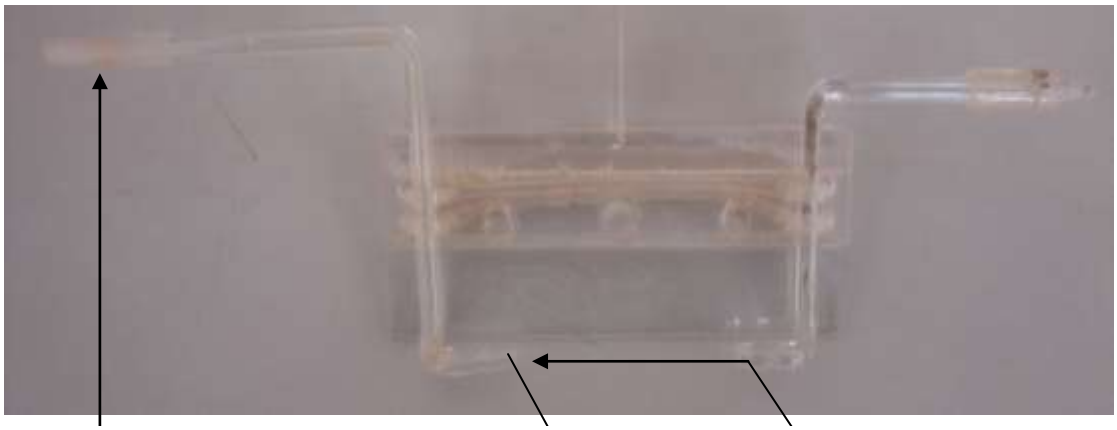

Figure 2: The apparatus with the two cannulas on each side as seen from the front.

Cannula which is connected by a tube to the pressure transducer

A piece of small bowel is placed between the two cannulas so that perfusion takes places in an oral to aboral direction.

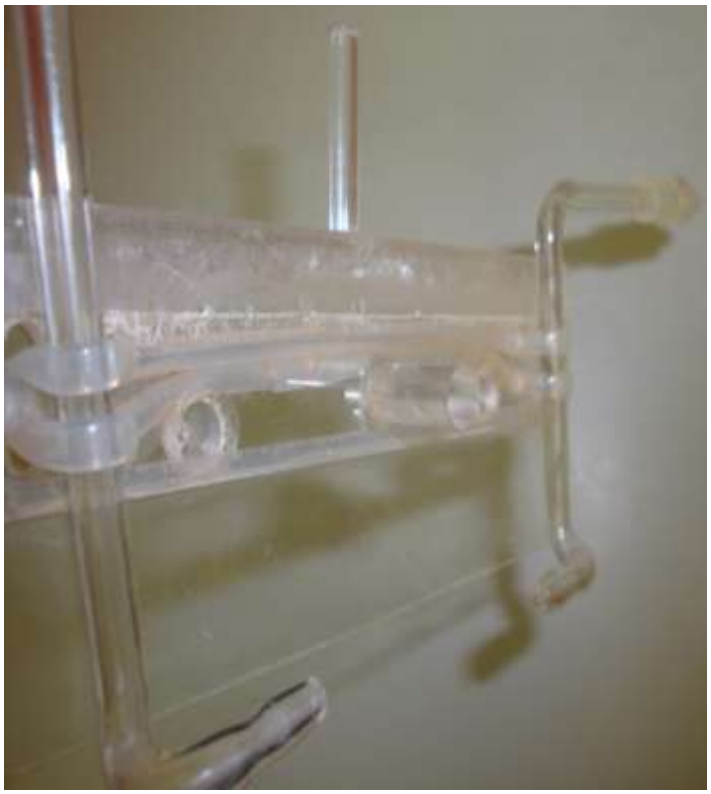

Figure 3: The apparatus with the two cannulas on each side from a different angle.

In half of the experiments, at first the aboral parts were divided into four segments, each one with an approximate length of 10 cm. Those four segments were tied with another cotton thread onto the holder. The oral end to the right cannula, the aboral to the left.

The holder with the intestinal segment attached was then moved to the organ bath filled with Tyrode solution. The organ bath itself was put in the thermostat bath, which was already heated. Through a tube the organ bath was oxygenated, keeping the intestinal segment vital. (16)

2.2 Experimental setup

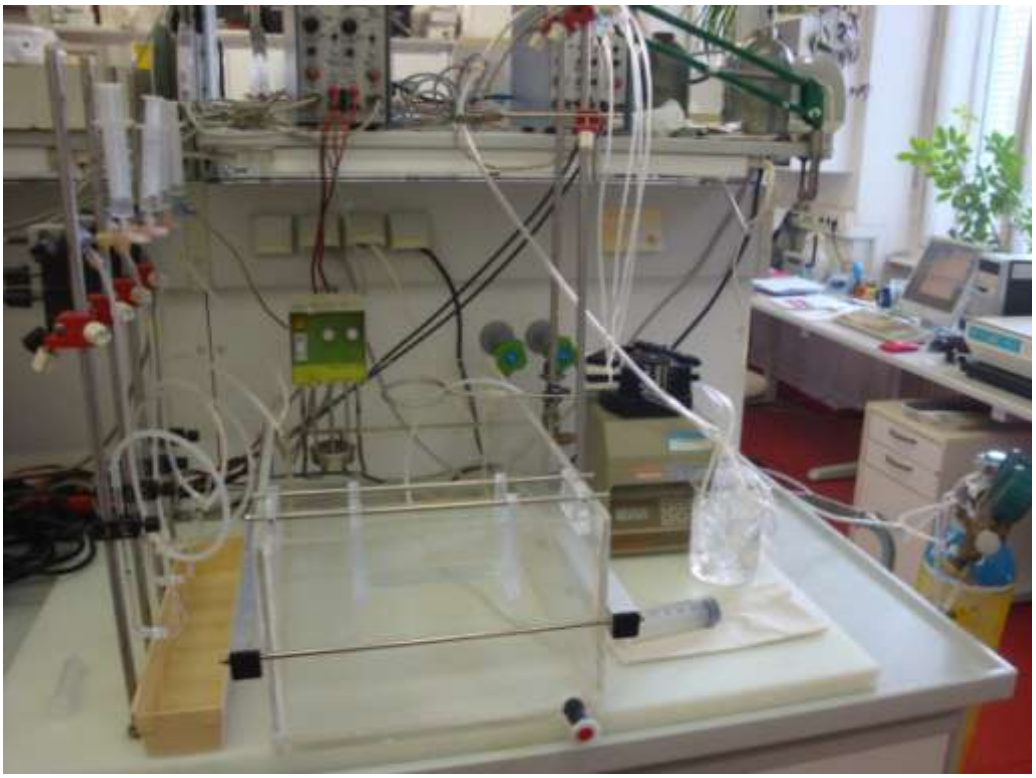

Figure 4: The experimental machine

The reservoir, the peristaltic pump, the organ bath within the thermostat bath and the pressure transducer made up the experimental setup.

Oxygenated Tyrode solution was pumped from a reservoir through the tubing at a rate of 0.5ml/minute by a peristaltic pump. The pump was connected by those tubes to the oral cannula, pumping the fluid through the lumen of the intestine.

At the junction between the tube and the cannula a connector to attach a syringe was affixed. This was used for flushing the intestine and thus getting rid of faeces and air bubbles, which would impair the measurement. (15)

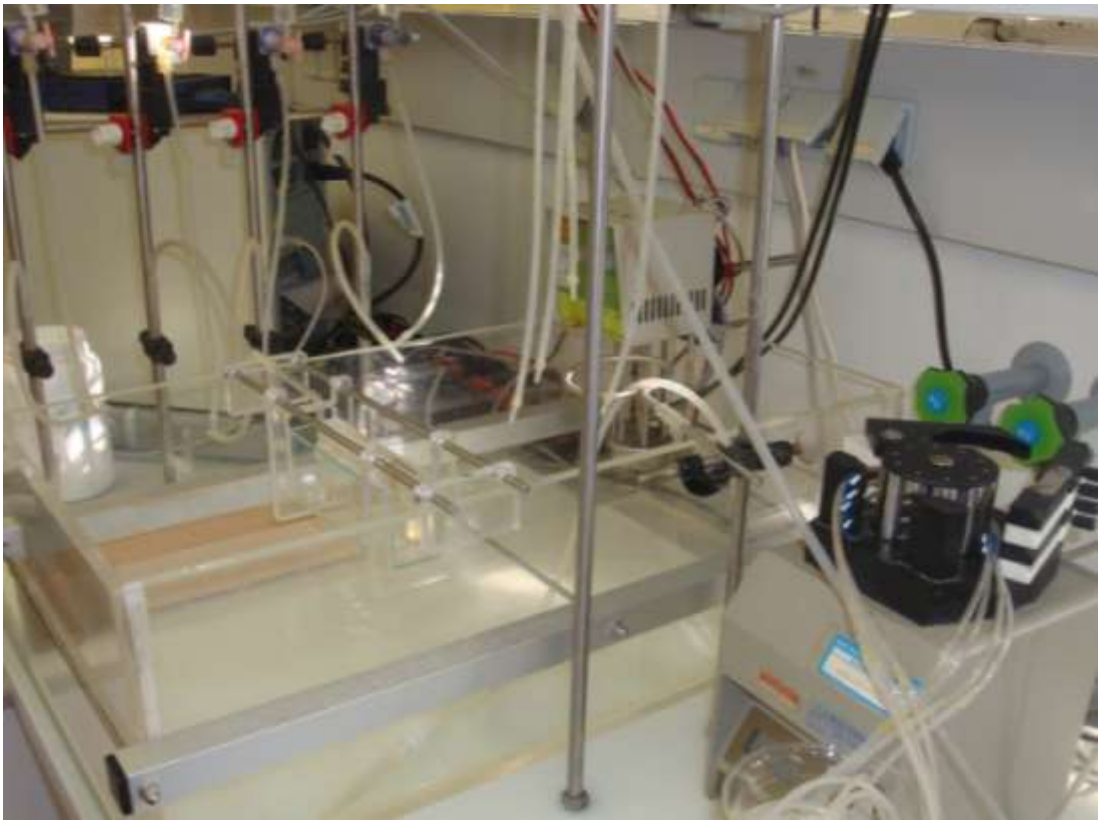

Figure 5: The setup from a different angle.

The intestinal segment together with its holder was placed in an organ bath filled with 30ml of Tyrode. (16) This was put in a thermostat bath filled with deionised water at 37°C.

From the apparatus the Tyrode solution flowed through the cannula of the aboral end to a pressure transducer. The signals from this pressure transducer were fed into a computer and digitised at a rate of 2Hz.
Recording and evaluation were performed with Peristal 1.0 software developed by Akos Heinemann. (15)

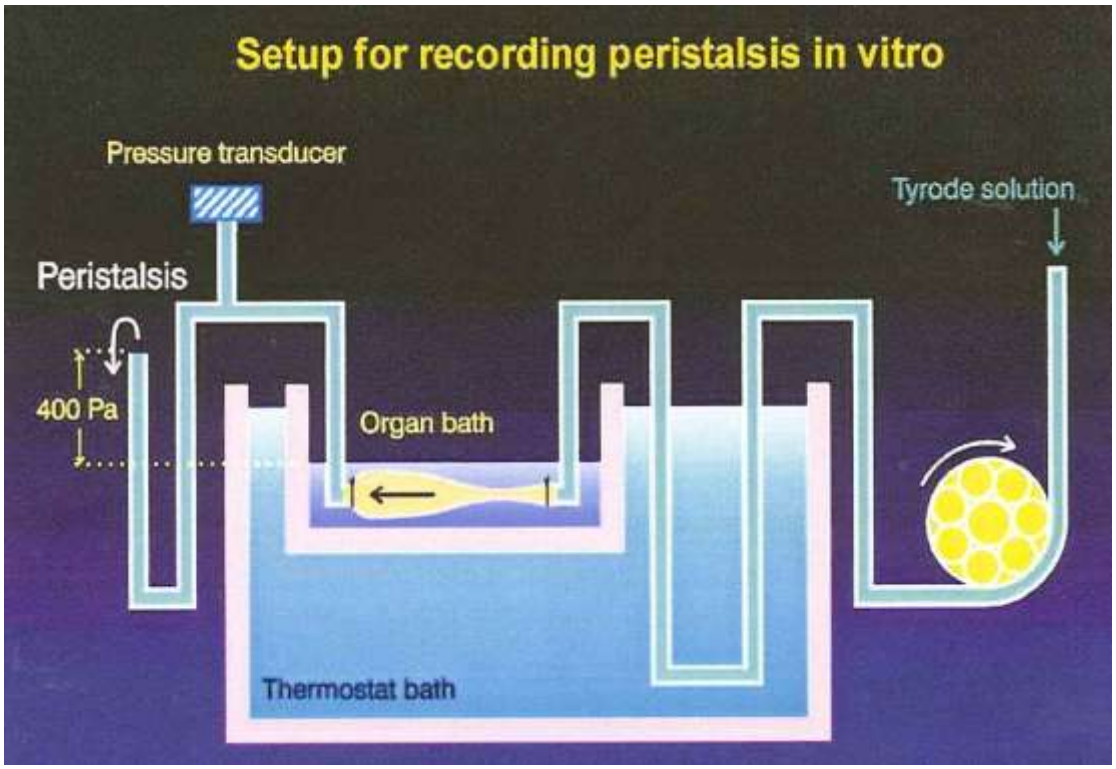

Figure 6: The setup for the evaluation of peristalsis of a segment from the ginea pig's small intestine. Perfusion takes place in a oral to aboral direction (provided by P. Holzer).

2.3 Experimental protocol

After all 4 segments had been set up as described above, each segment was carefully rinsed from the oral end with Tyrode by means of a syringe.

The pressure transducers were checked for bubbles once more. If one was detected, a syringe with deionised water was used to rinse the transducer. Then the intestinal segments were connected on the oral side to the tubing coming from the peristaltic pump, and on the aboral side to the tubing leading to the transducer. The outlet was adjusted to the level of fluid in the organ bath, allowing free outflow of the Tyrode solution.

After 20 minutes equilibration, the outlet was raised 4.1cm above the Tyrode level in the organ bath. Peristalsis was elicited by distending the gut wall. Tyrode solution could pass through this vertical outlet only if intraluminal pressure exceeded 400 Pa. After a threshold of 50-90 Pa had been reached a peristaltic reflex wave was triggered. (15,16) A sharp increase in pressure propelled the intraluminal content.

Figure 7: Pressure transducers

The experiment started after another 20 minutes, when regular peristaltic activity had been established. Cumulative dose response curves were constructed by adding increasing doses of test compounds every 15 minutes up to a maximum of 300µM. Experiments were terminated earlier if a lower concentration led to a complete blockade of peristalsis.

2.4 Evaluation and statistics

Four parameters were evaluated for every segment: the peristaltic pressure threshold, the maximal pressure, the base pressure, and the maximal acceleration. (16)

The peristaltic pressure threshold (PPT) refers to the pressure at which the peristaltic wave is initiated (Pa). *"Inhibition of peristalsis was associated with an increase in PPT, and abolition of peristalsis manifested itself in a lack of propulsive motility in spite of an intraluminal pressure of 400 Pa. Although in this case PPT exceeded 400 Pa, abolition of peristalsis was expressed quantitatively by assigning PPT a value of 400 Pa in order to obtain numerical results suitable for*

further statistical evaluation."[5] Maximal pressure (MP) is the pressure reached during a peristaltic wave. The pressure after evacuation of the intestinal segment is called base pressure (BP). Low baseline pressure depicts good emptying capacity of the intestinal segment during the peristaltic wave. The fourth index of effectiveness of peristalsis is acceleration, which describes the development of pressure during peristaltic waves ($\Delta Pa/s^2$). It *"is determined not only by the speed with which the muscle contracts, but also by the speed with which this contraction moves aborally to empty the segments."*[6](15,16)

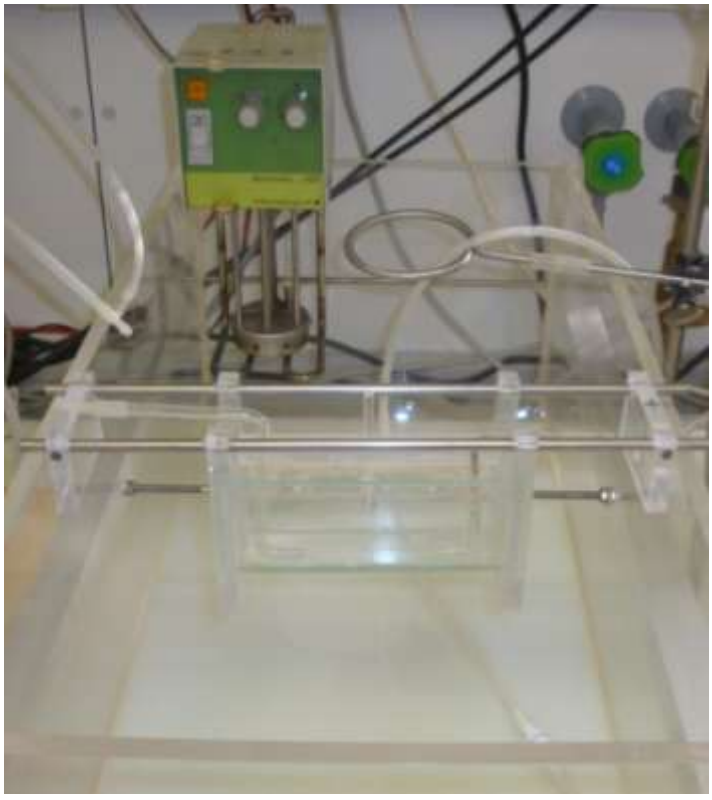

Figure 8: The organ bath. The holder will later be laid into the organ bath. 30ml of Tyrode solution and the chemical substances will be added.

[5] Shahbazian A, Heinemann A, Schmidhammer H, Beubler E, Holzer-Petsche U, Holzer P. Involvement of mu- and kappa-, but not delta-, opioid receptors in the peristaltic motor depression caused by endogenous and exogenous opioids in the guinea-pig intestine. Br. J. Pharmacol. 2002; 135(3):741–50.

[6] Holzer P, Shahbazian A, Painsipp E, Heinemann A. Evaluation of peristalsis in multiple segments of the guinea-pig isolated small intestine: optimisation of tissue use by refined in vitro methodology. Alternatives to laboratory animals : ATLA 2003; 31(4):419–27.

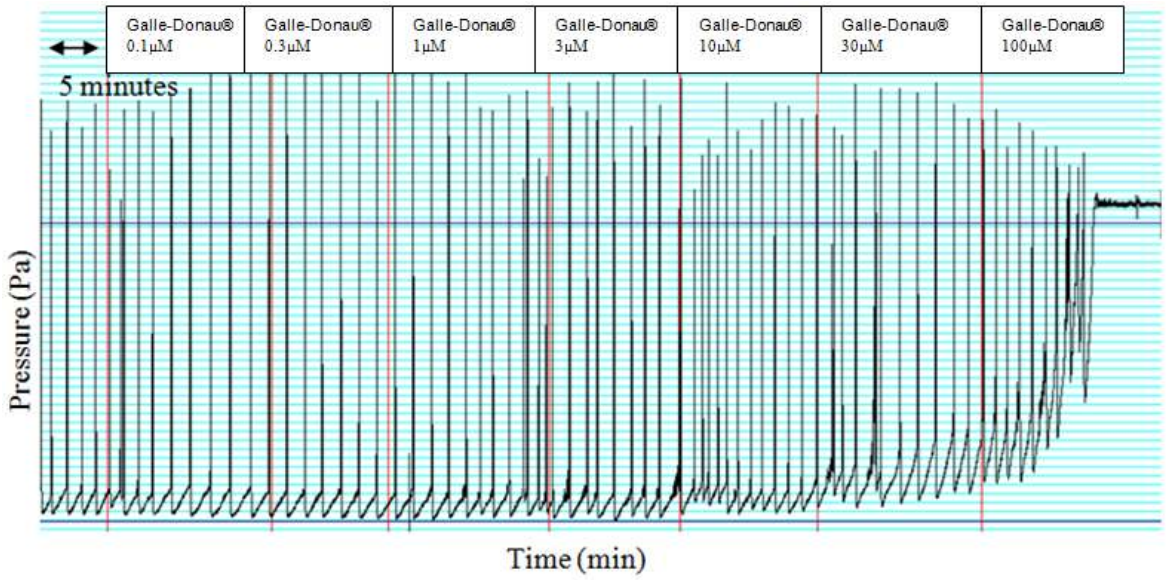

Figure 9: Peristaltic waves of an oral segment, after adding Galle-Donau®. The stated concentration of Galle-Donau® always refers to the dose of pTMC-N, the NAA concentration being 2.6 fold higher. On the right the horizontal tracing line signifies a loss of peristalsis. Peristaltic pressure threshold as well as base pressure increases, maximal pressure is reduced.

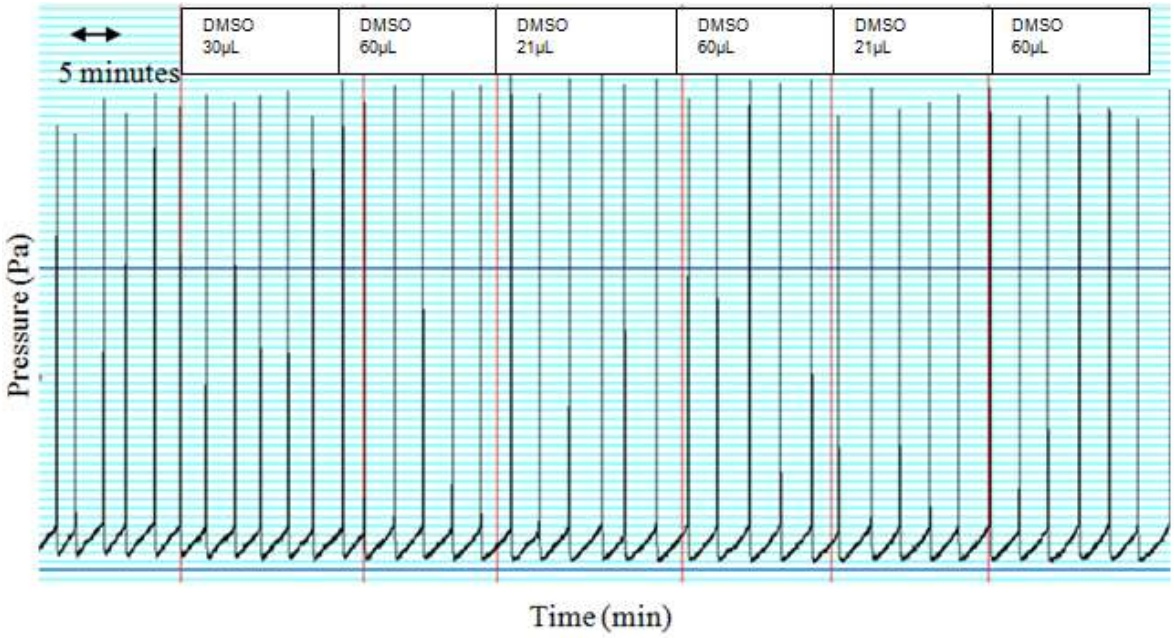

Figure 10: Peristaltic waves of an oral segment. The used substance was DMSO. In the first two additions, DMSO was diluted with deionised water at a ratio from 1:10. Then pure DMSO was used in concentrations as mentioned above. No loss of peristalsis or any irregularities are visible. All four parameters of peristalsis - peristaltic pressure threshold, base pressure, maximal pressure and acceleration - remain unchanged.

For evaluation, the last 3 peristaltic waves of each 15 minute period were saved. The arithmetic mean from those 3 values was calculated and fed into our statistics program.

For statistical analysis Sigma Plot 11 was used. The two way repeated measures ANOVA (one factor replication) was applied together with the Holm-Sidak test (all pairwise multiple comparison procedures), with an overall significance level of 0.05. Data was fitted a to sigmoidal curve, using Sigma Plot according to the formula

$$y = \min + \frac{\max - min}{1+10^{\,(logEC\;\;50-x)\;Hillslope}} .$$

2.5 Substances

For this experiment 4 substances were used: nicotinic acid, α-naphthylacetic acid (NAA), p-Tolylmethylcarbinol (pTMC), and p-Tolylmethylcarbinol-nicotinic acid ester (pTMC-N). They were dissolved in either deionised water or DMSO.

Nicotinic acid was ordered from Sigma-Aldrich Corporation, DMSO from Merck KGaA both located in Vienna.

α -naphthylacetic acid, pTMC, and pTMC-N, were acquired from Phafag Pharma Research & Trading GmbH, in Liechtenstein.

In order to mimic the administration of Galle-Donau® dragees NAA and pTMC-N were combined at a ratio of 2:1 on a weight basis, which corresponds to 2.6:1 on a molar basis. Therefore in the context of the present study, the name Galle-Donau® has to be understood as the combination of those two substances, without, however, the excipients contained in the original dragees.
A stated concentration of Galle-Donau® always refers to the dose of pTMC-N, the NAA concentration being 2.6 fold higher.

NAA was dissolved in 1,875 M NaOH at a concentration of 18.9 M.

Galle-Donau®, pTMC-N, as well as pTMC were dissolved in DMSO at concentrations of 10 mM, nicotinic acid in deionised water at 10 mM.

3 Results

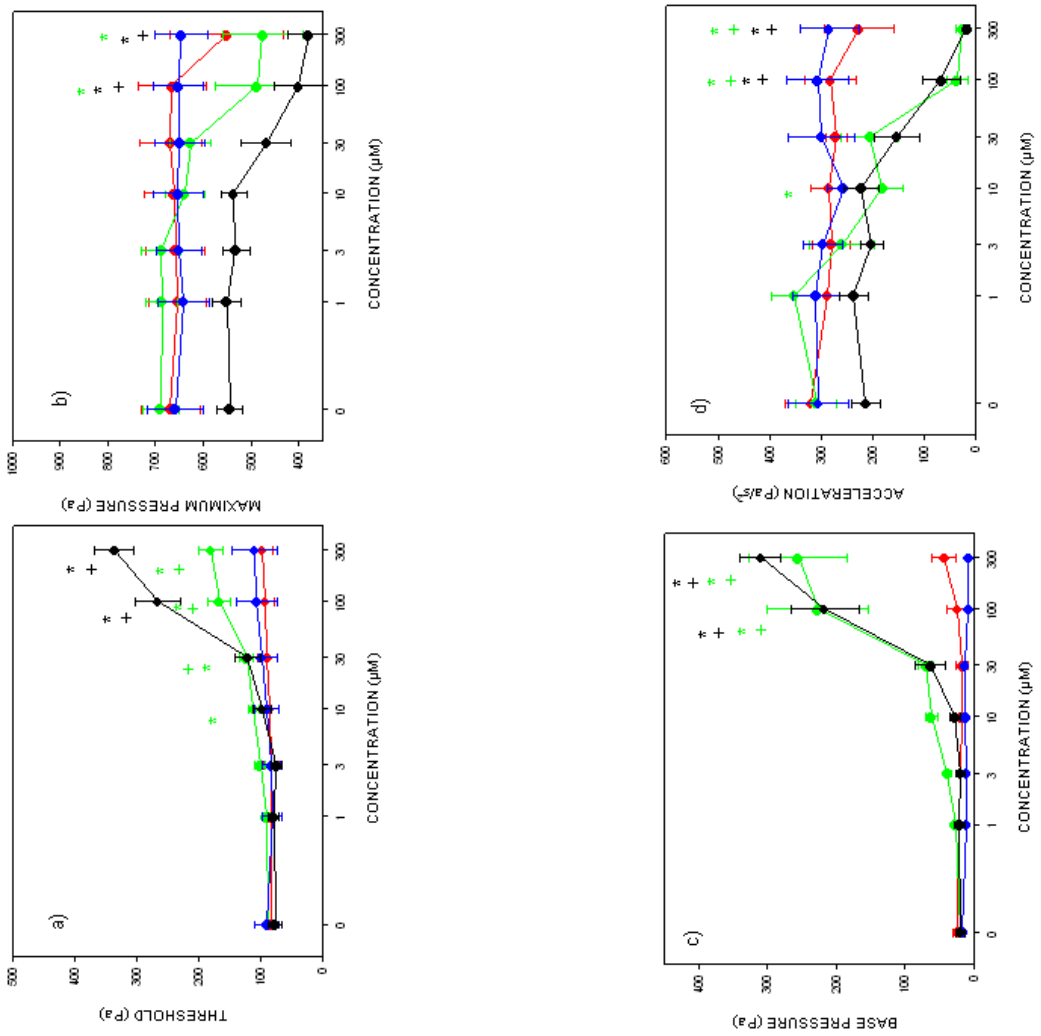

Figure 11: Influence of pTMC, pTMC-N, Galle-Donau® and of DMSO as their vehicle in oral segments of the small intestine. pTMC (red, n=6), pTMC-N (green, n=5), DMSO (blue, n=6), Galle-Donau® (black, n=7). The + marks a significant difference compared to DMSO at the same concentration, the * to 0 (value before addition of compound) as calculated by two way repeated measures ANOVA (one factor replication) followed by the Holm- Sidak test, with a significance level of 0.05. The values are depicted as $\bar{x} \pm$ SEM. a) depicts the values of the peristaltic pressure threshold b) of the maximal pressure c) of the base pressure and d) of the acceleration of the peristaltic wave.

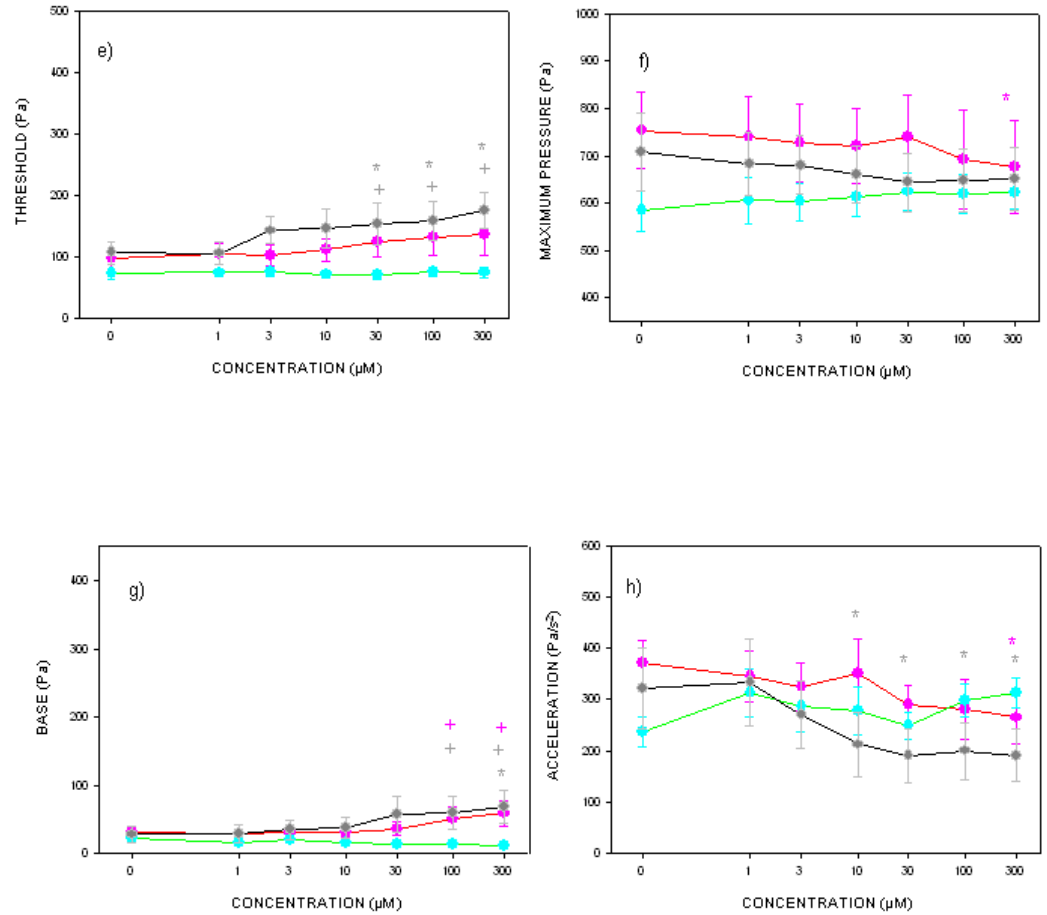

Figure 12: Influence of NAA, nicotinic acid, and deionised water as their vehicle in oral segments of the small intestine. NAA (grey, n=6), nicotinic acid (pink, n=7), and deionised water (blue, n=6). The + marks a significant difference compared to deionised water at the same concentration, the * to 0 (value before addition of compound) as calculated by two way repeated measures ANOVA (one factor replication) followed by the Holm- Sidak test, with a significance level of 0.05. The values are depicted as $\bar{x} \pm$ SEM. a) depicts the values of the peristaltic pressure threshold b) of the maximal pressure c) of the base pressure and d) of the acceleration of the peristaltic wave.

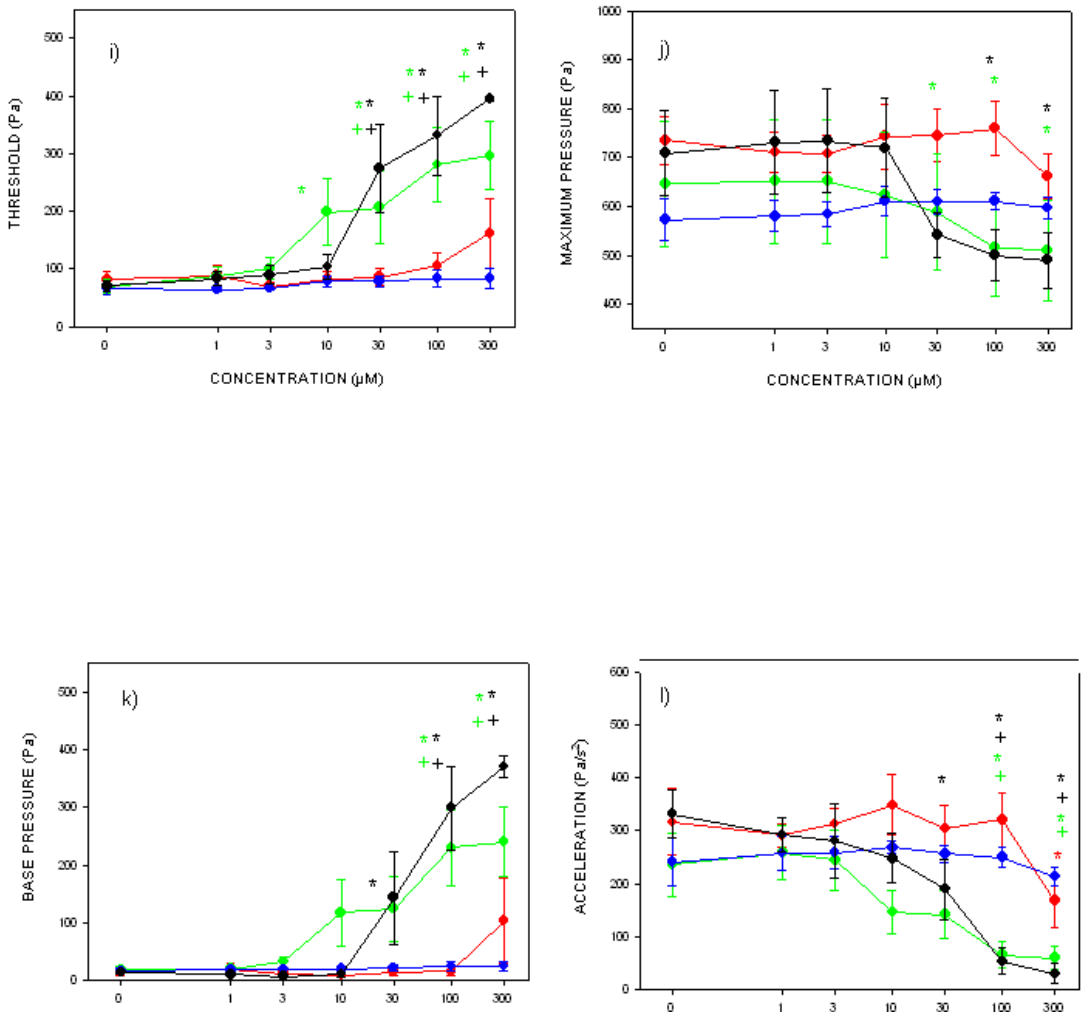

Figure 13: Influence of pTMC, pTMC-N, Galle-Donau® and of DMSO as their vehicle in aboral segments of the small intestine. pTMC (red, n=5), pTMC-N (green, n=6), DMSO (blue, n=6), Galle-Donau® (black, n=7). The + marks a significant difference compared to DMSO at the same concentration, the * to 0 (value before addition of compound) as calculated by two way repeated measures ANOVA (one factor replication) followed by the Holm- Sidak test, with a significance level of 0.05. The values are depicted as $\bar{x} \pm$ SEM. a) depicts the values of the peristaltic pressure threshold b) of the maximal pressure c) of the base pressure and d) of the acceleration of the peristaltic wave.

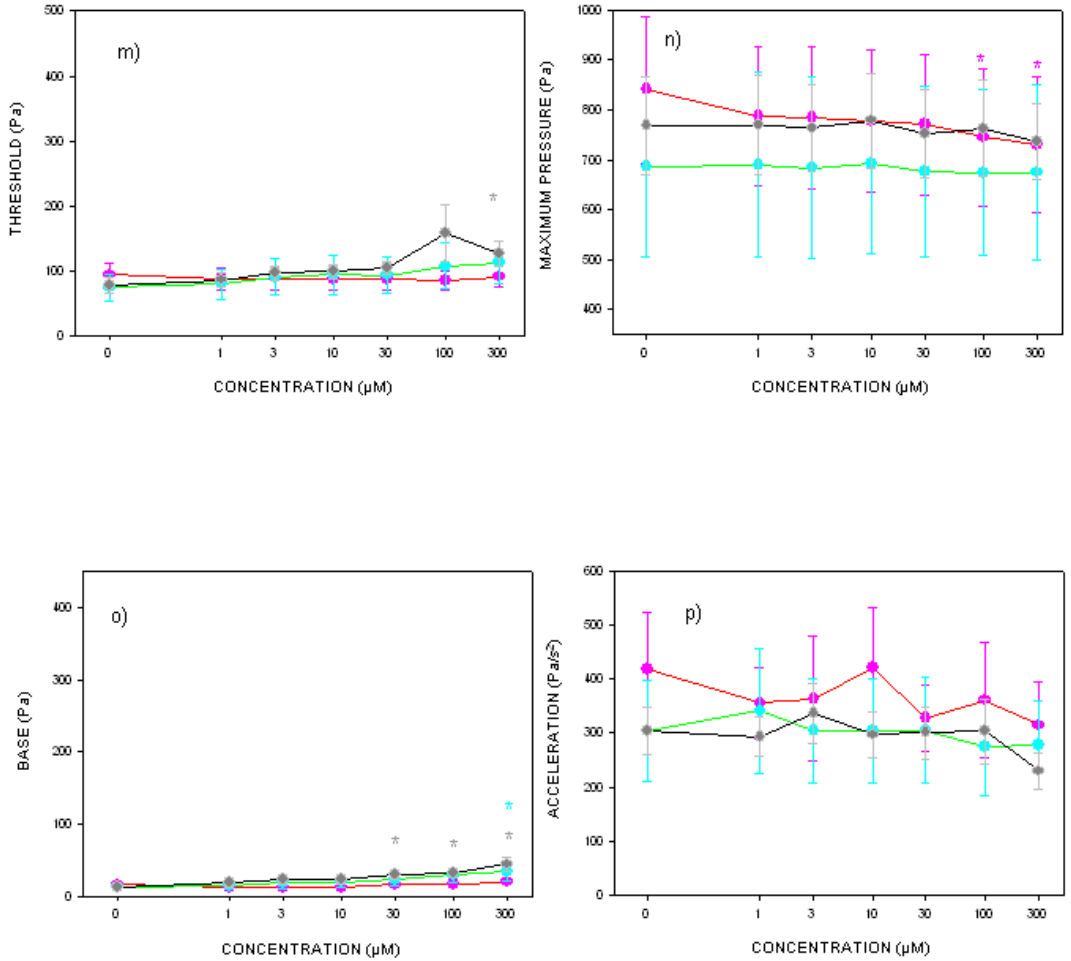

Figure 14: Influence of NAA, nicotinic acid, and deionised water as their vehicle in aboral segments of the small intestine. NAA (grey, n=7), nicotinic acid (pink, n=5), and deionised water (blue, n=6). The + marks a significant difference compared to deionised water at the same concentration, the * to 0 (value before addition of compound) as calculated by two way repeated measures ANOVA (one factor replication) followed by the Holm- Sidak test, with a significance level of 0.05. The values are depicted as $\bar{x} \pm$ SEM. a) depicts the values of the peristaltic pressure threshold b) of the maximal pressure c) of the base pressure and d) of the acceleration of the peristaltic wave.

3.1 Oral segments

Peristaltic pressure threshold (PPT) was significantly increased by pTMC-N at concentrations ≥ 10 µM. At concentrations of pTMC-N ≥30 µM, PPT was significantly higher than after addition of corresponding amounts of its vehicle, DMSO (Fig. 11a).
After ≥ 100 µM of Galle-Donau® was added to the organ bath, PPT significantly increased. These increases were also significantly higher than after addition of corresponding amounts of DMSO. DMSO, itself and pTMC had no effect (Fig. 11a).
NAA significantly increased PPT at concentrations ≥ 30 µM. PPT was significantly higher at concentrations of NAA ≥ 30 µM than after addition of corresponding amounts of deionised water (Fig. 12a).

Maximal pressure (MP) was significantly decreased by pTMC-N at concentrations ≥ 100 µM (Fig. 11b).
After addition of ≥ 100 µM of Galle-Donau® to the organ bath, MP significantly decreased. These decreases were also significantly higher than after addition of corresponding amounts of DMSO (Fig. 11b).
Nicotinic acid significantly decreased MP at a concentration of 300 µM (Fig. 12b).

Both pTMC-N and Galle-Donau® significantly increased BP at concentrations ≥ 100 µM. These increases were also significantly higher than after addition of corresponding amounts of DMSO to the organ bath (Fig. 11c).
At concentrations of ≥ 100 µM nicotinic acid and NAA slightly, but significantly increased BP compared with control segments. However, BP was significantly higher than before only after addition of NAA reaching a concentration of 300 µM (Fig. 12c).

The acceleration of the peristaltic pressure wave was significantly decreased by pTMC-N and Galle-Donau® at concentrations ≥ 100 µM. At these concentrations, acceleration was significantly lower than after addition of DMSO. Furthermore,

10µM pTMC-N also significantly reduced the acceleration compared with DMSO (Fig. 11d).
Nicotinic acid significantly decreased acceleration at a concentration of 30 µM. Acceleration was significantly lower at concentrations of NAA ≥ 10 µM (Fig. 12d).

3.2 Aboral segments

Peristaltic pressure threshold (PPT) was significantly increased by pTMC-N at concentrations ≥ 10 µM, and by Galle-Donau® at ≥ 30 µM. At ≥30 µM, pTMC-N and Galle-Donau® significantly increased PPT in comparison to corresponding amounts of its vehicle, DMSO (Fig. 13a).
NAA slightly, but significantly increased PPT at a concentration of 300 µM (Fig. 14a).

While pTMC-N significantly decreased maximal pressure (MP) at concentrations of ≥ 30 µM, Galle-Donau® showed the same effect at ≥ 100 µM (Fig. 13b).
Nicotinic acid slightly, but significantly decreased MP at a concentration of ≥ 100 µM (Fig. 14b).

Base pressure (BP) was significantly increased by pTMC-N and Galle-Donau® at concentrations ≥ 100 µM, also in comparison with corresponding concentrations of DMSO. In addition, 10 µM Galle-Donau® also significantly increased the BP (Fig. 13c).
NAA slightly, but significantly increased BP at concentrations ≥ 30 µM. A slight increase after the last addition of deionised water also reaches statistical significance (Fig.14c).

The acceleration of the peristaltic pressure wave was significantly decreased by pTMC-N at concentrations of ≥ 100µM by Galle-Donau® at concentrations of ≥ 30µM (Fig. 13d).
At a concentration of ≥100 µM of both substances, acceleration was significantly lower than after addition of DMSO (Fig. 13d).

Deionised water and substances dissolved in deionised water had no statistically significant effect on acceleration (Fig. 14d).

3.3 Oral vs. aboral segments

After administration of pTMC-N, no significant difference in peristaltic pressure threshold (PPT) between oral and aboral segments was found by the two way repeated measures ANOVA. When Galle-Donau® was administered, statistics showed at a concentration of 30 µM the PPT to significantly rise in aboral (192.5 ± 17.9 Pa) compared to oral segments (149.1 ± 15.2 Pa) (Fig. 11a, Fig. 13a).

There was no significant difference in maximal pressure between oral and aboral segments after pTMC-N administration. Nonetheless, after Galle-Donau® was added to the organ bath, maximal pressure was significantly higher in oral (486.9 ± 47.9 Pa) than in aboral (632.1 ± 56.7 Pa) segments at concentrations of 1, 3, and 10 µM (Fig. 11b, Fig. 13b).

No difference in base pressure between aboral and oral segments was discovered neither after adding pTMC-N, nor Galle-Donau® to the organ bath (Fig. 11c, Fig. 13c).

Also acceleration showed to be comparable between oral and aboral segments. However, aboral segments (203.4 ± 26.4 $\Delta Pa/s^2$) showed a higher initial acceleration than the oral (159.1 ± 22.2 $\Delta Pa/s^2$) ones before addition of Galle-Donau® (Fig. 11d, Fig. 13d).

Peristaltic pressure threshold (PPT)	pTMC-N		Galle-Donau®	
	Oral	aboral	oral	aboral
Minimum (Pa) 95% CI	76 -346 to 498.2	35 -511.4 to 580.7	80 51.27 to 109	82 16.11 to 148.5
Maximum (Pa) 95% CI	448 -716.2 to 1612	326 -34.26 to 685.4	347 278.2 to 416.3	369 298.6 to 440
Slope 95% CI	1 -2.047 to 3.131	1 -2.254 to 3.619	2 0.4667 to 3.605	3 -1.261 to 6.261
EC50 (µM) 95% CI	55 0.005274 to 5.700e+5	11 0.1245 to 1028	68 40.93 to 111.5	24 12.49 to 44.52

Table 1: Characteristics of the dose- response curves. Data were fitted to sigmoid curves by Sigma Plot 11.

No relevant difference between oral and aboral segments can be found. The confidence intervals (CI) for the data obtained for pTMC-N were large, indicating the great scatter of these data. The results of Galle-Donau® were more homogeneous, which is represented by a better fit. The slope of obtained with Galle-Donau® was much steeper than after administration of pTMC-N. EC50 was lower in aboral segments.

Maximal pressure	pTMC-N		Galle-Donau®	
	Oral	aboral	oral	aboral
Min (Pa) 95% CI	470 336.1 to 604.1	501 176. to 826.7	380 289.2 to 470.4	495 377.5 to 611.8
Max (Pa) 95% CI	673 600.6 to 746.2	651 455.8 to 846.7	544 485.6 to 602.1	732 615.7 to 849.2
Slope 95% CI	-3 -10.12 to 4.868	-2 -14.08 to 10.92	-2 -7.139 to 3.16	-4 -18.7 to 11.01
EC50 (µM) 95% CI	45 9.521 to 211.6	33 0.1874 to 5761	33 9.636 to 114	21 3.946 to 110.5

Table 2: Characteristics of the dose- response curves. Data were fitted to sigmoid curves by Sigma Plot 11.

As for PPT, no relevant difference between oral and aboral segments could be found. Data were more homogeneous for Galle-Donau® than for pTMC-N. The 95% confidence intervals of the minimum and maximum were overlapping for pTMC-N. Maximal pressure in segments treated with Galle-Donau® seemed to be higher aborally than in oral segments. The slope was always negative, indicating that MP decreased. EC50 was lower in segments treated with Galle-Donau®.

Base pressure	pTMC-N		Galle-Donau®	
	Oral	aboral	oral	aboral
Min (Pa) 95% CI	42 -8.146 to 91.87	-11 -326.8 to 304.8	21 -11.75 to 53.35	1 -64.94 to 66
Max (Pa) 95% CI	258 165.3 to 350	286 -131.6 to 703.8	328 241.5 to 415.5	377 245. to 509
Slope 95% CI	3 -1.633 to 7.552	1 -1.886 to 3.347	2 0.3371 to 3.632	2 -0.2949 to 3.78
EC50 (µM) 95% CI	56 20.14 to 153	23 0.7497 to 700.1	75 45.45 to 124.4	43 19.35 to 93.98

Table 3: Characteristics of the dose- response curves. Data were fitted to sigmoid curves by Sigma Plot 11.

For BP no relevant difference between oral and aboral segments could be found. Data were highly scattered, more so after pTMC-N than after addition of Galle-Donau®. The slope was highest in oral segments treated with pTMC-N and lowest in aboral segments treated with the same substance. EC50 was lower in segments treated with pTMC-N compared to Galle-Donau®.

Acceleration	pTMC-N		Galle-Donau®	
	Oral	aboral	oral	aboral
Min ($\Delta Pa/s^2$)	-1954	37	4	16
95% CI	-1.022e+6 to 1.018e+6	-179.7 to 254.3	-115.2 to 123.9	-113.6 to 146.1
Max ($\Delta Pa/s^2$)	9688	303	226	284
95% CI	-2.030e+7 to 2.032e+7	-69.51 to 675.1	178.8 to 273.5	216.2 to 352.8
Slope	-3e-2	-1	-2	-2
95% CI	-25.52 to 25.46	-3.217 to 1.688	-3.673 to 0.6427	-4.591 to 1.179
EC50 (µM)	2e-19	10	52	39
95% CI	0.000e+0 to +Inf	0.3355 to 307.8	16.09 to 167.7	13.01 to 118.2

Table 4: Characteristics of the dose- response curves. Data were fitted to sigmoid curves by Sigma Plot 11.

For acceleration, data seemed to be extremely scattered. No practically relevant difference could be evaluated by these data. All slopes were negative.

4 Discussion

4.1 Galle-Donau® dragees

Galle-Donau® dragees contain 37.50mg of p-Tolylmethylcarbinol nicotinic acid ester and 75 mg of α-naphthylacetic acid. Additionally, one can find 95.6 mg of lactose and 64.30 mg of saccharose. (13)

This drug is has multiple indications: *"functional disorders of the gall-bladder or the bile ducts without organic causes; inflammatory diseases of the entire biliary tract system; uncomplicated gall-stone troubles; cholestasis; gall deficiency symptoms after operative removal of gall-bladder; digestive troubles especially after increased consumption of fatty food, bloated feeling after rich food. obstipation and flatulence; improvement of the roentgenological examination of the biliary tract system both in case of oral as well as IV contrast media administration."*[7]

Galle-Donau® is a choleretic agent which causes an increase in bile production by stimulation of the liver cells. In a study with rabbits by Kobayashi et al. (17) Galle-Donau® dragees showed a bile-expelling effect by *"having [a] releasing effect on tonic terminal common bile duct (CBD) preparing a state of smooth excretion of bile and pancreatic fluids at the time of foods- loading together with a strong promoting effect of bile production.*[8]*"*

The effect of choleretic treatment with Galle-Donau® was evaluated in a prospective. non-randomised case control study by Kaserbacher et al. (18) with a *retrospective evaluation of pre-treatment period, evaluation of six months of treatment, open study after six month of treatment with evaluation of patients further treated and patients who discontinued treatment."*[9]

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[7] Summary of product characteristics; 2007. 2007 [cited 2009 Nov 16]. Available from: URL:http://www.phafag.at/files/attachments/1020/58071_GalleDonauFIEN.pdf.

[8] Kiso to Rinsho (The Clinical Report) 1974, 8(11):210-218

[9] Kaserbacher R, Propst A, Vogel W. Therapy of primary biliary cirrhosis with p-tolylmethylcarbinol nicotinic acid ester in combination with alpha-naphthylacetic acid. Wien. Klin. Wochenschr. 1996; 108(22):722–6.

Twelve female primary biliary cirrhosis (PBC) patients took Galle-Donau® dragees. Galle-Donau® dragees proved to decrease alkaline phosphatise, total bilirubin, unconjugated bilirubin, but not significantly, while triglycerides decreased significantly, but rose again after discontinuation of treatment. y-Glutamyl transpeptidase and IgM levels were not altered at all. Side effects included pruritus and sicca symptoms, but overall Galle-Donau® relieved symptoms of PBS and thus has become valuably for PBC therapy.

Inflammations of the biliary tract are effectively opposed by this drug. Bowel function, the concentration of antibiotics and gall contrast media - as well as its excretion - are enhanced by Galle-Donau® dragees. (13)

The dosage varies between one to 4 dragees, in case of fat digestion difficulties. Patients with chronic fat malabsorption can use Galle-Donau® dragees long-term, due to its low toxicity. This drug is applied orally. (13)

Side effects include flush, in patients diagnosed with hypersensitivity to pTMC-N, soft stool, diarrhea, and stomach ache. (13)

Galle-Donau® is a rather safe drug. Nonetheless, a study (13) on Donryu rats showed that toxic effects exist. α-naphthylacetic acid can cause acute toxicity with symptoms like tonic cramps, vomiting, spasms of the front legs and temporary ataxia after parenteral application. Subacute toxicity occurs only after an intake of a dose three times higher than recommended for patients.

Chronic use might temporarily elevate liver markers such as GOT and GPT, but has no other effects. No chronic toxicity is known to be caused by Galle-Donau® dragees. (13)

According to the official product characteristics (13) high dose administration of Galle-Donau® might lead to intrauterine death. No teratogenic effect could be detected.

Nonetheless, toxicity occurs at extremely high levels. The lethal dose for Galle-Donau® is 1841 mg/kg in mice and 5400 mg/kg in rats after oral administration. In comparison 1 Galle-Donau® Dragee contains 112.5mg. (13)

4.2 p-Tolylmethylcarbinol-nicotinic acid ester in combination with α-naphthylacetic acid

For the experiment we combined p-Tolylmethylcarbinol-nicotinic acid ester (pTMC-N) with α-naphthylacetic acid (NAA) in the same ratio as we can find it in Galle-Donau® dragees. pTMC was esterified to increase its bioavailability. Since esters tend to be cleaved very quickly in the intestine, we wanted to make sure to also test the cleavage products, nicotinic acid and pTMC. In case of a change in peristalsis during our experiment, this enabled us to identify the causative agent.

4.3 α-naphthylacetic acid

Also known as 1-Naphthaleneacetic acid or more commonly referred to as NAA. α-naphthylacetic acid is a colourless compound. (19)

Naphthylacetate is structurally similar to the peroxisome proliferator clofibrate. It has an effect on lipid metabolism and, through the human peroxisome proliferator-activated receptor (hPPAR), induces tumor cytostasis and differentiation. (20)

In a study of Höller et. al. (21) the toxicity and anti-inflammatory effects of α-naphthylacetic acid were measured. The lethal dose after one peroral application was 750 mg/kg. Nonetheless it has to be considered that one Galle-Donau® dragee contains only 112.5 mg. Signs of intoxications were lack of motion and convulsions. Respiratory depression and death followed soon.

4.4 p-Tolylmethylcarbinol and p-Tolylmethylcarbinol-nicotinic acid ester

pTMC-N was never used alone therapeutically because of its insolubility in water. It is not absorbed by the GI tract and was therefore esterified to upgrade its bioavailability. For use in Galle-Donau dragees it is esterified with nicotinic acid ester.

4.5 Nicotinic acid

Nicotinic acid is better known as Niacin or VitaminB3. This pyridine derivative is water soluble. A deficiency is linked to the disease pellagra. (22)

Nicotinic acid was approved in clinical practice as a lipid-modifying drug. Its medical value has been assessed by various studies throughout the years. Nicotinic acid is known and used for the elevation of high density lipoprotein (HDL) and can lower low density protein (LDL) and total cholesterol. A recent study by Bodor and Offermanns (23) suggested that nicotinic acid may be an old drug, but has a clinically strong effect. This is reasoned with its “strong HDL cholesterol-elevating effect”.

Nicotinic acid has several effects on lipid metabolism. Within minutes it decreases free fatty acid levels in the plasma. After hours VLDL and TGs diminish. It is believed that nicotinic acid lowers the amount of substrate for TG and VLDL production. LDL and HDL levels drop after administration of nicotinic acid for days. (23)

Recent studies (24,25) suggest that effects on VLDL and TG might be due to inhibition of diacylglycerol acyl transferase 2 and accelerating the intracellular degradation of apoprotein B. The explanation of how HDL is most likely to be increased is best described by a hypothesis based on the well-established inverse

correlation between TG levels and plasma HDL cholesterol concentrations mediated by Cholesterol ester transfer protein (CETP). (23)

Furthermore, nicotinic acid seems to enhance peroxisome proliferator-activated receptor-γ transcriptional activity in macrophages. (23)

Brown as well as white adipose tissues, dendritic cells, macrophages, monocytes, and neutrophils possess nicotinic receptors, referred to as GPR109A or HM74A in humans. *"GPR109A is coupled to Gi type G proteins. and its activation by nicotinic acid results in a G_i-mediated inhibition of adenylyl cyclase, resulting in a decrease in intracellular cyclic AMP levels."*[10] Increased β-adrenergic receptor activation and decreased phosphodiesterase activity increase cyclic AMP levels and thus enhance lipolysis. Additional, actions of GPR109A have to be evaluated in further studies. (23)

Diabetes mellitus type II patients might encounter dyslipidaemic changes since nicotinic acid causes increased insulin resistance. (23)

4.6 Motility effects

The aim of this work was to evaluate possible effects of Galle-Donau® on peristalsis of the small intestine in guinea pigs with a special focus on differences between oral and aboral segments.

The major results of our experiment are as follows: (1) Galle-Donau® has been shown to have a significant impact on the peristalsis in oral and aboral segments of the guinea pig's small intestine. (2) With the exception of a few slight statistical differences, our study has pointed out that peristaltic motor parameters were comparable between oral and aboral segments. (15)

[10] Bodor ET, Offermanns S. Nicotinic acid: an old drug with a promising future. Br. J. Pharmacol. 2008; 153 Suppl 1:S68-75.

4.6.1 Influence of Galle-Donau® on the peristalsis in oral and aboral segments of the guinea pig's small intestine

In all four parameters, in oral and aboral segments, Galle-Donau® has been showing to inhibit intestinal motility. pTMC-N, the ester of pTMC with nicotinic acid, showed similar effects.

Both substances, Galle-Donau® and pTMC-N, have proven to increase peristalsis pressure threshold (PPT) compared to 0 and their vehicle, DMSO.
As mentioned before, PPT refers to the pressure at which the peristaltic wave is initiated. An increase of PPT is associated with inhibition of peristalsis. PPT was elevated during contractions, while MP as well as acceleration were decreased. When peristalsis was blocked, the bowel gained intraluminal space and was not able to generate peristaltic waves.

However, these data strongly suggest that pTMC itself is not responsible for the change in peristalsis. Only at a concentration of 300 µM. pTMC decreased acceleration slightly, but significantly in aboral segments. All other parameters were not altered by addition of pTMC, neither in oral nor aboral segments. Thus, this substance did not have any practical effect on the peristalsis.

Nicotinic acid showed effects on the motility of the guinea pig's intestine. Nonetheless, they were weak. This substance never had an impact on PPT and seldom affected other parameters. However, its most prevalent side effects are cutaneous vasodilatation (flush), which can be prevented by additional administration of other drugs i.e. aspirin or slow increase in dosage. Flush is also a side effect of Galle-Donau® (23), supporting the thesis that the ester is quickly cleaved, freeing nicotinic acid. Hence, an in vivo effect of nicotinic acid, not measured with our methods, seems to be plausible.

In our experiment we also evaluated the influence of NAA. It showed a slight effect on peristalsis, statistically significant, but by far not as strong as that of Galle-Donau® or p-TMC-N. In vivo, its effect on the intestine's motility might be higher.

Being a choleretic agent, it causes an increased production of bile acids. Bile acids are known to have a laxative effect causing increased intestinal motility.

Höller et.al. (21) described a choleretic effect of NAA based on an experiment with male rats, who suffered from fistulas in common bile duct. Especially, when NAA was administered orally, the impact on the production of bile fluid was increased.

Hitzenberger and Klein (26) confirmed the finding of increased choleresis. In their study they proved that Galle-Donau® dragees cause real choleresis and not choledіuresis by measuring not only the volume of bile, but also its bilirubin content. The volunteers for this study were divided into three groups. Group 1 consisted of patients with no history of liver of gall bladder pathologies. Group 2 was made up of patients with previous cholecystectomy. The other volunteers suffered from various liver and gall bladder or duct diseases. No side effects were determined.

Another study also brought bile acid production and intestinal motility in correlation. Abrahamsson et al. (27) conducted a study evaluating an alteration of bile acid synthesis in constipated patients with irritable bowel syndrome and functional constipation. Symptom severity and frequency as well as the number of defecations in a 6 day period were recorded. Blood samples were taken to measure cholesterol, triglycerides and blood pressure. Colonic transit time was evaluated by fluoroscopy and 7α-hydroxy-4-cholesten-3-one (C4) and bile acid production. Lanosterold and β-sitosterold in plasma were determined from the levels in plasma. The results revealed a correlation of bile acid synthesis and oral-anal transit time (OATT) in constipated patients, but not in healthy volunteers or patients suffering from IBS-D.

Bile acids have a physiological laxative effect, and one would assume that C4 would be increased in patients with diarrhea and decreased in constipated people. Abrahamsson et al. (27) found though, that an increase of C4 at lunchtime occurred only in IBS-D and constipated patients with normal OATT. C4 was not increased at lunchtime in constipated, but also in an IBS-D patient with delayed

OATT. Thus, it has been suggested that changes in bile acid metabolism might play an important part in the pathophysiology of constipation.

Our experiment shows that alteration in peristalsis took place after administration of Galle-Donau®, but we cannot make any statements about a possible effect in IBS patients. Due to its inhibitory effect on intestinal motility, Galle-Donau® has potential to play an increasingly important role in the therapy of intestinal motility disorders in the future. Thus, clinical studies have to be performed with Galle-Donau® and its compounds in order to evaluate its further use and indications and verify our in vitro results.

There is no evidence that deionised water and DMSO could practically cause an alteration in peristalsis in the guinea pig's small intestine.

4.6.2 Comparability of oral and aboral segments of the small intestine

Only minor statistical differences between oral and aboral segments were found. These differences can be explained with the rather low number of experiments (n=85) and a large scatter in data. As a previous study of Holzer et. al. (15), evaluating the influence of morphine on the intestinal peristalsis of guinea pig's, has indicated, no differences in drug effects between oral or aboral segments could be found.

4.7 Conclusion

The work succeeded in evaluating the influence of Galle-Donau® and its compounds on the peristalsis of the guinea pig's small intestine. Alterations of the four parameters of intestinal motility were observed. We concluded that Galle-Donau® and pTMC-N caused an inhibition of peristaltic motility. According to our data, we also concluded that there was no relevant difference between oral and aboral segments of the small intestine. Thus, Galle-Donau® might play an

increasingly important role in the therapy of intestinal motility disorders in the future.

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