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TOMAS STENSTRÖM

Effects of luminal hypertonicity on some duodenal functions

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Author

Tomas Stenström

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Abstract

Background and aim: The duodenum is regularly exposed to contents with different osmolality, ranging from as low as 40 to as high as 1000 mOsmol/kg H_2O, depending what we drink and eat. Previous studies have examined the responses to a hypotonic milieu. The aim of the present study was to examine the duodenal responses to luminal hypertonicity. **Methods:** The proximal duodenum was cannulated and perfused with a 350 mM NaCl solution for 30 min and the effects on duodenal wall contractions, mucosal bicarbonate secretion, net fluid flux and mucosal permeability studied in anesthetized rats, in the absence and presence of the selective cyclooxygenase-2 (COX-2) inhibitor parecoxib. **Results:** Perfusion of the duodenum with 350 mM NaCl induced a delayed nine-fold increase in mucosal permeability in COX-2 inhibited animals but had no effect in controls or in animals that did not respond to parecoxib with duodenal contractions. Further, luminal hypertonicity induced net fluid secretion, inhibited parecoxib-induced duodenal motility and reduced duodenal mucosal bicarbonate secretion. No contractions were seen in controls. Luminal hypertonicity increased bicarbonate secretion and induced net fluid secretion. **Conclusions:** The duodenal responses to luminal hypertonicity varied considerable between controls and COX-2 inhibited animals. Luminal hypertonicity has dual effects on bicarbonate secretion. The stimulatory effect is passive and due to solvent drag, while the inhibitory effect is due to inhibition of motility.

Keywords

Duodenum, hypertonicity, cyclooxygenase, parecoxib, ^{51}Cr-EDTA permeability, fluid flux

Supervisors

Olle Nylander

Department of Neuroscience, Biomedical Centre, Uppsala University

Scientific reviewer

Lena Holm

Department of Medical Cell Biology, Biomedical Centre, Uppsala University

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Biology Education Centre Biomedical Centre Husargatan 3 Uppsala

Box 592 S-75124 Uppsala Tel +46 (0)18 4710000 Fax +46 (0)18 555217

EFFECTS OF LUMINAL HYPERTONICITY ON SOME DUODENAL FUNCTIONS

Sammanfattning

När vi äter och dricker utsätts tolvfingertarmen för stora variationer i osmolalitet (antal molekyler/kg H_2O). Intag av vatten utsätter tolvfingertarmens slemhinna för en låg osmolalitet ner mot 40 mOsm/kg H_2O medan en kolhydratrik förtäring såsom pasta eller juice ger upphov till en hög osmolalitet upp emot 1000 mOsm/kg H_2O. Studier har visat att dessa extrema miljöer, utan ett fungerande försvarssystem, kan ge upphov till skada på tarmslemhinnan. Tolvfingertarmen har ett genomsläppligt epitel i den mening att molekyler relativt lätt kan passera tarmslemhinnan mellan cellerna, vilket gör att tarmen snabbt och enkelt kan reglera osmolaliteten och därmed förhindra skada. Osmolaliteten regleras via absorption respektive sekretion av vatten och elektrolyter, främst Na^+ och Cl^-, beroende på den rådande miljön i tarmen. Tarmen strävar hela tiden efter att ha en luminal osmolalitet lika med den i blodplasma (300 mOsm/kg H_2O).

Man har tidigare studerat vad som händer då tolvfingertarmen exponeras för lösningar med låg osmolalitet. Syftet med den här undersökning var dock att se hur tolvfingertarmen påverkades av en hög osmolalitet. En omfattande bukoperation utfördes på sövda råttor där ett 3 cm långt segment av främre tolvfingertarmen isolerades. Detta segment genomspolades med en saltlösning på 700 mOsm/kg H_2O och effekterna på tolvfingertarmens motorik, epitelets genomsläpplighet, vätskeflöde samt bikarbonatsekretion studerades.

TOMAS STENSTRÖM

Examensarbete 20 p i Molekylär bioteknikprogrammet,
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GLOSSARY

Bilateral cervical vagotomy
Bilateral cervical vagotomy is surgical cutting of the two vagus nerves in the cervical vertebra. The vagus nerve is one of the paired cranial nerves. Vagal output comprises a significant part of the total parasympathetic activity and among other things innervates the GI tract. A vagotomy blocks the vagus nerve by surgical cutting of the nerve. “Cervical” means neck and “bilateral” refers to the two vagus nerves.

Bolus
A bolus is a large dose of a drug that is given at the beginning of treatment to quickly raise blood-level concentrations.

Carotid artery
Carotid artery refers to the major artery in the head and neck. The artery of interest in this study is the left common carotid artery, which is a large artery in the neck.

Cauterization
Cauterize is a procedure to stop bleeding by burning the body tissue. In this study a cauterization was performed to prevent bleeding in connection to the incision of the duodenal wall.

Chyme
Chyme is the liquid mixture of ingested food and drink, hydrochloric acid and various gastrointestinal enzymes. The chyme, made in the stomach, move through the pylorus into the duodenum and then continue further down in the gastrointestinal system.

Duodenum
Duodenum is the first and shortest part of the small intestine. Duodenum is connected proximal to the stomach and distal to the jejunum.

Femoral artery
The femoral artery is the large artery in the thigh.

Laparotomy
A laparotomy is a type of an abdominal surgery. To get access into the abdominal cavity the abdomen is opened by an incision through the wall.

Osmolality
The osmolality is the concentration of solutes present in the solution and is expressed as mOsm/kg H_2O. The osmolality of blood plasma is 300 mOsm/kg H_2O. A solution with an osmolality equal to blood plasma is said to be an isotonic solution. Solutions with higher or lower osmolality are hypertonic or hypotonic compared to blood plasma.

Secretagogue
A hormone that is secreted is referred to as a secretagogue.

Submucosal and myenteric plexuses
The enteric nervous system (ENS) is the nervous system that innervates the gastrointestinal (GI) tract. In the wall of the GI tract the ENS forms two layers of neurons: the *submucosal plexus* that is found between the submucosa and the circular muscle layer, and the *myenteric plexus* that lies between the circular and longitudinal muscle layer.

1 INTRODUCTION

The food and drink that we ingest every day are very different concerning content, pH, osmolality and temperature. The epithelium lining the upper gastrointestinal (GI) tract, from mouth to small intestine, must always be prepared to handle these provocations without getting injured and, furthermore, be able to distinguish harmless molecules from those which constitute a potential threat to the integrity of the mucosa. The duodenum is equipped with different sensory receptors that continuously monitor the chemical (pH, osmolality, nutrients) and the physical (pressure) milieu. When these "sensory" receptors are stimulated information is transmitted as action potentials in primary afferent neurons to the "command central" of the enteric nervous system (ENS), the nervous system of the gut. The ENS, together with the sympathetic and parasympathetic nervous systems, constitutes the autonomic nervous system (ANS). The "command central" of the ENS is a mesh of interneuron's that form "microcircuits" in the submucosal and myenteric plexuses. The interneurons in turn regulate the activity in the motor neurons thereby controlling gastrointestinal motility, absorption and secretion as well as blood flow. Two important excitatory neurotransmitters in the ENS are acetylcholine, which stimulates smooth muscle contraction and the release of enteric hormones and vasoactive intestinal peptide (VIP), a potent secretagogue and inhibitor of motility. Another essential neurotransmitter is nitric oxide (NO), which like VIP, inhibits muscle contractions [**1, 2**] but has many other actions as well.

The GI tract has vital functions such as digestion of food and absorption of nutrients, electrolytes, vitamins and water but it also has several important defence mechanisms. The duodenum is regularly exposed to luminal content with varying osmolality. Drinking water will expose the duodenal mucosa to an extreme hypotonic milieu, i.e., 40-50 mOsmol/kg H_2O [**3**] while intake of a carbohydrate-rich meal like pasta creates a hypertonic luminal content [**4**]. One important function of the duodenum is to adjust the osmolality of the luminal content to that of blood plasma [**5**].

There are two pathways through which molecules can move across the intestinal epithelium: the transcellular and the paracellular pathway. The transcellular route requires carrier proteins or ion channels in the plasma membrane and the transport is strictly regulated. Solutes and water can also permeate the epithelium in the spaces between the enterocytes, i.e., paracellularly. The tight junction is the rate-limiting barrier for the passive movement of ions

and water through the paracellular pathway. The permeability of this junction is not static but can be modulated by physical, pathological and physiological events [6]. Neurotransmitters, hormones and paracrine factors may affect tight junction permeability via receptor-mediated changes in second messengers. This raises the possibility that the intestinal epithelium is capable of short-term modulation of the junctional barrier.

One unique quality, that distinguishes the duodenum from other segments of the GI tract, is its "leakiness", meaning that transepithelial movement of water and solutes can occur through paracellular pathways [7, 8]. This feature is probably the main reason why the duodenum, compared to other parts of the GI tract, efficiently can regulate luminal osmolality.

The duodenal mucosal permeability can be assessed via measurements of the blood-to-lumen clearance of the radioactive molecule ^{51}chromium labelled **e**thylene**d**iamine**t**etraacetic **a**cid (^{51}Cr-EDTA). The technique is well characterized and was proven to have many benefits. First, the ^{51}Cr-EDTA is a hydrophilic chemically stable, non-toxic chelate, with a molecular weight of 360 and a radius of 7 Å [9, 10]. Second, ^{51}Cr-EDTA is not metabolized nor taken up by cells and the transport, from blood to lumen, is blood flow independent [11, 12, 13]. Studies have shown that the rate-limiting barrier of the ^{51}Cr-EDTA movement from blood to lumen is the intestinal epithelium [14]. Taken together, these features suggest that ^{51}Cr-EDTA is an excellent probe for assessing changes in paracellular permeability *in vivo*.

Recently it was shown that perfusion of the rat duodenum with hypotonic solutions increases the blood-to-lumen clearance of ^{51}Cr-EDTA in an osmolality-dependent manner. A very good correlation was found between the increase in mucosal permeability to ^{51}Cr-EDTA and increases in perfusate osmolality and it was proposed that the hypotonicity-induced increase in mucosal permeability facilitates the adjustment of luminal osmolality [15]. Interestingly, the hypotonicity-induced increase in mucosal permeability is enhanced by inhibition of cyclooxygenase-2 [16] and reduced by tetrodotoxin, 5-HT_3 and nicotinic acetylcholine receptor antagonism strongly suggesting physiological regulation of this process [ ].

Gastrointestinal wall contractions are an essential function of the gut and contribute in several ways to facilitate digestion and absorption in the small intestine. Muscle contractions mix the luminal content with enzymes and juices and move the chyme anally at a pace optimal for digestion and absorption. Principally, there are two types of wall contractions, each

characterized by a unique pattern, i.e. the fed and interdigestive motility pattern, respectively. The "fed" pattern is organized irregular contractions. Between meals, or in connection to fasting, an interdigestive pattern is at work. This motility pattern is known as the migrating motor complex, MMC, and is characterized by a long period of physiological ileus, i.e., no motor activity followed by a period of irregular contractions and ending with intense, phasic contractions that persist for only some minutes. As previously described, the motility in the gastrointestinal tract is supervised and regulated by the ENS [**17**].

In the present study a segment of the proximal duodenum was prepared for luminal perfusion in anesthetized rats *in vivo*. An important question in this context is whether the abdominal trauma affects the function of the duodenum. Abdominal surgery is known to inhibit the GI motility, called postoperative or "paralytic" ileus, and is a common cause for prolonged hospital visits. Prostaglandins seem to play an important role in causing paralytic ileus [**18, 19**]. The enzyme responsible for the prostaglandin production is cyclooxygenase (COX). Actually, there are two isoforms of the enzyme, COX-1 and COX-2. COX-1 is present in all cells and participates in the regulation of various physiological processes [**20, 21**]. The COX-2 isoform, which is mainly inducible, has been proposed to constitute an important element of the body's alarm system that is brought into action in response to mechanical injury and inflammation [**22**]. Today it is possible to selectively block either COX-1 or COX-2 [**23**]. Recent studies in the anesthetized rat strongly suggests that inhibition of COX-2 changes duodenal physiology in a manner which, compared to untreated ones, better resembles the situation in fasted conscious rats [**23**]. Furthermore, previously studies have shown that short-term inhibition of COX-2 does not induce mucosal damage in the gastrointestinal tract [**24, 25**]. For these reasons some animals in the present investigation was treated with the selective COX-2 inhibitor parecoxib to prevent an exaggerated synthesis of endogenous prostaglandin caused by abdominal surgery.

The aim of the present study was to examine the duodenal responses to luminal hypertonicity. The proximal duodenum was perfused with a 350 mM NaCl solution and the effects on duodenal wall contractions (motility), mucosal bicarbonate secretion, net fluid flux and mucosal permeability was examined in anesthetized rats. The osmolality of the hypertonic solution (700 mOsmol/kg H_2O) was chosen from data in rats demonstrating an osmolality of the luminal content in duodenum of 680 mOsmol/kg H_2O after an ordinary meal [**26**]. Another aim was to learn the surgical procedure for preparing a duodenal-perfusion model in the

anesthetized rat and to gain knowledge about the methodology used to assess some common physiological parameters *in vivo*.

2 MATERIALS AND METHODS

The Uppsala University Ethics Committee for Animal Experiments approved all the experimental methods involving the rats.

2.1 ANIMALS

All experiments were performed on male F1 hybrids of Lewis-Dark Agouti rats (Biomedical Centre, Uppsala, Sweden) weighing 200-300g. Rats in groups of two or more were kept under constant conditions with 12/12h light/dark cycle and with a room temperature at 21°C. They had continuously free access to standardized pellet food and water. Before surgery the rats were fasted for approximately 16 hours but had free access to water. The animals were anesthetized by an intraperitoneal injection of Inactin® (Na-5-ethyl-1-(1´-methyl-propyl)-2-thiobarbituric acid), a long acting thiobutabarbital in the rat, at a dose of 120 mg/kg body weight (wt). To avoid unnecessary stress the regular keeper at the Animal department anesthetized the animals. Within thirty minutes after the injection the surgery was initiated.

2.2 SURGICAL PROCEDURE

During the surgery and the following experiment the rat was placed in a supine position on a heating pad connected to a rectal thermometer. The heating pad together with a lamp maintained body temperature around 37.5°C. To facilitate breathing a tracheotomy was performed and a PE200 cannula inserted into the trachea. The femoral and carotid arteries were catheterized with PE-50 polyethylene cannulas containing heparinized NaCl solution (12.5 IU Heparin/L isotonic NaCl). The femoral artery was used for recordings of systemic arterial blood pressure and the carotid artery for blood withdrawals. For infusion of ^{51}Cr-labeled EDTA and for infusion of drugs and electrolytes the right and left femoral veins were catheterized, respectively. For both veins a PE-50 polyethylene cannula containing a 150 mM NaCl solution (isotonic saline), was used.

Subsequently, a laparotomy was performed, i.e., the abdomen was opened by an incision of approximately 10 cm through the midline, *linea alba*. To prevent bililary-pancreatic juice from entering the duodenum the common bile duct was catheterized with a thin cannula (PE-10) and fixed with ligatures close to its entry into the duodenum. Silicon tubing was

guided into the mouth down through the oesophagus and the stomach via pylorus into the duodenum. 3-5 mm distal to the pylorus the tubing was fixed with ligatures. Approximately 3 cm distal to the pylorus an incision of duodenum was made and a PE-320 cannula was inserted and fixed with ligatures. The silicon tubing was connected to a peristaltic pump (Gilson Minipuls 3, Villiers, Le Bel France) for luminal perfusion with saline during the whole experiment. To prevent bleeding the exposed part of duodenum was cauterized. During the laparotomy the duodenum and other organs were treated very gently and constantly rinsed with warm saline (37°C). Before the abdominal cavity was closed with sutures the isolated part of duodenum was stretched and fixed in a way to eliminate the risk of twisting and twining during the perfusion with saline. To prevent heat loss a plastic foil was laid on the wound. In Figure 1 the arrangement of the experiment is illustrated.

2.3 EXPERIMENTAL PROCEDURE

During the hour that followed completion of surgery, the rat was allowed to recover from the operation thereby stabilizing cardiovascular, respiratory, and gastrointestinal functions. During this stabilizing period the duodenum was perfused with a 150 mM NaCl solution (isotonic saline) at a rate of approximately 0.4 mL/min.

At least 40 minutes (40-60 min) before the experiment began an intravenous bolus of 75 µCi ^{51}chromium-labeled **e**thylene**d**iamine**t**etraacetate **a**cid (^{51}Cr-EDTA) at a volume of 0.2 mL was injected and this was followed by a constant intravenous infusion of 50 µCi ^{51}Cr-EDTA per hour (1 mL/hour).

During the experiment, three blood samples, approximately 0.2 mL each, were taken at times: -10, 50 and 130 min, in relation to commencement of the experiment. The blood samples were centrifuged and 50 µL plasma was used for radioactivity determination. The blood volume loss, in connection to the blood withdrawal, was compensated for by injecting 0.2 ml of a 7 % bovine albumin solution (diluted in saline). Before putting the rat to death, with an intravenous injection of 1 mL saturated KCl, the abdomen was opened and the examined part of duodenum was exteriorized and saved for wet weight and length determination.

The duration of the experiment was 130 min and the effluent was collected in 13 vials, one for every 10-min period. Two additional 10-min samples were taken to determine the flow of the peristaltic pump alone. Throughout the experiment the perfusion rate was set to 0.4 mL/min.

Before use, all solutions including drugs, NaCl and albumin solution were preheated (37°C) in a water bath.

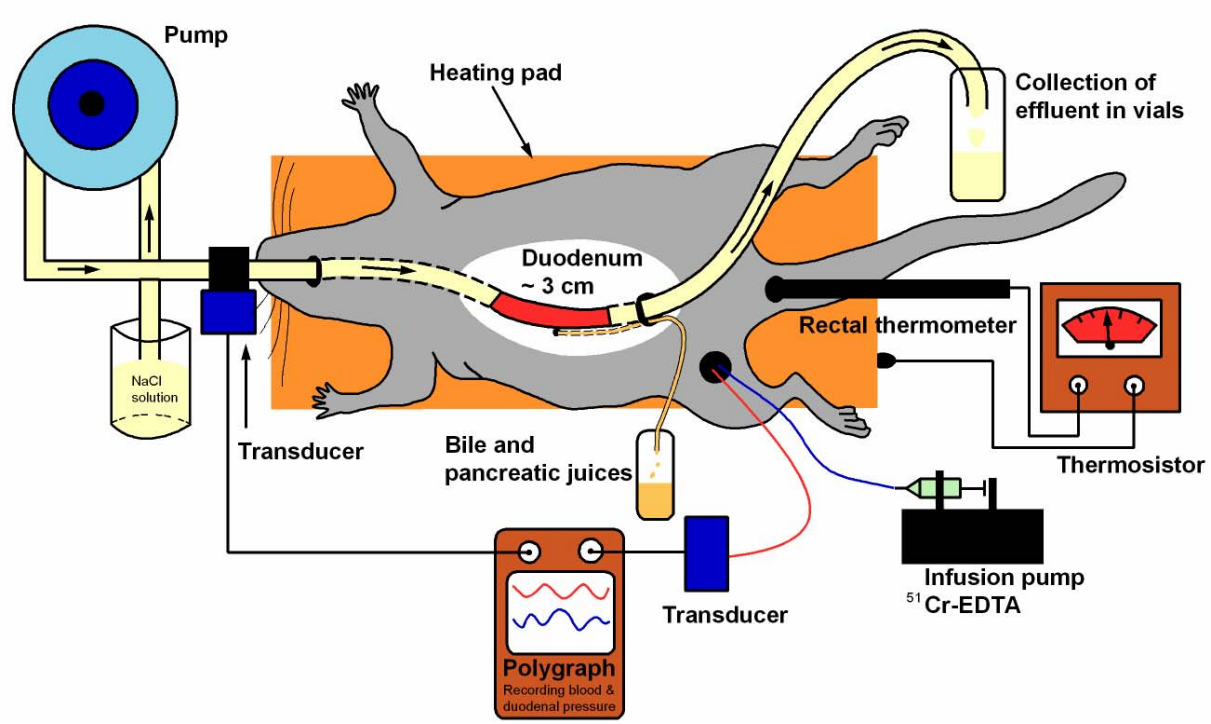

FIGURE 1 The arrangement of the experiment. The rat was in a supine position and the isolated part of duodenum was continuously perfused with warm saline. The effluent was collected in vials for further measurements. Blood and duodenal pressure were visualized via a polygraph.

2.4 PROTOCOL

2.4.1 Control Animals

During the first 70 minutes of the experiments the duodenum was perfused with 150 mM NaCl solution (isotonic saline, ~300 mOsm/kg H_20). Thereafter the duodenum was perfused for 30 min with a 350 mM NaCl solution (~700 mOsm/kg H_2O), referred as the hypertonic period. During the final 30 minutes of the experiment, the duodenum was again perfused with isotonic saline.

2.4.2 Parecoxib Treated Animals

For animals in this group the same procedure previously described was applied but they were also given the selective cyclooxygenase-2 inhibitor parecoxib (Dynastat®/Rayzon/Xapit). Parecoxib, dissolved in saline, was given as an intravenous bolus at a dose of 5 mg/kg 20 minutes after start of the experiment.

2.5 ANALYSIS

2.5.1 Measurement of Duodenal Contractions

To assess duodenal wall contractions a pressure transducer coupled to a polygraph (model 7D, Grass Instruments, Quincy MA) was connected to the inlet perfusion tubing, via a T tube. Since the peristaltic pump delivers a constant flow a rise in intraluminal pressure must be caused by an increase in resistance, which in turn is affected by the radius of the duodenum. Hence, contractions of the circular musculature decrease the radius and increases resistance. When the rise in intraduodenal hydrostatic pressure was large enough (> 2mm Hg above baseline) it was defined as a duodenal muscle contraction.

2.5.2 Measurement of Duodenal Mucosal Bicarbonate Secretion

The rate of duodenal mucosal bicarbonate secretion (DMBS) was measured by back titration of the perfusate to an end point pH of 4.8. The perfusate was titrated by 10 mM HCl using a pH-stat (Schott Titroline) together with nitrogen gassing (100% N_2), used as mixer. The pH electrode was calibrated with standard buffers before every experiment. The DMBS was expressed as micromoles of the base secreted per cm of intestine wet tissue length per hour ($\mu mol \cdot g^{-1} \cdot h^{-1}$).

2.5.3 Measurement of Mucosal Permeability

To determine the radioactivity in plasma about 0.2 mL of blood was withdrawn from the animal and centrifuged. 50 µL of plasma was removed and put in a vial for analysis of radioactivity. The plasma from 3 blood samples and 13 luminal samples were counted in a gamma counter (model 1282, Compugamma CS, PerkinElmer Life Science, Wallac, Upplands Väsby, Sweden). A linear regression analysis of the three plasma values was performed to get a corresponding plasma value for each effluent sample. To determine the blood to lumen clearance the following formula was used:

$$\mathbf{Clearance}_{^{51}Cr-EDTA}(mL \cdot \min^{-1} \cdot 100 g^{-1}) = \frac{\text{activity}_{\text{perfusate}}(cpm \cdot mL^{-1}) \cdot \text{perfusion rate}_{\text{pump}}(mL \cdot \min^{-1})}{\text{activity}_{\text{plasma}}\ (cpm \cdot mL^{-1}) \cdot \text{weight}_{\text{wet tissue}}(g)} \cdot 100$$

2.5.4 Measurement of Fluid Flux

To assess the effluent weight vials with and without effluent were determined. A precision scale was used and the mean of the following two to three effluent was calculated: sample 1 and 2 (0-20 min), sample 3 and 4 (30-40 min), samples 5, 6 and 7 (50-70 min), samples 9 and

10 (90-100 min) and samples 12 and 13 (120-130 min). Sample 8 and 11 were disregarded due to mix-up of solutions.

To compensate for the higher density of the hypertonic saline a ratio of the isotonic and the hypertonic saline was calculated. One mL of isotonic or hypertonic solution was pipetted in Eppendorf tubes and their weight determined on a precision scale. For each solution twenty samples were pipetted. The highest and lowest weight value for each solution was omitted and on this basis a ratio was calculated (data not shown). The density of the isotonic solution was set to 1 and the volume could be set equal to the weight. The ratio was calculated to 1.0105 and used to correct the weight for the hypertonic solution.

The peristaltic pump volume for 10 minutes ($volume_{pump}$) was measured and subtracted from each effluent when determining the net fluid flux. Determination of the net fluid flux was done using the following formula:

$$\textbf{Fluid flux}_{net}(mL \cdot h^{-1} \cdot g^{-1}) = \frac{\left[\text{volume}_{perfusat}(mL \cdot 10\,\text{min}^{-1}) - \text{volume}_{pump}(mL \cdot 10\,\text{min}^{-1})\right] \cdot 6}{\text{weight}_{wet\ tissue}\ (g)}$$

Changes in net fluid flux (Δ Fluid $flux_{net}$) of the hypertonic samples compared with the isotonic group directly before (sample 5, 6 and 7) was determined by subtracting the mean value of the fluid flux for the hypertonic group with the isotonic group as the following formula:

$$\Delta\ \textbf{Fluid flux}_{net}(mL \cdot h^{-1} \cdot g^{-1}) = \text{Fluid flux}_{net}\ (\text{mean 9 \& 10}) - \text{Fluid flux}_{net}\ (\text{mean 5, 6 \& 7})$$

2.6 STATISTICAL ANALYSIS

If nothing else is said, values are presented as mean ± standard error of the mean (mean ± SEM). SEM was calculated using the following formula:

$$SEM = \frac{SD}{\sqrt{n}},\ SD = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})}{n-1}}$$

The statistical significance comparisons were evaluated via an unpaired Student's t-test with a two-tail P value. $P < 0.05$ was considered significant. The statistical calculations were performed with the software Graphpad Instat 3.

2.7 CHEMICALS

KCl, NaCl and Bovine albumin was obtained from Sigma Chemicals (St. Louis, MO, USA). Saline solutions were made with deionized Millipore® water. A stock solution of 1M HCl (Titrosol®, E.Merck, Germany) was used and diluted with deionized Millipore® water to achieve the proper concentration of 10 mM. Parecoxib (Dynastat®) and ^{51}Cr-EDTA was purchased from Pharmacia, Sweden and PerkinElmer Life Sciences Inc. Boston, MA, USA respectively. Inactin was obtained from RBI, Natick, MA and Heparin from Kabi Vitrum, Stockholm, respectively.

3 RESULTS

During the experiments the body temperature remained stable at 37.5 ± 5 ºC, while the blood pressure dropped from approximately 110 ± 10 mm Hg to 80 ± 10 mmHg. The perfusion rate (~0.4 mL/min) and the intestinal wet weight (~0.18 g) and length (~2.7 cm) were approximately the same for every group of animals (data not shown).

3.1 CONTROL ANIMALS

A total of nine control experiments were performed and six of them were used when computing the results. The other three had to be omitted due to the bad health of these rats.

No duodenal muscle contractions were obtained in control animals (data not shown). The blood-to-lumen clearance of ^{51}Cr-EDTA (Fig. 4) remained stable throughout the entire experiment, apart from a discernible but insignificant increase ($p>0.05$) seen immediately after the change to luminal hypertonicity.

Duodenal mucosal bicarbonate secretion (DMBS) was stable during the control period with a mean of 7.4 ± 0.4 $\mu mol \cdot cm^{-1} \cdot h^{-1}$ and increased ($p<0.01$) to 12.1 ± 1.6 $\mu mol \cdot cm^{-1} \cdot h^{-1}$ in response to the hypertonic solution (350 mM NaCl). After cessation of the perfusion with the hypertonic solution DMBS decreased to 9.0 ± 1.0 $\mu mol \cdot cm^{-1} \cdot h^{-1}$ and approached the value during the control period (Fig. 3).

During the control period the net fluid flux remain steady, demonstrated in Fig. 4, with a negative value (-2.16 ± 0.25 ml · g^{-1} · h^{-1}) indicating net fluid absorption. Changing the intraluminal milieu from ~300 mOsmol/kg H_2O to ~700 mOsm/kg H_2O induced a positive net fluid flux (1.09 ± 0.74 ml · g^{-1} · h^{-1}), i.e., a net fluid secretion. During the final 30 min perfusion with isotonic saline net fluid flux again became negative (-0.59 ± 0.52 ml · g^{-1} · h^{-1}). The change in net fluid flux in response to 350 mM NaCl was 3.03 ± 0.83 ml · g^{-1} · h^{-1} (Fig 5). Knowing the net volume secreted and the increase in the rate of luminal alkalinization, the concentration of bicarbonate in the net fluid secreted could be calculated and found to be 24 ± 10 mM.

3.2 PARECOXIB-TREATED ANIMALS

Not all animals responded to parecoxib with duodenal contractions. For that reason two subgroups of the parecoxib-treated animals were made, i.e., “responder” and “non-responder”.

3.2.1 Responders

Eight of eleven experiments were successful and used for further analysis.

All animals in this group obtained muscle contractions within 10 minutes after injection of parecoxib, a selective cyclooxygenase-2 inhibitor. Contractions, as shown in Fig. 2, occurred as complexes and these were repeated once every tenth min. Luminal perfusion with the hypertonic solution caused an immediate stimulatory effect on motility, usually manifested by increases in the amplitude of the contractions. This was followed by a marked decrease in duodenal motility for the remaining hypertonic period. During the subsequent isotonic saline perfusion period contractions re-occurred.

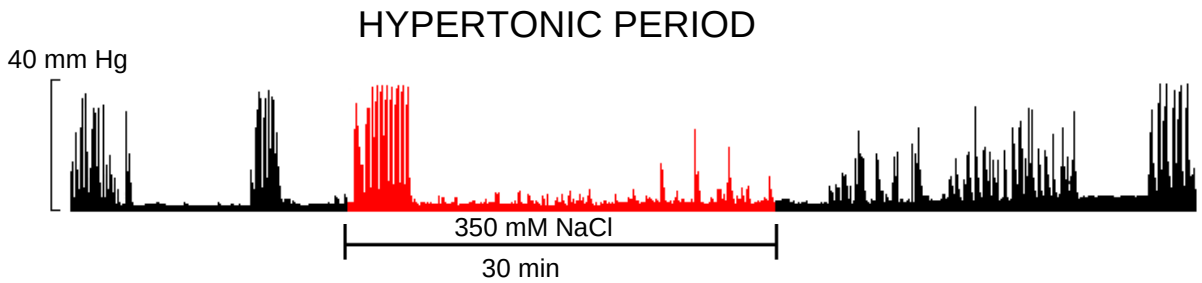

FIGURE 2 The intraluminal pressure, i.e. the motility, from a parecoxib treated animal and the inhibitory effect of the hypertonic exposure. The illustrated experiment is representative for the whole group.

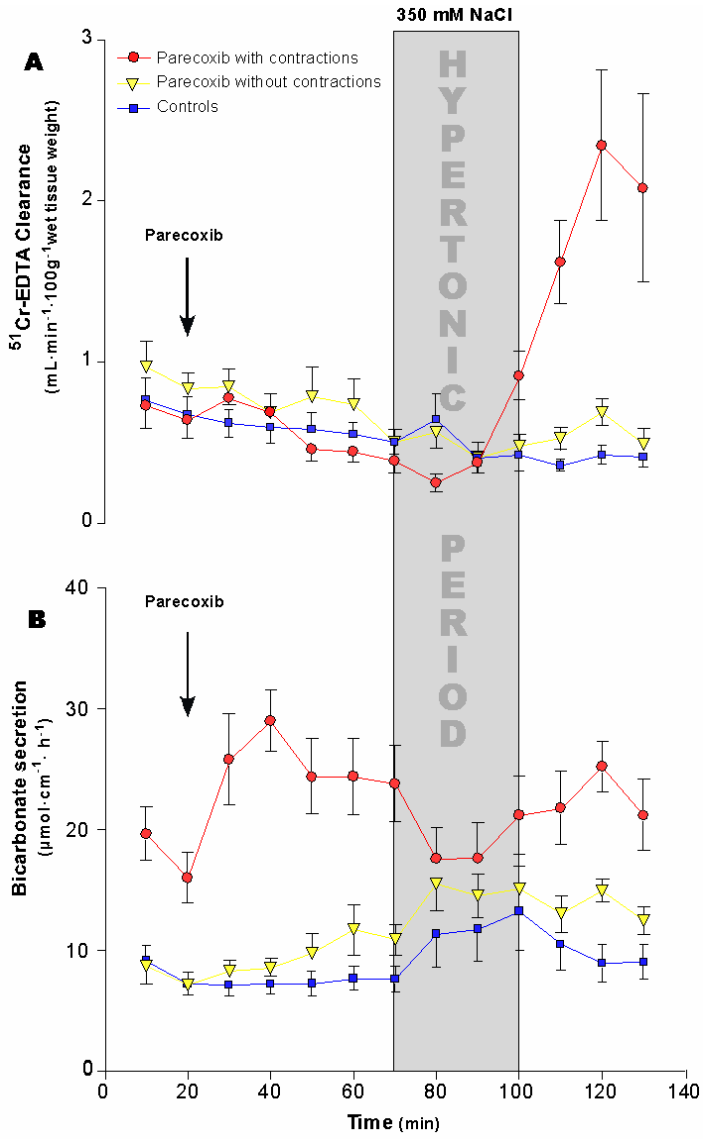

FIGURE 3 Effects of luminal perfusion of the duodenum with a hypertonic saline solution on the blood-to-lumen clearance of ^{51}Cr-EDTA (A) and duodenal mucosal bicarbonate secretion (B) in: controls (■ , n = 6) and parecoxib treated animals with (● , n = 8) and without (▽, n = 4) contractions. Parecoxib was given intravenously at a dose of 5 mg · kg^{-1} at the time 20 min (depicted by the arrows in the figure). The hypertonic period (350 mM NaCl solution), is illustrated by the shaded area. Values are shown as mean ± SEM.

Parecoxib induced a brief insignificant increase in ^{51}Cr-labeled EDTA clearance followed by a decrease to a value not different from that obtained in the control animals (Fig. 3). During the perfusion with the hypertonic solution a tiny decrease in ^{51}Cr-labeled EDTA clearance was obtained but this was followed by a gradual increase in clearance which culminated during the subsequent perfusion with isotonic saline at a value approximately 9.3 fold higher than basal (from 0.25 ± 0.06 to 2.34 ± 0.47 mL· min^{-1} · 100g^{-1}). A decrease in mucosal permeability was discernible during the last 10 minutes.

Parecoxib stimulated DMBS (Fig. 3) and increased the fluid secretion (Fig.4). The increased fluid secretion was transient and was followed by a value not different from zero. Perfusion of the duodenum with the hypertonic solution decreased DMBS while it at the same time induced net fluid secretion. After cessation of the hypertonic perfusion the DMBS returned to pre-hypertonic levels. A positive value of fluid flux was obtained for the final isotonic period, indicating net secretion of fluid, but was much smaller compared to the secretion reached during the hypertonic period (4.65 ± 0.71 and 1.58 ± 0.66 ml · g^{-1} · h^{-1}, respectively). The change in net fluid flux in response to 350 mM NaCl was 4.79 ± 0.92 ml · g^{-1}·h^{-1}, illustrated in Fig. 5.

3.2.2 Non-responders

This group was unsuccessful in the sense that parecoxib did not induce duodenal contractions (data not shown).

Parecoxib did not affect basal ^{51}Cr-EDTA clearance (Fig. 3). Luminal perfusion of the duodenum with 350 mM NaCl did not affect ^{51}Cr-EDTA clearance. The large increase in ^{51}Cr-EDTA clearance obtained after cessation of the perfusion with the hypertonic solution in parecoxib-treated animals was not seen in these animals.

Parecoxib did not affect the basal net fluid flux (Fig. 4). Perfusion of the duodenum with the hypertonic solution induced net fluid secretion. The change in net fluid flux in response to 350 mM NaCl was similar to that in controls (Fig. 5).

A tendency of a slight and gradual increase in DMBS was seen after the injection of parecoxib (Fig. 3). A significant increase in DMBS was observed in response to 350 mM NaCl.

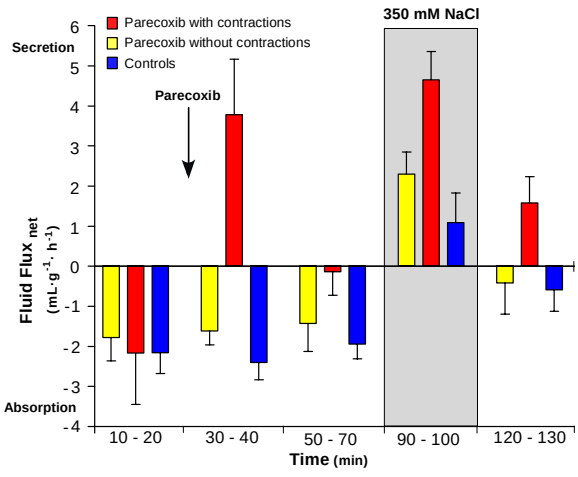

FIGURE 4 Luminal perfusion of the duodenum with 350 mM NaCl and its effect on net fluid flux across the duodenal mucosa in: controls (■ , n=6) and parecoxib treated animals with (■ , n=8) and without (□ , n=4) contractions. Parecoxib was given intravenously at a dose of 5 mg · kg^{-1} 20 min after start of effluent collection (depicted by the arrow in the figure). The hypertonic period is illustrated by the shaded area. Values are shown as mean ± SEM.

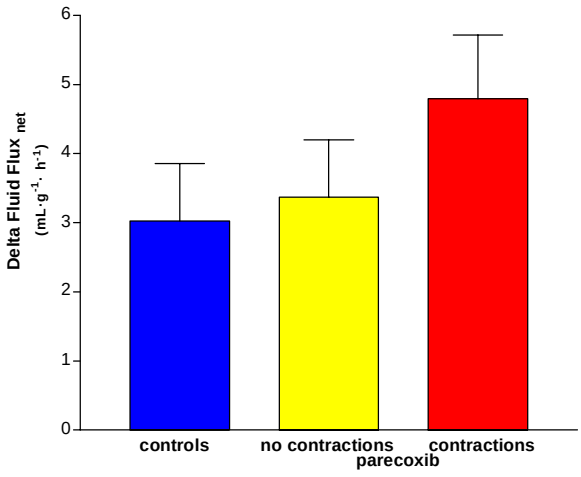

FIGURE 5 The change in net fluid flux in response to luminal hypertonicity (350 mM NaCl solution) in: controls (■ , n=6) and parecoxib treated animals with (■ , n=8) and without (□ , n=4) contractions. Values are shown as mean ± SEM.

4 DISCUSSION

As already mentioned in the introduction, the duodenum is regularly exposed to contents with different osmolality, ranging from as low as 40 to as high as 1000 mOsmol/kg H_2O depending what we drink and eat [**3**, **4**]. It is well known that isolated cells "burst" due to osmosis when put into a beaker of plain water. Conversely, when exposed to a hypertonic solution cells collapse. So, the question is how the duodenal epithelium can maintain its integrity when exposed to such great variations in luminal osmolality? This is incompletely understood but suggests that the duodenal epithelium possesses properties enabling rapid and effective adjustment of luminal osmolality.

Previous experiments in this laboratory have examined the duodenal responses to a hypotonic milieu. The results suggest that the osmolality-adjusting process is not only due to passive transport of ions and water through "leaky" paracellular shunts but also involves quite dramatic changes in duodenal mucosal permeability. The hypotonicity-induced increase in duodenal mucosal permeability is rapid and distinct and, furthermore, permeability returns to pre-hypotonic levels only 20 min after cessation of the perfusion with the hypotonic solution [**15**].

In the present investigation we examined the effects of luminal hypertonicity on some duodenal functions. More specifically, the duodenum was perfused with a 350 mM NaCl solution in anesthetized rats *in vivo* and effects on mucosal permeability, duodenal mucosal bicarbonate secretion (DMBS), motility and net fluid flux across the duodenal mucosa studied.

The basal blood-to-lumen clearance of ^{51}Cr-EDTA was approximately the same in every group. However, in response to luminal hypertonicity, the ^{51}Cr-EDTA clearance differed considerably between controls and parecoxib treated animals with contractions. A maximal 9.3 fold increase was seen in the parecoxib-treated animals with contractions compared to the steady value obtained in control animals. The magnitude of the hypertonic-induced increase in permeability was very similar to that in animals perfused with 50 mM NaCl [**15**]. It should be noted though, that the maximal increase in clearance occurred during the perfusion with the hypotonic solution but after the cessation of the perfusion with the hypertonic solution. This clearly suggests that luminal hypertonicity affects mucosal permeability by a different

mechanism than luminal hypotonicity. Interestingly, luminal hypertonicity or hypotonicity had no, or only minor effects, on ^{51}Cr-EDTA clearance in control animals suggesting that endogenous prostaglandins inhibit both responses.

Is the hypertonicity-induced increase in mucosal permeability due to a physiological mechanism or does the increase reflect mucosal damage? The mucosal permeability did not return to control levels before termination of the experiment. For that very reason two additional experiments were done where the perfusion period with isotonic saline, following that with 350 mM NaCl, was extended to 60 minutes. Data from these experiments showed that the hypertonicity-induced increase in ^{51}Cr-EDTA clearance returned to control values 40 min after cessation of the hypertonic saline perfusion (unpublished observations). Although these data do not exclude the possibility of mucosal damage as an explanation for the increase in ^{51}Cr-EDTA clearance, it seems unrealistic that injury would heal completely within a time period of 20 min. Furthermore, previous studies [**26**] have shown that in connection to a normal meal, the duodenum is exposed to an osmolality equal to 700 mOsm/ kg H_2O, which indicate that the duodenum is prepared to handle such a high luminal osmolality. Preliminary results from our laboratory indicate that the hypertonicity-induced increase in duodenal mucosal permeability is not due to the high sodium chloride concentration but rather to the high luminal osmolality [Nylander, personal communication]. The reason for believing so is that perfusion of the duodenum with orange juice (~ 700 mOsmol/kg H_2O) produced exactly the same response as 350 mM NaCl. Hence, it seems very unlikely that the hypertonicity-induced increase in duodenal mucosal permeability is due to mucosal injury. However, to be absolute sure whether or not luminal hypertonicity induces mucosal injury, a histological examination of the duodenal mucosa needs to be done. In that case the duodenum would be exteriorized and fixed at the same time as ^{51}Cr-EDTA clearance reaches its maximal level, i.e., 20 min after the perfusion with the hypertonic saline. Further, it would be interesting to perform an experiment with a hypertonic perfusion period of one hour to see what happens with the mucosal permeability.

Nylander *et al*., 2003, showed that a high mucosal permeability may increase the ability of the duodenum to adjust luminal hypotonicity to that of blood plasma by boosting the transfer of NaCl into the luminal solution [**15**]. Likewise, if the hypertonic-induced increase in mucosal permeability is a physiological response, the function could be to facilitate the adjustment of luminal osmolality. However, this seems a bit far-fetched considering the fact that the

permeability change occurred after cessation of the perfusion with the hypertonic perfusion. The permeability response may instead reflect a defence mechanism, i.e., increased paracellular secretion of isotonic fluid that would dilute the ”irritant” in the bulk solution.

When the duodenum is exposed to a hypertonic milieu a difference in osmolality between the lumen and blood is created, forcing water to move into lumen by osmosis. Furthermore, the high luminal sodium chloride concentration increases the movement of NaCl into the mucosa. These two processes would bring the osmolality of the luminal fluid closer to isotonicity.
In parecoxib treated animals, with contractions, the hypertonic saline perfusion decreased DMBS and increased fluid secretion. The induction of water secretion was anticipated, which thus provides a means to adjust the hypertonic milieu to an isotonic one. The decrease in duodenal bicarbonate secretion in response to luminal hypertonicity was less expected. In contrast, luminal hypertonicity increased DMBS in controls and in animals that did not respond to parecoxib with contractions. One reasonable explanation for this increase in DMBS is solvent drag. The difference in osmolality between the lumen and interstitium possibly creates a flow of water through the paracellular pathways.

With regard to the paracellular route there are data in the literature implicating the existence of intercellular pore-systems of different sizes in the small intestinal mucosa [**27**]. Morphological analysis of the small intestinal mucosa [**28**] as well as functional studies [**29, 30**] indicate that the smallest pores are localized in the villous tip epithelium while the larger ones are confined to the basal part of the villous or the crypts. Hence the fraction of fluid that crosses the duodenal mucosa via the paracellular route, in response to the hypertonic solution, may do so in shunts that are impermeable to ^{51}Cr-EDTA []. This probably explains why there is no correlation between the hypertonicity-induced net fluid flux and ^{51}Cr-EDTA clearance. However, the bicarbonate ions possibly are small enough to pass through these paracellular pathways. Therefore, as the water molecules flow through the pore, the solutes dissolved in this fluid, including HCO_3^-, will follow provided their reflection coefficient is well below 1 [**31, 32**]. Supporting the view, that the hypertonicity-induced increase in DMBS is a solvent drag phenomenon is the finding that the concentration of bicarbonate in the fluid secreted turned out to be 24 mM, which is exactly the same as in blood plasma.

However, an important question still remains, why does luminal hypertonicity decrease DMBS in parecoxib-treated animals with contractions? Well, this is probably related to the

existence of a close relationship between duodenal motility and DMBS. Nylander and Sababi [**33**] have shown that elevation of the intraluminal pressure increases the DMBS by a mechanism inhibited by the nicotinic acetylcholine receptor antagonist hexamethonium and bilateral cervical vagotomy. Furthermore, previous studies [**34**] and the result of the present investigation show that induction of duodenal motility stimulates DMBS. Blocking nicotinic receptors and mucosal nerves by lidocaine can prevent the motility-induced increase in DMBS strongly suggesting that motility increases DMBS by activating a neural reflex [**34**]. Of great interest in this context is the finding that the hypertonic solution, except for a very brief time in the beginning of the perfusion, markedly diminished duodenal motility. This may well explain why luminal hypertonicity decreases DMBS in parecoxib-treated animals. Most likely, however, there is a solvent drag effect also in the parecoxib treated animals with contraction but this effect is probably masked by the larger decrease in secretion caused by inhibition of duodenal motility. Some relevant experiments to do in the future would be to prolong the perfusion period with the hypertonic solution and perhaps also to increase the osmolality of solution further because that may completely inhibit duodenal motility.

One may ask why duodenal motility is inhibited by luminal hypertonicity. One explanation is that the decrease in DMBS facilitates the adjustment of luminal osmolality by reducing the output of ions into the luminal solution. This seems unlikely, however, because inhibition of motility possibly decreases the osmolality-adjusting capability by reducing the mixing and stirring of the luminal fluid. This in turn could reduce the functional surface area for secretion of water and absorption of NaCl.

It is interesting to note that luminal hypertonicity give rise to the same response on motility in COX-inhibited animals as HCl and capsaicin, a neurotoxin present in red pepper [**35**]. Capsaicin stimulates vanilloid receptors, which is a subtype of nociceptors. Nociceptors are activated by injury and inflammation [**36**]. The possibility thus exists that the hypertonicity-induced inhibition of motility is part of a mucosal defence mechanism. Inhibition of motility possibly reduces the risk that the “irritant species” in the luminal solution, in this case the high concentration of NaCl, reaches deeper parts of the mucosa.

Some animals did not respond to parecoxib with duodenal contractions. A more traumatic operation that activates additional defence mechanisms, such as increased production of nitric oxide (NO), is a likely explanation [**37**]. Another explanation may be that rats respond to

abdominal surgery differently, although this seems less likely considering the data from more experienced personal. The results from the "non-responding" parecoxib group were quite different from those in the "responding" group but very similar to the results generated from control animals. For instance, luminal hypertonicity did not affect mucosal permeability but increased DMBS in the "non-responding" parecoxib group despite inhibition of COX-2. Considering these data it is very important for the researcher to know that just because parecoxib does not induce any effects in their anesthetized rat model, this does by no means exclude endogenous prostaglandins from participating in causing postoperative ileus.

One reason for this can be that the activity of the enteric nervous system was affected in connection to the abdominal surgery. The surgery was not properly performed and the trauma that followed was so big that the enteric nervous system was affected and thereby induced a paralytic state.

The use of non-selective cyclooxygenase (COX) inhibitors (non-steroid anti-inflammatory drugs, NSAID) to prevent prostaglandin synthesis in association with extensive abdominal surgery may be controversial. The main reason is that NSAID:s have undesirably side effects in the gastrointestinal tract, such as mucosal lesions and local bleedings. However, Pihl and Nylander [**23**] recently investigated the effects of some selective and non-selective COX inhibitors and how they affected the duodenal motility, DMBS, mucosal permeability and fluid flux. These results were then compared to untreated animals with and without spontaneous contractions. Taken together, the results from this study indicated that parecoxib-treated animals, better than untreated ones, resembled conscious fasted rats, at least with regard to the above mentioned functions. Previous studies have also shown that the selective COX-2 inhibitor parecoxib will not, in contrast to NSAIDs, induce mucosal damage [**24, 25**].

In vivo studies are often very complex and it is important to have a sensitive and reproducible method with as few sources of errors as possible. First, the surgery performed is very complicated, at least for an inexperienced student like me. For this reason it is hard to know to what extent the surgery affects the physiology of the individual animal. Furthermore, do all animals respond to surgery in a similar way? Moreover, can results obtained from an inexperienced surgeon really be compared with those generated by an experienced and skilful one? Probably not. How great the difference can be is illustrated in the present study where some animals responded to the parecoxib administration with induction of duodenal motility

while others did not. Therefore, to obtain consistent data with limited variations, it is preferably to use experienced and skilful rat surgeons, instead of novice ones, when making *in vivo* studies like this.

The investigator's dream would be to use healthy conscious and freely moving animals. However, this is impossible and therefore, one needs to strive mimicking the normal physiological state as much as possible. One such step may be to pre-treat animals undergoing extensive abdominal surgery with parecoxib.

4.1 CONCLUSIONS

Luminal hypertonicity induced a delayed 9.3 fold increase in the mucosal permeability in COX-2 inhibited animals but did not affect this parameter in controls or in those animals that did not respond to parecoxib with duodenal contractions. Whether this increase in permeability reflects disturbance of mucosal integrity or a physiological mechanism is not known and therefore warrants further research. Further, luminal hypertonicity inhibited parecoxib-induced duodenal motility and as a consequence of this, reduced duodenal mucosal bicarbonate secretion. In contrast, luminal hypertonicity increased duodenal mucosal bicarbonate secretion in control animals and in those that did not respond to parecoxib with duodenal contractions. This effect is most probably due to a solvent drag phenomenon since the concentration of bicarbonate in the net fluid secreted was about 24 mM. Taken together, the duodenal responses to luminal hypertonicity varied considerable between controls and COX-2 inhibited animals with contractions while the responses obtained in controls and COX-2 inhibited animals without contractions were very similar.

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