Transepithelial ion transport in the stomach of pigs exposed to gastric ulcer conditions

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APPLICATION FOR PRODUCERS

By increasing our understanding of gastric ulcers, ongoing research in this area will help in developing effectic diagnosic, prevention and management strategies.

SUMMARY

Gastric ulcers in pigs increase significantly when stressful events occur. However, the exact causes of these gastric ulcerations remain unclear. Gastric acid production is known to be influenced by anionic secretion. An Ussing chamber study was conducted to evaluate the agonist-induced electrogenic secretory responses in the different sections of the stomach of pigs exposed to gastric ulcers. Changes in short-circuit current (Isc) in pigs euthanized 48h after an out-of-feed event significantly decreased following the activation of secretion via the adrenergic agonist in the cardia, and the cholinergic agonist in the pars and pylorus. There were no significant secretory responses in the other segments. Since bumetanide failed to inhibit the basolateral cotransporter 1 (NKCC1), the changes in the stimulatory Isc could be attributed to bicarbonate secretion.

"Gastric ulcers are accountable for about 1 to 2% of mortality among market hogs. "

INTRODUCTION

Gastric ulcers are prevalent in modern, intensive pig production, posing challenges to growing pigs and resulting in financial losses. Gastric ulcers are accountable for about 1 to 2% of mortality among market hogs. Most factors associated with gastric ulcers are linked to an increase in gastric acid secretions in the stomach or a decrease in the capacity of the mucus to provide protection.

Gastric acid secretion, mainly hydrochloric acid, is secreted from gastric parietal cells located in the stomach and helps in food digestion and controls harmful bacterial pathogens. The disruption (activation or inhibition) of gastric secretion in the stomach has been suggested to cause gastric ulcers.

This study, therefore, aimed to investigate electrogenic secretory response in the 4 regions of the pig stomach to determine its contribution to gastric secretion and ulcers when pigs are fed diets containing air-classified pea starch (ACPS) diets with an out-of-feed event.

We hypothesized that pigs fed 40% ACPS diets and exposed to an out-of-feed event would have increased electrogenic secretory response.

EXPERIMENTAL PROCEDURES

Pigs were fed diets with 40% air-classified pea starch (particle size 10-20 µm) and exposed to an out-of-feed event, both recognized as potential factors contributing to gastric ulcer formation.

Sixteen pigs (initial BW of 90.6±2.2 kg), were fed for 14d and divided into 4 treatment groups (control, 24h, 48h, 72h). The control group had feed throughout the trial period. For the treatment groups, feed was removed on d13 at 7 am and reintroduced on d14 at 7 am. After the "out-of-feed" event, feed was reintroduced and pigs were selected and euthanized after 24h, 48h and 72h to make up the 3 treatment groups. All animals were stunned with a captive bolt shot and stomach tissue was then harvested immediately after slaughtering for Ussing chamber studies. The stomach was opened by an incision along the greater curvature, rinsed with chilled (4°C) Krebs Ringer Bicarbonate buffer (pH 7.4), and transported immediately to the laboratory.

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Upon arrival in the laboratory, the four main segments; pars oesophagea, cardia, fundus and pylorus, had the muscle layers removed (stripped) from the peritoneum with forceps. Twelve Ussing chambers (Figure 1) were available for each animal with 3 chambers per section. Pieces of the stripped segments were mounted on 1.5 cm^2 Ussing chamber inserts and inserted into the Ussing chamber. Each chamber contained 5 ml Krebs Ringer Bicarbonate buffer solution on each side. Transepithelial potential differences were short-circuited to 0 mV 3M KCl salt bridges connected to Ag-AgCl electrodes. The ends of the salt bridges were located near the tissue on both the mucosal and serosal sides.

Figure 1. Ussing chamber systems set up in the lab.

The mounted tissues were allowed to equilibrate with the buffer for about 20 minutes before the addition of drugs. The resistance and tissue viability were determined using a 1mV pulse every 30 seconds. The baseline short-circuit current (Isc) and potential difference (PD) values were measured after equilibration. The resistance was also calculated using Ohm's law.

After equilibration and when a steady state was reached, an adrenergic agonist, isoproterenol (10 µM), was added to the serosal side to increase cyclic AMP (cAMP) and stimulate cAMP-dependent channels (such as CFTR; cystic fibrosis transmembrane conductance regulator), followed by the addition of 100 µM carbachol, a cholinergic agonist, to the serosal side of the tissue after a steady state has been reached. After a steady state had been reached, 10 µM forskolin and 1mM of 1M 3-isobutyl-1-methylxanthine (IBMX) were added to the mucosal and serosal sides of the tissue. This was aimed to cause an irreversible and sustained elevation in cAMP to fully activate cAMP-activated secretion. When a steady state was reached, 0.1 mM bumetanide was added to the serosal side to inhibit the serosal Na+/K+/2Cl- co-transporter 1 (NKCC1). Electrophysiological responses were expressed as the differences (ΔIsc) between the initial and minimal or maximal Isc values with the addition of an activator or inhibitor, respectively.

RESULTS AND DISCUSSION

Changes in short-circuit current (Isc) in pigs euthanized 48h after the out-of-feed event significantly increased to 17.9±2.02 µA/cm2 from 4.8±1.6 µA/cm2 in the control group following the activation of secretion via the adrenergic agonist (isoproterenol) in the cardia (Fig. 2) suggesting an increase in the anionic transport through CFTR.

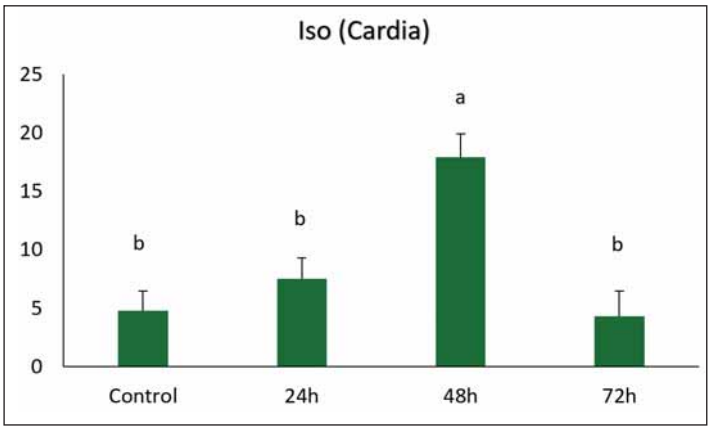

Figure 2. Changes in Isc response in the cardia to the addition of ion channel activator, isoproterenol (P<0.05).

Following the inclusion of carbachol, there was an increase in calcium-activated Isc cholinergic activation in the pars (Fig. 3) and pylorus (Fig. 4) tissues and a tendency to increase in the cardia (Fig. 5) of the 48h group of pigs.

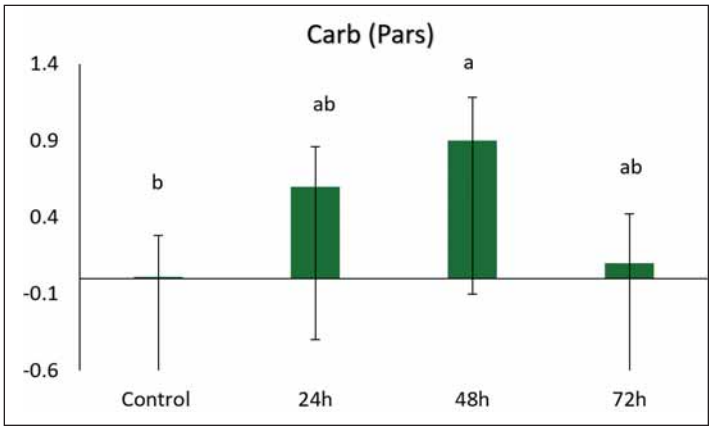

Figure 3. Changes in Isc response in the pars oesophagea to the addition of ion channel activator, carbachol (P<0.05).

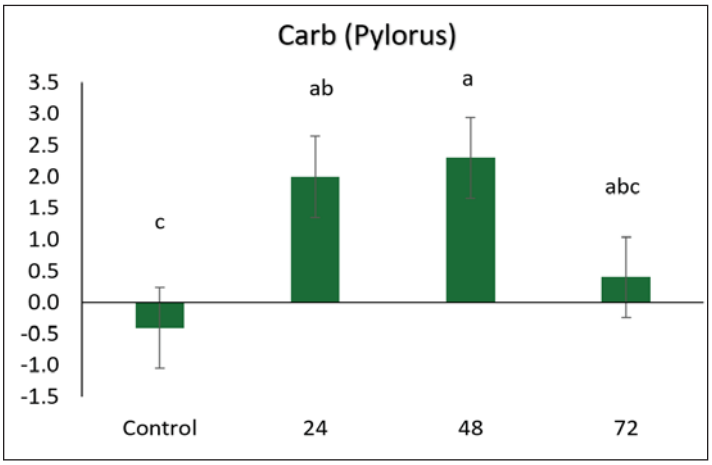

Figure 4. Changes in Isc response in the pylorus to the addition of ion channel activator, carbachol (P=0.58).

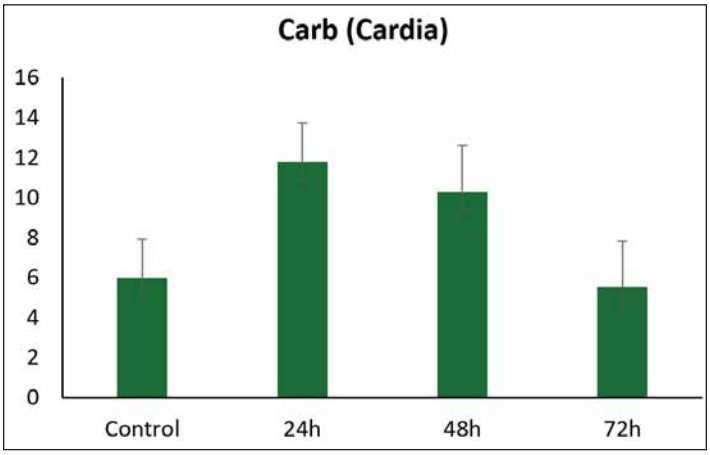

Figure 5. Changes in Isc response in the cardia to the addition of ion channel activator, carbachol (P=0.09).

Carbachol binds to both nicotinic and muscarinic acetylcholine receptors, which leads to an intracellular influx of Ca2+. This triggers a rapid activation of calcium-activated ion channels. No significant Isc changes were detected when bumetanide, an inhibitor of NKCC1 (a basolateral cotransporter) was added to the pars and cardia (Fig. 6). This suggested that the anionic secretion could be linked to bicarbonate (HCO3-) since CFTR is also a bicarbonate channel.

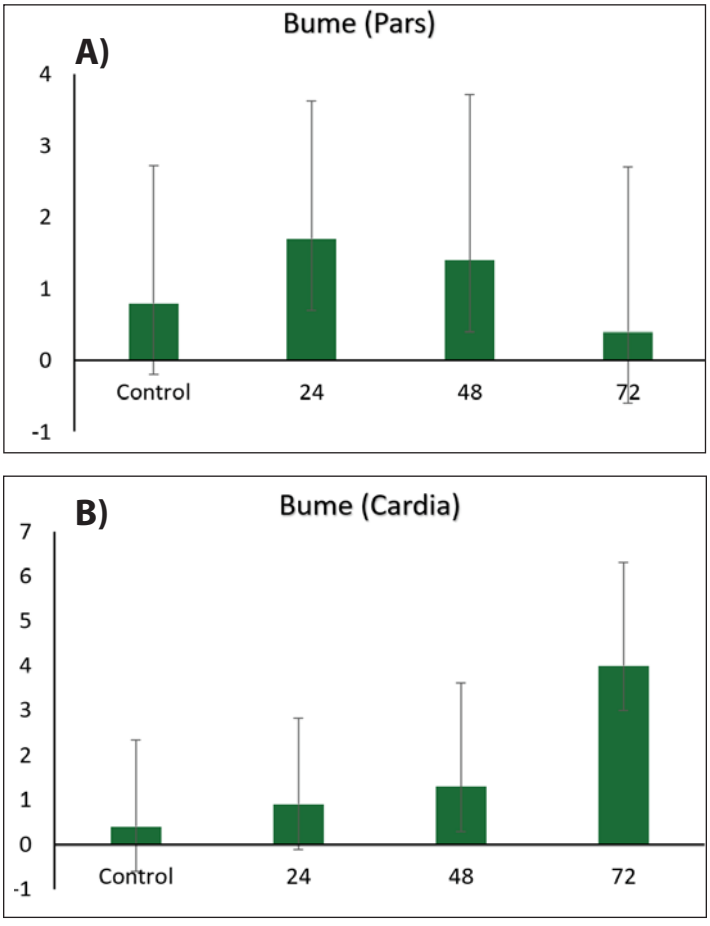

Figure 6. Changes in Isc response in the pars oesophagea (A) and cardia (B) to the addition of NKCC1 inhibitor bumetanide (P>0.10).

There were no significant secretory responses in the other segments and other pharmacological drugs added. Additionally, there were no significant differences between the control (no out-of-feed event) and the 24h and 72h (after out-of-feed event) in any of the parameters measured. Secretory responses were not compared between segments.

IMPLICATIONS

We demonstrated that 48 h after an out-of-feed event a significant increase in electrogenic anionic secretory response occurs in pigs fed with 40% ACPS diets. We also report for the first time that during gastric ulcer conditions, the pars oesophagea becomes an actively secreting tissue which sheds light on the complex mechanisms involved in the pathophysiology of gastric ulcers and may have implications for the diagnosis and treatment of this condition.

ACKNOWLEDGEMENTS

Financial support for this project was provided by the Government of Saskatchewan. The authors would also like to acknowledge the strategic program funding to Prairie Swine Centre provided by Sask Pork, Alberta Pork, Ontario Pork, the Manitoba Pork Council, and the Saskatchewan Agriculture Development Fund. In addition, we wish to acknowledge the support of the production and research technicians at Prairie Swine Centre that make it possible to conduct this research.