Smooth muscle electromyographic measurements with pigs in a stress model

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Abstract: Stress adversely affects physiological processes in animals, including gastrointestinal motility, potentially reducing digestion efficiency. Electromyography (EMG) is a suitable method for monitoring smooth muscle activity, allowing separate measurements of the heart and digestive organs (stomach, small intestine, large intestine). This study aimed to investigate whether stress alters the smooth muscle activity of the digestive tract using electromyographic measurements. We conducted the experiment on Topigs x Duroc barrows (n = 4), weighing 30 kg. Stress induction was performed with a single intravenous ACTH injection (10 µg/kg), which dose corresponds to a mild stress effect. Electromyographic signals were continuously recorded for 8 hours both in control and stress conditions. The measurements were divided into 30-minute intervals, and the smooth muscle electric potential (mV) was determined for each organ. Statistical comparisons between control and stress-induced states were made using paired Student's t-test. The results revealed a post-stress reduction in smooth muscle activity across all organs. The decrease was statistically significant in the small and large intestine, while in the stomach, reduced activity was observed 5 hours after stress induction. In conclusion, even mild stress decreases the smooth muscle activity of digestive organs, reducing gastrointestinal motility and potentially impairing digestion efficiency.

Keywords: pig, electromyography, smooth muscle, stress

Introduction

Under intensive production conditions, pigs are subjected to both prolonged and more intense short-term stress, which can affect their overall well-being. High levels of stress and reduced welfare negatively impact key factors in pig farming, including animal performance, reproductive capacity, immunity, and meat quality (Smulders et al., 2006). Animal welfare laws are becoming increasingly strict, primarily due to public concerns and criticism of animal husbandry, but scientific experiments conducted on farm animals and methodology applied to collect data are also under discussion. This opens new areas of scientific research, such as incorporation of non-invasive sampling methods and techniques and their validation in scientific experiments (Sciascia and Metges, 2023).

Non-invasive methods for measuring muscle activity are collectively referred to as electromyography (EMG). Among these, electrocardiography (ECG) is used to monitor cardiac activity, while electrogastrography (EGG) is suitable for examine the gastrointestinal tract (Fonyó, 2011). The smooth muscle tissues that make up the gastrointestinal organs have their own pacemaker cells, the interstitial cells of Cajal. These cells play a key role in the generation and propagation of the electrical signal during the contraction of the smooth muscles of the gastrointestinal organs. Interstitial cells of Cajal generate slow wave electrical impulses, which can be measured by the electrical and mechanical activity of the smooth muscle cells, and the frequency value of the waves generated can also be determined (Sanders et al., 2006). Based on this frequency value, with the appropriate settings the holter device measures only the signals of the smooth muscles, not the striated muscles,

Electromyographic measurements have a long history in human studies. The first electromyographic measurements started in the 1920's (Alvarez, 1922). The method was first applied with humans' stomach activity measurements, in the 1960's continued with the gastrointestinal tract's motility measurements. In lot of pharmacological preclinical studies, the electromyographic and electro-gastrographic measurements were used on model animals, mostly on rats or pigs (Szűcs et al., 2018, Bures et al., 2013, Bures et al., 2020). The adaptation of the electromyographic measurements for pigs has been completed, with the first studies published by Nagy et al. (2021). Based on the experimental series, it was concluded that the method is suitable for examining smooth muscle tissue activity in the gastrointestinal tract (stomach, small and large intestine) in awake growing pigs.

The number of publications is very low reporting the gastrointestinal segment's motility of the animals during stress. In rats, it has been confirmed that stress induces alterations in the motility of the stomach and small intestine, as well as in the electrical impulses generated in the smooth muscle tissue of the gastrointestinal tract (Pribék et al., 2021). According to our knowledge, there has been no previous study conducted with electromyographic or electrogastrographic measurements in a stress model with pigs. Therefore, the aim of the experiment was to investigate whether adrenocorticotropic hormone (ACTH) induced, standardized stress modifies the smooth muscle activity in different segments of the digestive tract, and if this can be confirmed by electromyographic measurements.

Materials and Methods

Animals, housing and preparation

The experiment was conducted in December 2023, at Hungarian University of Agriculture and Life Sciences, Department of Farm Animal Nutrition. To the experiment we involved 4 growing barrows (Topigs x Duroc, 30 kg). The animals were kept individually in a concrete floored stall and fed two times a day at 8 a.m. and 3 p.m., access to drinking water was ad libitum.

In order to be able to monitor the gastrointestinal motility during non-stress condition and ACTH induced stress, the animals were carefully prepared. For a period of one month, we got the animals used to human presence and handling, and to wear a vest that holds the wires and the holter necessary for electromyographic measurements.

Stress model

A self-control study was designed, each animal was its own control. To perform this, we measured the animals for two days, the first day was the control day without stress, while on stressed day the animals got ACTH injection (Synacthen 0.25 mg/1 ml) through permanent vein catheter intravenously at a dose of 10 µg per kilogram of body weight. By that, the stress induction was standardized.

Electromyographic measurements

For electromyographic measurements we used the data collecting holter device from MSB-Met Kft., which is capable of gathering data using a wire connected to two electrodes placed on the surface of the skin. After shaving and cleaning the required area, two self-adhesive electrodes were applied to the skin. One electrode on the left side was placed near to the heart, right behind the front left leg. The other electrode on the right side was placed on the fold of skin connecting the pig's thigh and lower abdomen.

The measurements lasted for 9 hours from which 8 hours were analysed.

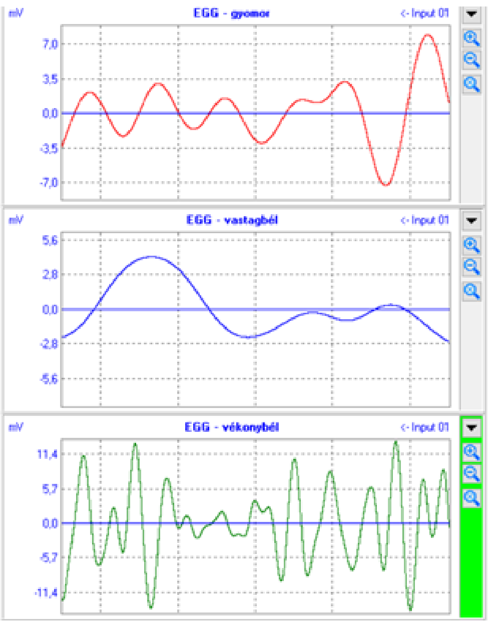

Figure 1 Example of wave graphs of stomach (gyomor), small intestine (vékonybél), large intestine (vastagbél) in the EasyChart softver

For data analysis we used the EasyChart software. The measured 8 hours were divided into 30 minute intervals. From the 8 hours long analysis we assigned the two main intervals: between the timepoint of the ACTH injection and 2 hours after, while the other interval was around the feeding, 30 minutes before feeding and one hour after.

For data analysis, the baseline was set to zero in the software, and then, using average cycle amplitude analysis, we obtained the potential of the smooth muscle tissue in millivolts for the selected organ over the specified 30-minute intervals.

Statistical analysis

Statistical analysis was performed with paired Student's t-test, comparing data of 30 minute intervals of the control day with data of the stress induction's day of each animal's own.

Results and discussion

Results of the smooth muscle electric potential changes in different segments of the gastrointestinal tract right after the stress induction compared to the pairwise periods in non-stressed day are shown in Figure 2.

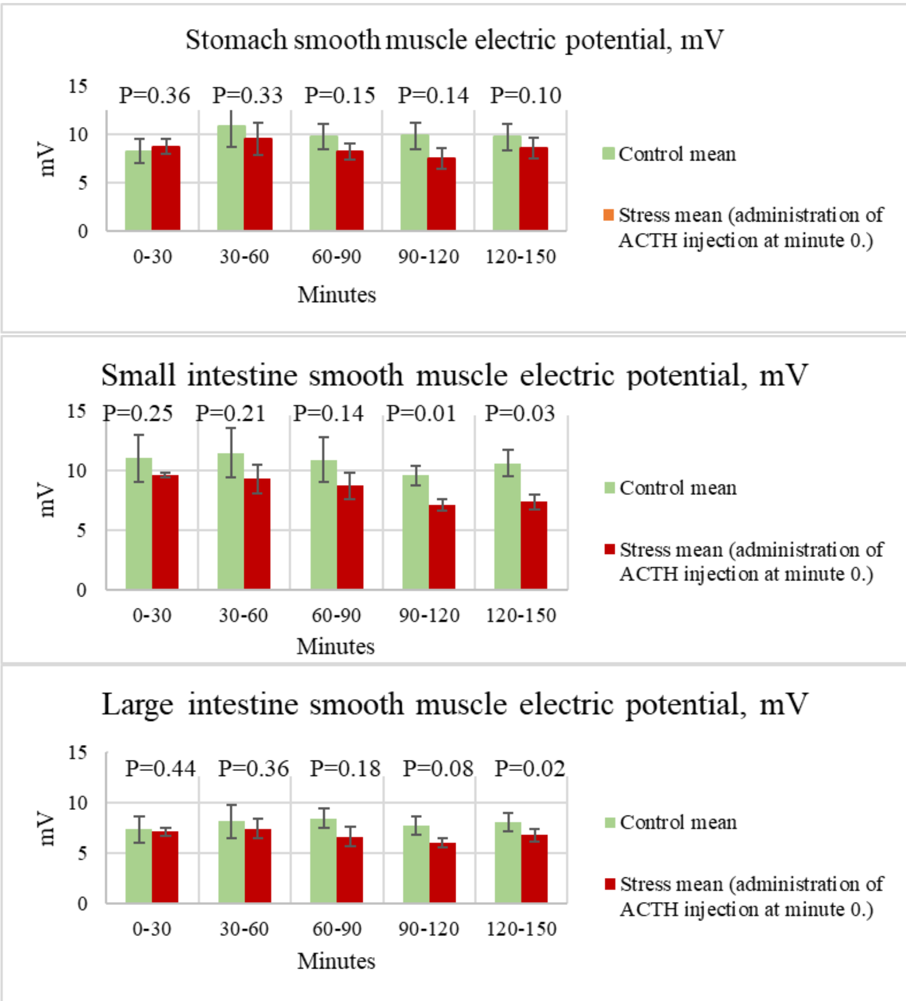

Figure 2 Means with standard errors of stomach, small intestine, and large intestine smooth muscle electric potential in millivolts in each 30 minutes intervals post stress, n = 4

In the stomach the smooth muscle activity was statistically the same in non-stressed and stressed day right after the ACTH injection, and slightly reduced between 120–150 minutes post-stress ($P = 0.10$). In small intestine between 90 and 150 minutes the electric potential of smooth muscle was lower in stress day compared to the non-stress day ($P < 0.05$). The smooth muscle activity in large intestine tended to be lower on stressed day between 90 and 120 minutes ($P = 0.08$) and was significantly lower between 120 and 150 minutes post stress induction ($P < 0.05$). We checked the activity of smooth muscle of GIT around the feeding time as well, which was approximately 4 hours post-stress. Data of stress induced day was compared to the pairwise periods in non-stressed day (Figure 3). The smooth muscle activity in stomach was significantly lower ($P < 0.01$) on stress induced day between the 60th and 90th minutes after feeding. It was not the case in small intestine where the difference was just numerical but not confirmed by statistics ($P > 0.10$) around the feeding. In large intestine the smooth muscle activity tended to be lower between 30 minutes before the feeding timepoint and 30 minutes after ($P = 0.09$).

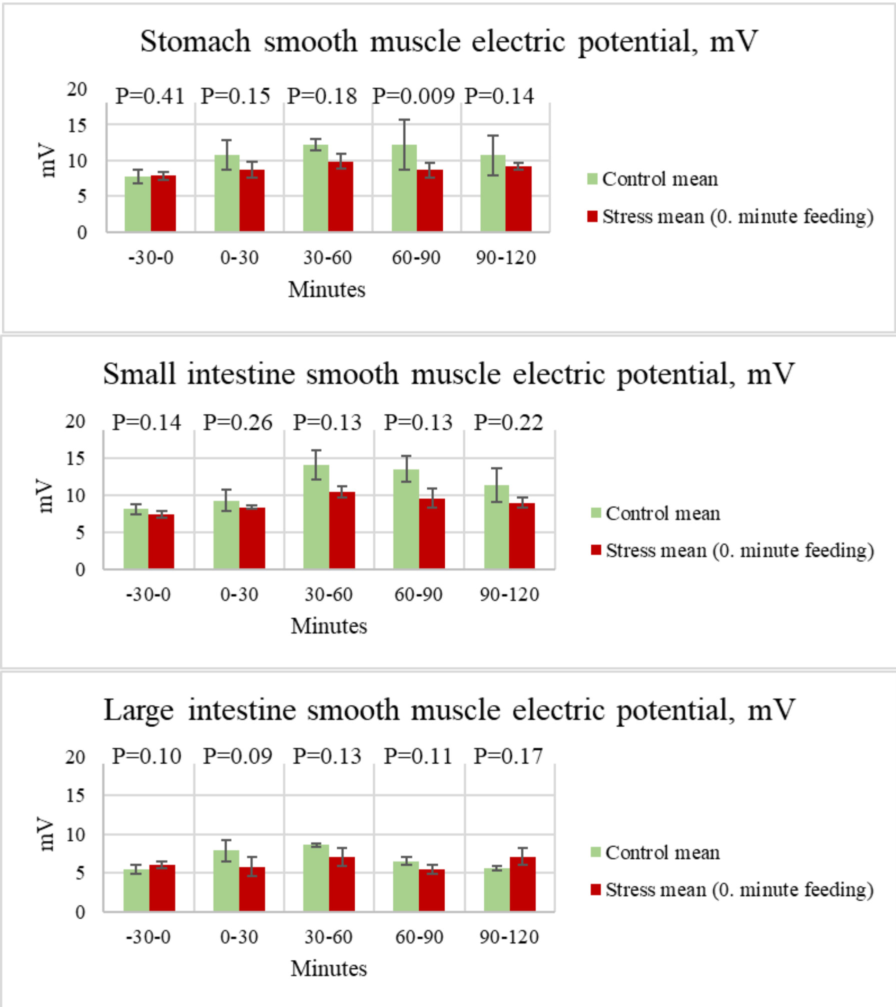

Figure 3 Means with standard errors of stomach, small intestine, and large intestine smooth muscle electric potential in millivolts 30 minutes before feeding and 120 minutes after feeding, n = 4

Conclusions

Based on the experiment's results it can be concluded that electromyographic measurements can be used in a stress model on awake pigs and the suppressed activity of the digestive organs can be measured with it. It has been confirmed that even mild stress reduces the smooth muscle activity of digestive organs, thereby decreasing the gastrointestinal motility, which in turn could modify the digestion processes.

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References

Alvarez, W. C. (1922): The electrogastrogram and what it shows. Journal of American Medical Association, 78, 1116–1118. http://doi.org/10.1001/jama.1922.02640680020008

Bures, J., Kvetina, J., Radochova, V., Tacheci, I., Peterova, E., Herman, D., Dolezal, R., Kopacova, M., Rejchrt, S., Douda, T., Sestak, V., Douda, L., Karasova, J. Z. (2020): The pharmacokinetic parameters and the effect of a single and repeated doses of memantine on gastric myoelectric activity in experimental pigs. Plos One. 15(1), e0227781. https://doi.org/10.1371/journal.pone.0227781.

Bures, J., Kvetina, J., Pavlik, M., Kunes, M., Kopacova, M., Rejchrt, S., Jun, D., Hrabinova, M., Kuca, K., Tachecí, I. (2013): Impact of paraoxon followed by acetylcholinesterase reactivator HI-6 on gastric myoelectric activity in experimental pigs. Neuroendocrinology Letters, 34(Suppl 2), 79–83. 24362097

Fonyó, A. (2011): Az orvosi élettan tankönyve. Medicina Könyvkiadó Zrt. 708 p.

Martens, B. M. J., Noorloos, M., de Vries, S., Schols, H. A., Bruininx, E. M. A. M., Gerrits, W. J. J. (2019): Whole digesta properties as influenced by feed processing explain variation in gastrointestinal transit times in pigs. The British Journal of Nutrition, 122(11), 1242–1254. https://doi.org/10.1017/S0007114519002198

Nagy, K., Fébel, H., Bazar, G., Grosz, G., Gáspár, R., Szűcs, F. K., Tóth, T. (2021): Non-invasive smooth muscle electromyography (SMEMG) as a novel monitoring technology of the gastrointestinal tract of awake, free-moving pigs-A pilot study. PloS One, 16(9), e0257311. https://doi.org/10.1371/journal.pone.0257311

Pribék, I. K., Szűcs, K. F., Süle, M., Grosz, G., Ducza, E., Vigh, D., Tóth, E., Janka, Z., Kálmán, J., Datki, Z. L., Gáspár, R., Andó, B. (2021): Detection of acute stress by smooth muscle electromyography: A translational study on rat and human. Life sciences, 277, 119492. https://doi.org/10.1016/j.lfs.2021.119492

Sanders, K. M., Koh, S. D., Ward, S.M. (2006): Interstitial cells of cajal as pacemakers in the gastrointestinal tract. Annual review of physiology, 68, 307–343. https://doi.org/10.1146/annurev.physiol.68.040504.094718

Sciascia, Q. L., Metges, C. C. (2023): Review: Methods and biomarkers to investigate intestinal function and health in pigs. Animal : an international journal of animal bioscience, 17(Suppl 3), 100860. https://doi.org/10.1016/j.animal.2023.100860

Smulders, D., Verbeke, G., Mormède, P., Geers, R. (2006): Validation of a behavioral observation tool to assess pig welfare. Physiology & Behavior, 89(3), 438–447. https://doi.org/10.1016/j.physbeh.2006.07.002

Szűcs, K.F., Grosz, G., Süle, M., Sztojkov-Ivanov, A., Ducza, E., Márki, A., Kothencz, A., Balogh, L., Gáspár, R. (2018): Detection of stress and the effects of central nervous system depressants by gastrointestinal smooth muscle electromyography in wakeful rats. Life sciences, 205, 1–8. https://doi.org/10.1016/j.lfs.2018.05.015

Wilfart, A., Montagne, L., Simmins, H., Noblet, J., Van Milgen, J. (2007): Effect of fibre content in the diet on the mean retention time in different segments of the digestive tract in growing pigs. Livestock Science, 109, 27–29. https://doi.org/10.1016/j.livsci.2007.01.032