

## Use of Electrogastrography in Preclinical Studies of Cholinergic and Anticholinergic Agents in Experimental Pigs

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### Summary

Electrogastrography (EGG) is a non-invasive method for the assessment of gastric myoelectrical activity. Porcine EGG is comparable with human one. The purpose of this study was to evaluate the effect of atropine and neostigmine on the EGG in experimental pigs. Adult female pigs were administrated atropine (1.5 mg i.m., n=6) and neostigmine (0.5 mg i.m., n=6) after the baseline EGG, followed by a 90-min trial recording (MMS, Enschede, the Netherlands). Running spectral analysis was used for the evaluation. The results were expressed as dominant frequency of slow waves and EGG power (areas of amplitudes). Neostigmine increased continuously the dominant frequency and decreased significantly the EGG power. Atropine did not change the dominant frequency significantly. However, atropine increased significantly the EGG power (areas of amplitudes) from basal values to the maximum at the 10-20-min interval. After that period, the areas of amplitudes decreased significantly to the lowest values at the 60-90-min interval. In conclusion, cholinergic and anticholinergic agents affect differently EGG in experimental pigs.

### Key words

Electrogastrography • Experimental pigs • Atropine • Neostigmine

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### Introduction

Surface electrogastrography (EGG) is a non-invasive method for clinical assessment of gastric myoelectrical activity (Chen 1994, Jackson *et al.* 2000, Parkman *et al.* 2003, Koch and Stern 2004, Bureš *et al.* 2007, 2008). Our group has demonstrated that EGG is also reliable and feasible in experimental pigs (Varayil *et al.* 2009, Květina *et al.* 2010, Tachecí *et al.* 2013). Porcine EGG is fully comparable with that recorded in healthy humans (Varayil *et al.* 2009, Tachecí *et al.* 2013). Previously, we studied EGG in experimental pigs under different conditions, like general anesthesia (Tachecí *et al.* 2013), volume challenge (Tachecí *et al.* 2014), atropine administration (Bureš *et al.* 2014a) or the effect of different prokinetics (Varayil *et al.* 2009, Tachecí *et al.* 2011, Douda *et al.* 2014). Other EGG studies in experimental setting (in pigs or dogs) are quite exceptional so far (Mintchev *et al.* 1993, Koenig *et al.* 2009, O'Grady *et al.* 2009).

Our current research has been focused on pharmacokinetics and gastrointestinal motor effects of novel acetylcholinesterase modulators in experimental pigs (Bureš *et al.* 2013, 2014b, Kuneš *et al.* 2014, Žďárová Karasová *et al.* 2013). The aim of this study was

to evaluate the impact of basic cholinergic and anticholinergic agents on porcine EGG in a standardized protocol.

## Material and Methods

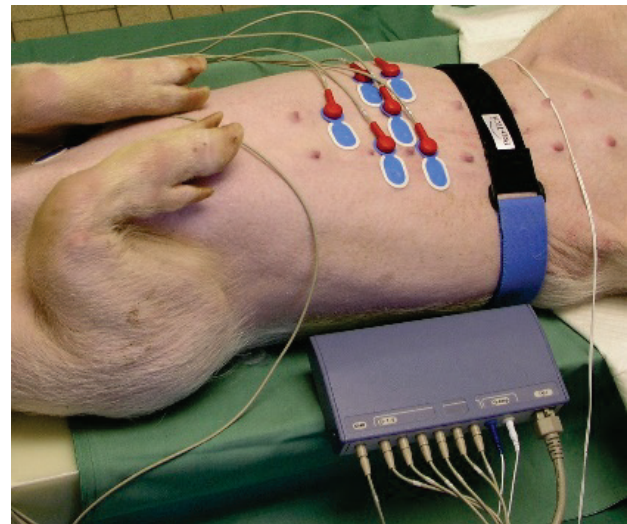
### Animals and study design

Six experimental mature female pigs (*Sus scrofa f. domestica*, hybrids of Czech White and Landrace breeds; 3-4-months old; mean weight  $31.2 \pm 2.1$ , median 30.9 kg) entered the study twice. All pigs were consecutively given atropine and neostigmine, always after a one-week washout period. Animals were fed twice a day (standard assorted food A1) and were allowed free access to water. All EGG recordings were performed under general anesthesia in the morning after 24 h of fasting. Intramuscular injections of ketamine (20 mg per kg; Narkamon, Spofa, Praha, Czech Republic) and azaperone (2.2 mg per kg; Stresnil, Janssen Animal Health, Saunderton, UK) were used as an introduction. General anesthesia was carried out by isoflurane (Flurane, Abbott, Queenborough, UK) that was delivered by mask: inhalation 2 % isoflurane in medicinal oxygen (2 liters per min). A 10-min baseline EGG was recorded 20 min after general anesthesia started. After the baseline period animals were administrated atropine (1.5 mg i.m.; *Atropini sulfas monohydricus*; Biotika Bohemia) and after a one-week washout period they were administrated neostigmine (0.5 mg i.m.; *Neostigmini metilsulfas*; Hoechst). After the baseline, EGG followed by a 90-min trial recording in both groups.

### Electrogastrography

We used our own methods of porcine EGG described elsewhere (Tacheci *et al.* 2013). Briefly, six active self-adhesive high-quality electrodes were placed on the upper part of the abdomen, the 7th electrode (basal) was placed left of the middle sternum (Fig. 1). Electrodes were arranged for mutual bipolar recording. A special abdominal belt (respiratory sensor) was used to identify possible artefacts due to breathing and body movements (see Fig. 1). Surface cutaneous EGG was recorded using an Electrogastrography Stand Alone System (MMS – Medical Measurement Systems B.V., Enschede, the Netherlands). This highly sophisticated device has appropriate amplifiers and filters so that it is able to execute, secure and process the 50 to 500  $\mu$ V EGG signal that ranges from 1 to 15 cycles per min (cpm). The EGG recording is filtered digitally to remove

unwanted frequencies such as 0.5-cpm ultraslow pattern and respiratory or cardiac rhythms. MMS software (version 8.19) was used to assess EGG recordings. Running spectral analysis was used for the evaluation (Koch and Stern 2004). Results were expressed as dominant frequency of slow waves of EGG recordings. EGG power was assessed as areas of amplitudes. Individual one-min intervals were used for all evaluation.



**Fig. 1.** Detailed view of the placement of 7 electrodes and a special abdominal belt (functioning as a respiratory sensor).

### Statistical analysis

The data were analyzed using SigmaStat software (Version 3.1, Jandel Corp., Erkrath, Germany). Standard normal distribution of data was assessed first. Particular EGG parameters before and after the drug administration were tested. Number of one-min EGG intervals was different in particular groups that is why non-paired t-test was tried. If normality testing failed, non-parametric Mann-Whitney rank sum test continued.

### Ethics

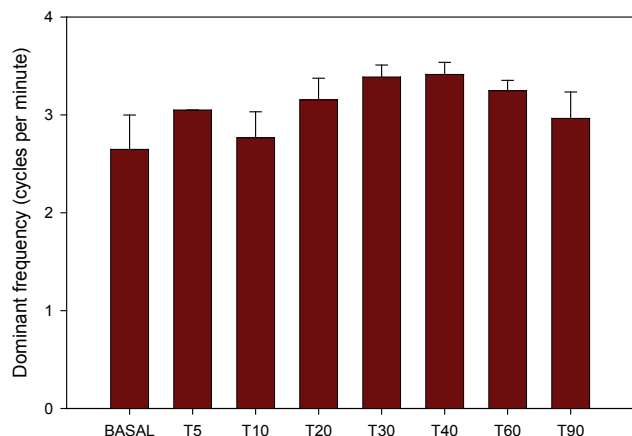
The Project was approved by the Institutional Review Board of the Animal Care Committee of the University of Defence, Faculty of Military Health Services, Hradec Kralove, Czech Republic, Protocol Number 14/12 (2012). Animals were held and treated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe, 2009).

## Results

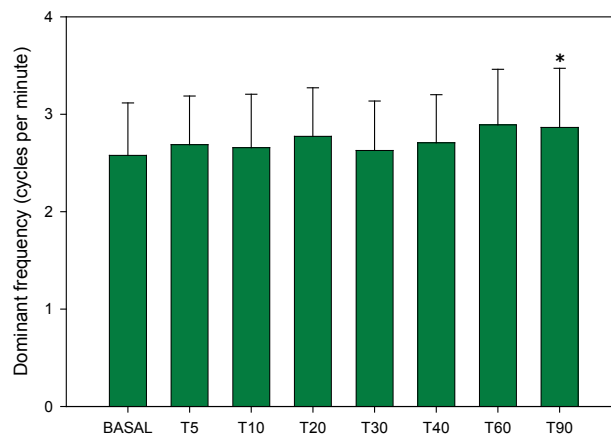
Basic results are shown in Figures 2-5.

Neostigmine increased continuously the dominant frequency from basal EGG  $2.58 \pm 0.54$  cpm up to  $2.86 \pm 0.61$  at the 60-90-min interval ( $p < 0.001$ ). Neostigmine decreased significantly the EGG power ( $594.3 \pm 853.8 \mu\text{V}^2$  at the 5-10-min interval,  $p = 0.036$ , throughout to the 60-90-min interval,  $117.6 \pm 157.5 \mu\text{V}^2$ ;  $p < 0.001$ ). Atropine did not change the dominant

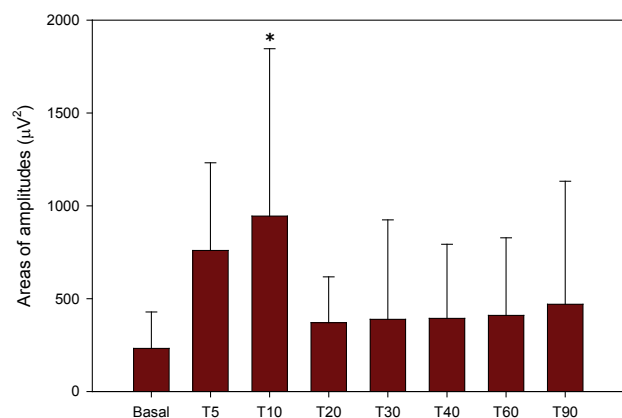
frequency significantly. However, atropine increased significantly the EGG power (areas of amplitudes) from basal values ( $232.5 \pm 195.9$ ) to the maximum at the 10-20-min interval ( $945.0 \pm 901.2 \mu\text{V}^2$ ;  $p < 0.001$ ). After that period, the areas of amplitudes decreased significantly to the values of  $409.8 \pm 417.9 \mu\text{V}^2$  at the 60-90-min interval ( $p = 0.001$ ).



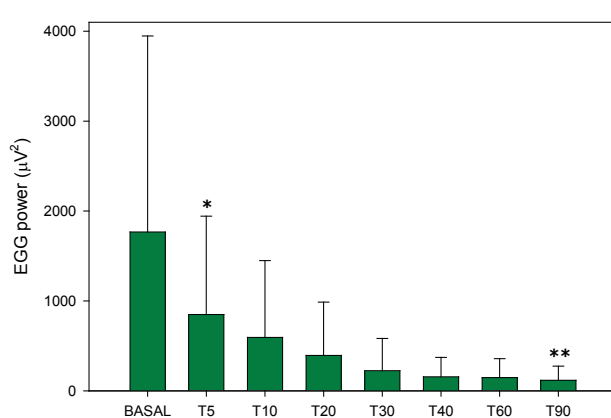
**Fig. 2.** Effect of atropine on dominant frequency in porcine electrogastronomy. Basal: a 10-min basal EGG recording before i.m. administration of atropine. T5: EGG recording at time interval between the 1<sup>st</sup>-5<sup>th</sup> min after i.m. administration of atropine; T10: interval 6-10 min; T20: 11-20 min; T30: 21-30 min; T40: 31-40 min; T60: 41-60 min; T90: 61-90 min



**Fig. 3.** Effect of neostigmine on dominant frequency in porcine electrogastronomy. Basal: a 10-min basal EGG recording before i.m. administration of neostigmine. T5: EGG recording at time interval between the 1<sup>st</sup>-5<sup>th</sup> min after i.m. administration of neostigmine; T10: interval 6-10 min; T20: 11-20 min; T30: 21-30 min; T40: 31-40 min; T60: 41-60 min; T90: 61-90 min. \*  $p < 0.001$



**Fig. 4.** Effect of atropine on EGG power (areas of amplitudes) in porcine electrogastronomy. Basal: a 10-min basal EGG recording before i.m. administration of atropine. T5: EGG recording at time interval between the 1<sup>st</sup>-5<sup>th</sup> min after i.m. administration of atropine; T10: interval 6-10 min; T20: 11-20 min; T30: 21-30 min; T40: 31-40 min; T60: 41-60 min; T90: 61-90 min. \*  $p < 0.001$



**Fig. 5.** Effect of neostigmine on EGG power (areas of amplitudes) in porcine electrogastronomy. Basal: a 10-min basal EGG recording before i.m. administration of neostigmine. T5: EGG recording at time interval between the 1<sup>st</sup>-5<sup>th</sup> min after i.m. administration of neostigmine; T10: interval 6-10 min; T20: 11-20 min; T30: 21-30 min; T40: 31-40 min; T60: 41-60 min; T90: 61-90 min. \*  $p = 0.036$ , \*\*  $p < 0.001$

## Discussion

Our current study brought new important data on the impact of basic cholinergic and anticholinergic agents on porcine EGG. Pigs can be used in various preclinical

experiments as an omnivorous representative due to their relatively very similar gastrointestinal functions compared to humans (Kararli 1995, Suenderhauf and Parrott 2013). Neostigmine is a parasympathomimetic that acts as a reversible acetylcholinesterase inhibitor. By

interfering with the breakdown of acetylcholine, neostigmine indirectly stimulates both nicotinic and muscarinic receptors. Its half-life in humans is about 50-90 min (Neostigmine Drug Information, 2015). Atropine, an anticholinergic agent, is a competitive antagonist for the muscarinic acetylcholine receptors. Its half-life in humans is about 2 h (Atropine Drug Information, 2015). However, very little is still known about the effect of cholinergic and anticholinergic agents on myoelectrical activity of the stomach. Kaneko *et al.* (1995) found that both vagal and non-vagal cholinergic activity influenced postprandial EGG (mostly amplitudes) in healthy volunteers. Katoh *et al.* (2003) studied the effect of glucagon and scopolamine butylbromide. The peak power amplitudes significantly decreased and dominant frequency increased in both groups. Parkman *et al.* (1999) studied low doses of atropine and bethanechol in humans. Ten healthy adult volunteers received intravenous bolus of 0.6 mg atropine and then a 15-min low-dose i.v. infusion (by rate 0.25 mg/h). In that setting, atropine caused a slight increase in gastric myoelectrical activity by EGG. Bethanechol slightly increased the amplitude, but slightly decreased the frequency of gastric myoelectrical activity by EGG (Parkman *et al.* 1999). However, EGG recording lasted only 15 min in the Parkman's study. Authors did not mention body weight of volunteers. If we assume that mean body weight was about 75 kg, they used 5-times lower doses than we did.

We have shown different early impact of atropine and neostigmine. Atropine produced a significant initial increase of the EGG power (with maximum at 10 min after i.m. administration) with a subsequent gradual decrease. Neostigmine caused a significant continuous decrease of EGG power compared to the basic recording. This different effect illustrates the possible vagal and non-vagal cholinergic impact in the control of gastric myoelectric activity in experimental pigs. It has been known that cholinergic stimulation increases slow wave frequency (Koch and

Stern 2004). Kim *et al.* (2003) studied muscarinic regulation of pacemaker frequency in murine gastric interstitial cells of Cajal and they found that acetylcholine increased the frequency of slow waves in gastric muscles. High concentrations of carbachol may block the entrainment of pacemaker currents (Kim *et al.* 2003). Neostigmine, as an indirect stimulator of nicotinic and muscarinic receptors, increased significantly the dominant frequency in our current study, but still within normal range, while atropine did not reveal any significant effect in this aspect. Nevertheless, we are aware of possible drawbacks of the study and that is why it is necessary to interpret our results with cautions. There is a great inter-individual variability of EGG in particular pigs. To reduce this impact and to minimize possible bias we related all trial parameters to basal values before the study drugs administration. Large amount of data acquired from one-minute intervals allowed reliable statistical analysis. We applied a standardized protocol using the same isoflurane general anesthesia. Thus our results could be considered as credible.

## Conclusions

Both cholinergic and anticholinergic agents affect differently EGG in experimental pigs. These basic data are mandatory for the proper future evaluation of preclinical gastrointestinal motor effects of novel acetylcholinesterase modulators in experimental pigs.

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

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