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ORIGINAL ARTICLE

Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs

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Abstract

OBJECTIVES: Tacrine was the first acetylcholinesterase inhibitor approved for therapy of Alzheimer's disease. It has currently been withdrawn in some countries mostly due to the risk of hepatotoxicity and might be replaced by its derivate 7-methoxytacrine (7-MEOTA). The aim of this study was to assess the impact of these two compounds on gastric myoelectrical activity by means of surface cutaneous electrogastrography (EGG). **METHODS:** Twelve pigs (*Sus scrofa f. domestica*, weighing 30–35 kg) entered the study. A single dose of tacrine (200 mg i.m., n=6) or 7-MEOTA (200 mg i.m., n=6) was administrated. All EGG recordings were performed under general anaesthesia in the morning after 24 hours of fasting. Basal (30 minutes) and study recordings (150 minutes) were accomplished using an EGG stand (MMS, Enschede, the Netherlands). Results were expressed as dominant frequency of gastric slow waves, power analysis (areas of amplitudes) and power ratio assessment (ratio of the areas of amplitudes after and before study drug administration). **RESULTS:** Tacrine decreased EGG dominant frequency 10 minutes after its administration (from basal 3.1±0.6 to 2.8±0.6 cycles per minute; p=0.014). Tacrine induced a non-significant 60-minute increase of the power (with maximal value 493±533 μV^2 at 20 minutes) and power ratio (with maximal value 2.04±3.4 at 10 minutes). Tacrine caused substantial gastric arrhythmia. 7-MEOTA did not influence dominant frequency of gastric slow waves significantly. 7-MEOTA caused a short-term late increase of the power ratio at 60 minutes (6.3±11.2; p=0.003). Blood cholinesterase activity did not correlate with any EGG parameter either after tacrine or 7-MEOTA at any time. **CONCLUSIONS:** Tacrine and 7-MEOTA have different impacts on EGG. Tacrine decreased dominant frequency and induced long-lasting gastric arrhythmia. 7-MEOTA caused a short-term late increase of the EGG power in experimental pigs.

Abbreviations

7-MEOTA	- 7-methoxytacrine (7-methoxy-tetrahydroaminoacridine)
EGG	- electrogastrography
i.m.	- intramuscular
NMR	- nuclear magnetic resonance
p.o.	- peroral

INTRODUCTION

Tacrine (9-amino-1,2,3,4-tetrahydroacridine) was the first acetylcholinesterase inhibitor approved for therapy of Alzheimer's disease. The beneficial action of tacrine seen in some patients suffering from Alzheimer's disease is probably due to its complex action. Besides inhibiting acetylcholinesterase and butyrylcholinesterase, which has been reported to compensate cholinergic deficit, tacrine exerts effects on other cholinergic structures, ion channels, and the monoaminergic systems (reviewed by Soukup *et al.* 2013). Likely, 7-MEOTA (7-methoxy-tetrahydroaminoacridine) is pharmacologically in the same way as tacrine, but less toxic (reviewed by Soukup *et al.* 2013). Tacrine has dose limiting side effects, including diarrhoea, nausea, vomiting and abdominal discomfort. It has currently been withdrawn in some countries mostly due to the risk of hepatotoxicity (O'Hara *et al.* 2000; Patocka *et al.* 2008; Press *et al.* 2015) and might be replaced by its derivate 7-MEOTA as a single compound (Bajgar *et al.* 1995; Pohanka *et al.* 2008; Korabecny *et al.* 2010; Soukup *et al.* 2013) or together with donezepil (Korabecny *et al.* 2014; Sepsova *et al.* 2015). To the best of our knowledge, no study was published so far to explain the myoelectrical basis of possible nausea and vomiting associated with tacrine administration.

Surface electrogastrography (EGG) is a non-invasive method for the functional assessment of gastric myoelectrical activity (Chen *et al.* 1994; Parkman *et al.* 2003; Koch *et al.* 2004, Bureš *et al.* 2008; Jonderko *et al.* 2014). Our group has demonstrated that EGG is also reliable and feasible in experimental pigs (Tacheci *et al.* 2011; Tacheci *et al.* 2013; Bures *et al.* 2014). Porcine EGG is fully comparable with that recorded in healthy humans, i.e. its basic myoelectrical pattern, dominant frequency and amplitudes (Varayil *et al.* 2009; Tacheci *et al.* 2013).

Our current research has been focused on pharmacokinetics and gastrointestinal motor effects of novel acetylcholinesterase modulators in experimental pigs (Bures *et al.* 2013, 2014, Kuneš *et al.* 2014; Zdarova Karasova *et al.* 2013).

The aim of this study was to assess the impact of tacrine and 7-MEOTA on gastric myoelectrical activity by means of surface cutaneous electrogastrography (EGG) in fasting experimental pigs.

MATERIAL AND METHODS

Animals

Twelve experimental mature female pigs (*Sus scrofa f. domestica*, hybrids of Czech White and Landrace breeds; 3–4-month old; weighing 30–35 kg, mean 33.8±1.8 kg) entered the study. Animals were fed twice a day (standard assorted food A1) and were allowed free access to water. All EGG recordings were performed under general anaesthesia in the morning after 24 hours of fasting. Intramuscular injections of 5% 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 anaesthesia was carried out by isoflurane (Flurane, Abbott, Queenborough, UK) that was delivered by mask: inhalation 2% isoflurane in medicinal oxygen (2 litres per minute).

Drugs

A single dose of tacrine (200 mg, n=6) or 7-MEOTA (200 mg, n=6) was administrated i.m. (right leg). Tacrine (tetrahydroaminacrine hydrochloride hydrate) was purchased from Sigma-Aldrich (St. Louis, MO, USA), 7-MEOTA was synthesised at the Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Hradec Králové (Czech Republic) and its purity (>99%) was characterised using melting point and NMR spectra (Varian Mercury VX BB 300 or Varian S500, Varian Comp. Palo Alto, USA).

Cholinesterase activity measurement

Cholinesterase (acetylcholinesterase and butyrylcholinesterase) activity was determined in whole blood. Acetylcholine iodide was added as a substrate and liberated acetic acid was titrated with sodium hydroxide using an automatic titrator in potentiostatic mode. All chemicals used in this determination were of analytical purity and were purchased from Sigma-Aldrich spol. s r.o. (Prague, Czech Republic). Whole blood (1 mL) was added to the solution of sodium chloride (0.0154 mol.L^{-1}; 9 mL) in deionized water. Acetylcholine iodide (1.5 mol.L^{-1}; 0.010 mL) was added as a substrate and liberated acetic acid was titrated with 0.01 mol.L^{-1} sodium hydroxide using an automatic titrator (Titrando 842, Metrohm, Czech Republic) in the pH-stat mode (pH 7.4) at 37 °C under nitrogen atmosphere. The slope of the linear part of the time dependence of used sodium hydroxide represents the activity of cholinesterases (erythrocyte acetylcholinesterase and plasma butyrylcholinesterase) in whole blood. Calculations were performed using software Microsoft Excel 2007 (Redmont, WA, USA) and GraphPad Prism version 5.02 for Windows, GraphPad Software, San Diego, CA, USA (www.graphpad.com) (Hrabinova *et al.* 2006; Musilek *et al.* 2007; Kuča *et al.* 2013).

Electrogastrography

Our own methods of porcine EGG was used, described elsewhere (Tachecí *et al.* 2013). Briefly, six active self-adhesive electrodes were placed on the upper part of the abdomen, the 7th electrode (basal) was placed left of

the middle sternum. A special abdominal belt (respiratory sensor) was used to identify possible artefacts due to breathing and body movements. Surface cutaneous EGG was recorded using an Electrogastrography Stand Alone System (MMS – Medical Measurement Systems B.V., Enschede, the Netherlands). Basal (30 minutes) and study recordings (150 minutes) were accomplished. MMS software (version 8.19) was used to assess EGG recordings. Running spectral analysis based on Fourier transform was used. Results were expressed as dominant frequency of gastric slow waves, power analysis (areas of amplitudes) and power ratio assessment (ratio of the areas of amplitudes after and before study drug administration).

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).

Statistical analysis

The data were analysed using SigmaStat software (Version 3.1, Jandel Corp., Erkrath, Germany). Descriptive statistics, Fisher's exact test, unpaired t-test, Mann-Whitney rank sum test and Pearson product moment correlation test were used.

RESULTS

Tacrine decreased EGG dominant frequency 10 minutes after its administration (from basal 3.1±0.6 to 2.8±0.6 cycles per minute; p=0.014). Tacrine induced a non-significant 60-minute increase of the power (with maximal value 493±533 μV^2 at 20 minutes) and power ratio (with maximal value 2.0±3.4 at 10 minutes). Tacrine caused substantial gastric arrhythmia. 7-MEOTA did not influence dominant frequency of gastric slow waves significantly. 7-MEOTA caused a non-significant short-term late increase of the power (from basal 618.3±747.3 to 2540.2±6130.3 μV^2 at 90 minutes) and a significant increase of the power ratio at 60 minutes (6.3±11.2; p=0.003), see Figures 1–6 for details.

Maximal inhibition of blood cholinesterase activity was recorded after 10 minutes, being about 50% stronger after administration of tacrine compared to 7-MEOTA (Figures 7 and 8). Blood cholinesterase activity did not correlate with any EGG parameter either after tacrine or 7-MEOTA at any time (data not displayed).

DISCUSSION

To the best of our knowledge, this is the first study evaluating the effect of tacrine and 7-MEOTA on EGG. So that it was not possible to compare our results with other human or experimental studies. Recommended therapeutical doses of tacrine in humans are 10 mg orally four-times per day at the beginning (for

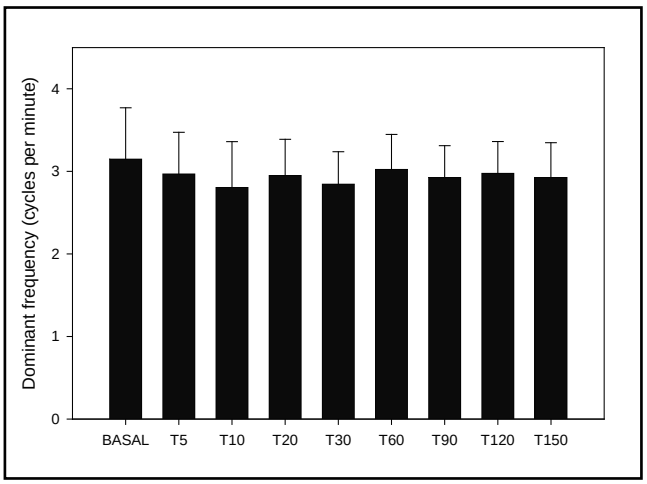

Fig. 1. Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs. Porcine electrogastrography. Dominant frequency before and after a single dose of tacrine (200 mg i.m.), mean ± standard deviation. BASAL: a 30-minute basal EGG recording before i.m. administration of tacrine. T5: EGG recording at time interval between the 1st–5th minute after i.m. administration of tacrine; T10: interval 6–10 min.; T20: 11–20 min.; T30: 21–30 min.; T40: 31–40 min.; T60: 41–60 min.; T90: interval 61–90 min.; T120: interval 91–120 min.; T150: interval 121–150 min. after i.m. administration of tacrine.

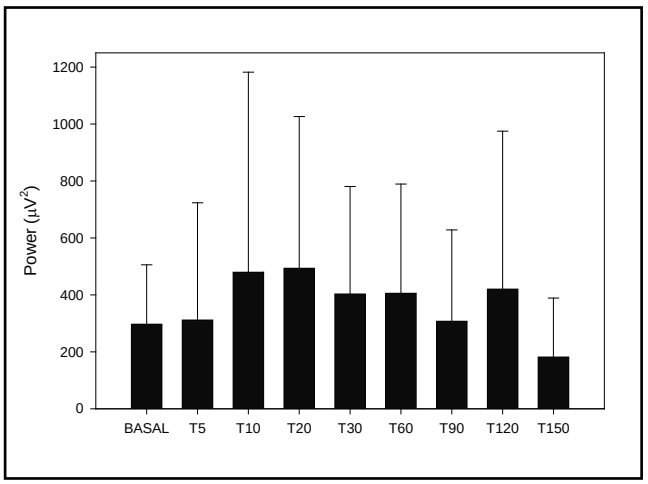

Fig. 2. Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs. Porcine electrogastrography. Power (areas of amplitudes) before and after a single dose of tacrine (200 mg i.m.), mean ± standard deviation. BASAL: a 30-minute basal EGG recording before i.m. administration of tacrine. T5: EGG recording at time interval between the 1st - 5th minute after i.m. administration of tacrine; T10: interval 6–10 min.; T20: 11–20 min.; T30: 21–30 min.; T40: 31–40 min.; T60: 41–60 min.; T90: interval 61–90 min.; T120: interval 91–120 min.; T150: interval 121–150 min. after i.m. administration of tacrine.

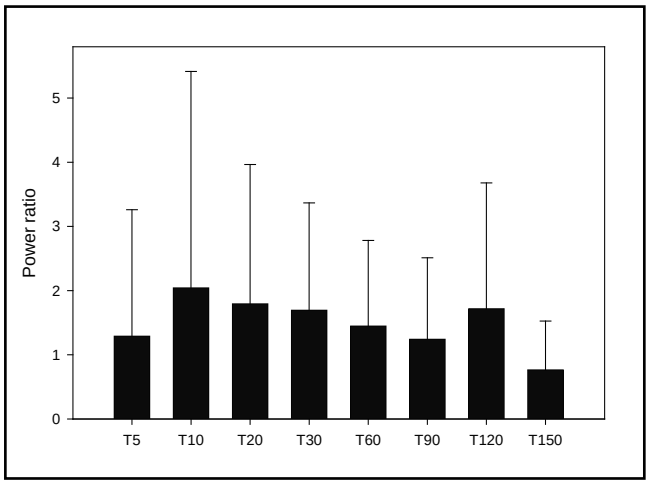

Fig. 3. Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs. Porcine electrogastrography. Power ratio (fraction of the areas of amplitudes after and before a single dose of tacrine (200 mg i.m.), mean ± standard deviation. T5: power at time interval between the 1st–5th minute after i.m. administration of tacrine divided by basal power; T10: the same fraction at interval 6–10 min.; T20: 11–20 min.; T30: 21–30 min.; T40: 31–40 min.; T60: 41–60 min.; T90: interval 61–90 min.; T120: interval 91–120 min.; T150: the same fraction at interval 121–150 min.

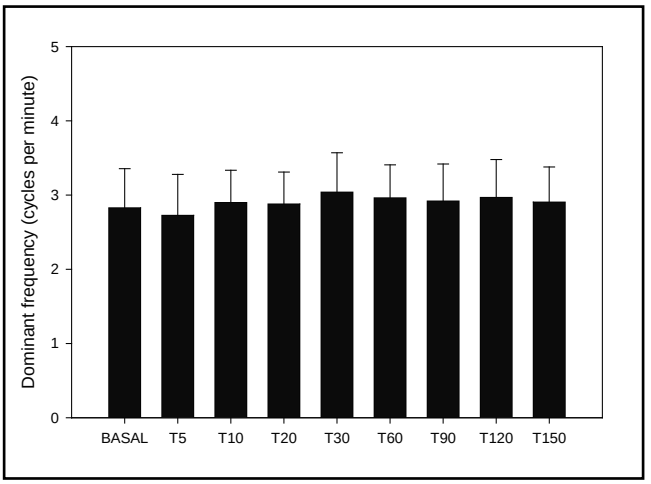

Fig. 4. Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs. Porcine electrogastrography. Dominant frequency before and after a single dose of 7-MEOTA (200 mg i.m.), mean ± standard deviation. BASAL: a 30-minute basal EGG recording before i.m. administration of 7-MEOTA. T5: EGG recording at time interval between the 1st–5th minute after i.m. administration of 7-MEOTA; T10: interval 6–10 min.; T20: 11–20 min.; T30: 21–30 min.; T40: 31–40 min.; T60: 41–60 min.; T90: interval 61–90 min.; T120: interval 91– 120 min.; T150: interval 121–150 min. after i.m. administration of 7-MEOTA.

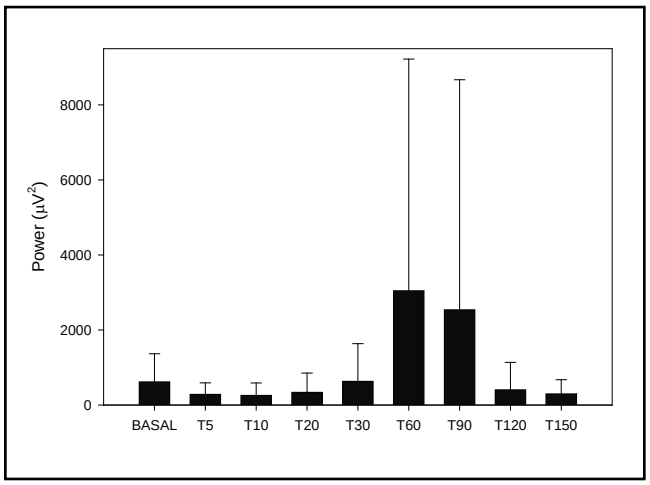

Fig. 5. Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs. Porcine electrogastrography. Power (areas of amplitudes) before and after a single dose of 7-MEOTA (200 mg i.m.), mean ± standard deviation. BASAL: a 30-minute basal EGG recording before i.m. administration of 7-MEOTA. T5: EGG recording at time interval between the 1st–5th minute after i.m. administration of 7-MEOTA; T10: interval 6–10 min.; T20: 11–20 min.; T30: 21–30 min.; T40: 31–40 min.; T60: 41–60 min.; T90: interval 61–90 min.; T120: interval 91–120 min.; T150: interval 121–150 min. after i.m. administration of 7-MEOTA.

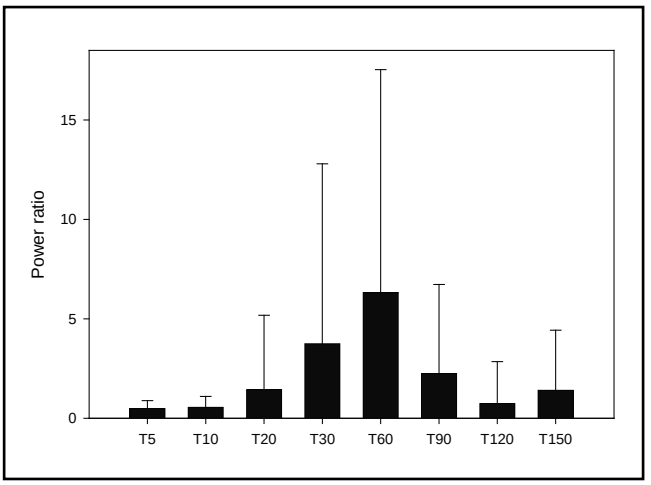

Fig. 6. Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs. Porcine electrogastrography. Power ratio (fraction of the areas of amplitudes after and before a single dose of 7-MEOTA (200 mg i.m.), mean ± standard deviation. T5: power at time interval between the 1st–5th minute after i.m. administration of 7-MEOTA divided by basal power; T10: the same fraction at interval 6–10 min.; T20: 11–20 min.; T30: 21–30 min.; T40: 31–40 min.; T60: 41–60 min.; T90: interval 61–90 min.; T120: interval 91–120 min.; T150: the same fraction at interval 121–150 min.

six weeks) with gradual increase up to 160 mg per day orally. 7-MEOTA was tested in 48 young healthy volunteers in a single-dose pharmacokinetic study with either peroral or intramuscular administration. The dose of 7-MEOTA was 2, 4 or 8 $mg.kg^{-1}$ body weight p.o. or 0.5, 1 or 2 $mg.kg^{-1}$ body weight i.m. Cholinergic adverse effect was seen in two healthy volunteers (4%) with blood concentration exceeding 1,500 $\mu g.L^{-1}$ (Filip *et al.* 1991).

Following oral administration of tacrine the drug is rapidly and well absorbed with peak plasma concentrations (Cmax) achieved within 1/2 to 3 hours (after a

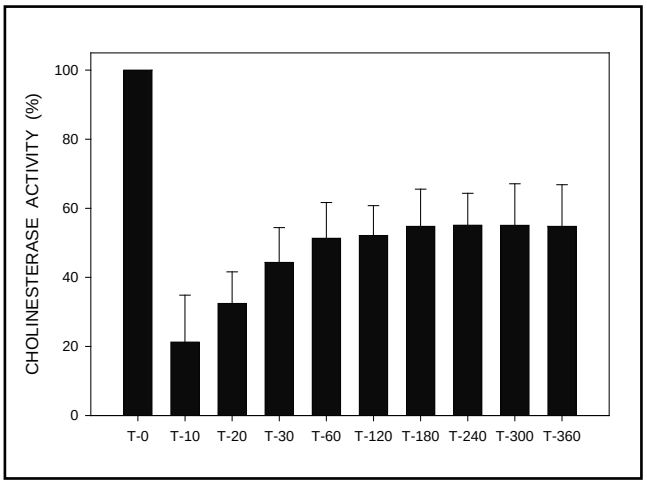

Fig. 7. Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs. Inhibition of blood cholinesterase activity before (T-0; 100%) and after administration of tacrine (200 mg i.m.), mean ± standard deviation. T-10: 1st–10th minute interval after the administration; through up to T-360: interval between the 301st–360th minute after the administration Significant difference between T-0 and T-10, T-20 or T-30 (p=0.020).

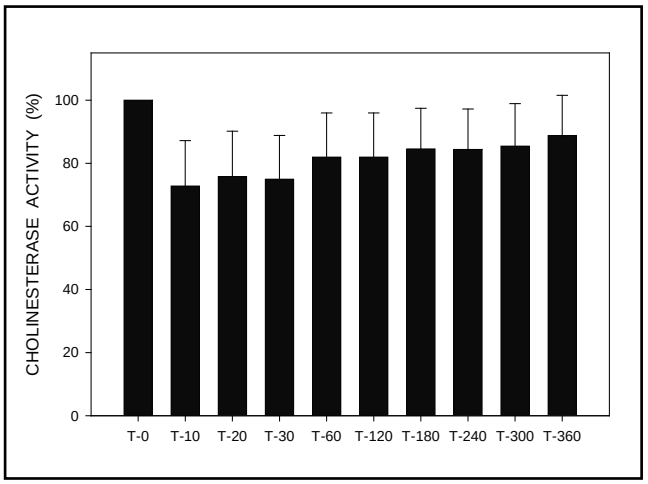

Fig. 8. Impact of tacrine and 7-methoxytacrine on gastric myoelectrical activity assessed using electrogastrography in experimental pigs. Inhibition of blood cholinesterase activity before (T-0; 100%) and after administration of 7-MEOTA (200 mg i.m.), mean ± standard deviation. T-10: 1st–10th minute interval after the administration; through up to T-360: interval between the 301st– 360th minute after the administration. Significant difference between T-0 and T-10, T-20 or T-30 (p=0.020).

single dose of 20 to 50 mg). The elimination half-life of tacrine was short, 1.5 to 2.5 hours after single oral and intravenous doses and 2.9 to 3.6 hours after multiple oral doses (Madden *et al.* 1995). In 7-MEOTA, its Tmax were 4 hours (after peroral use) and 1 hour (after i.m. administration), the half-time (T1/2) was 8.7±3.9 hours and 6.5±5.8 hours in case of peroral and intramuscular administration, respectively (Filip *et al.* 1991).

In an *in vitro* study, 7-MEOTA acted as a stronger inhibitor of acetylcholinesterase compared to tacrine (Pohanka *et al.* 2008). We were not able to confirm these *in vitro* results in our porcine study. Maximal inhibition of blood cholinesterase activity was recorded 10 minutes after study drugs administration, being about 50% stronger after administration of tacrine compared to 7-MEOTA.

Tacrine and 7-MEOTA surprisingly revealed different mode of gastric myoelectric action. Tacrine displayed more harmful impact on myoelectric activity, causing long-lasting gastric arrhythmia. We can hypothesise that this action is mediated by hydroxylated metabolites of tacrine, similarly as in its hepatic toxicity (Patocka *et al.* 2008). 7-MEOTA, probably due to the different metabolism (Soukup *et al.* 2013), does not exert this side effect. In our current study, 7-MEOTA, unlike tacrine, caused a significant late increase of the power ratio at 60 minutes. Different pharmacokinetics, interaction with muscarinic and nicotinic receptors and possible impact on the brain-stomach neurohumoral axis might, at least partly, explain this interesting difference in the mode of gastric action.

We are fully aware of possible limits of our study. First of all, it was difficult to choose the optimal dose of study drugs. As there is no linear toxicity, it was not possible to set isotoxic or even isoeffective amount. We decided same dosage of 200 mg as both compounds have comparable molecular weight and lipophilicity. Body weight of experimental animals was comparable in both groups, too. The decided amounts were derived from upper dose limits recommended for tacrine and upper doses of 7-MEOTA tested in healthy volunteers previously (Filip *et al.* 1991). There is a great interindividual variability of tacrine and 7-MEOTA pharmacokinetics and their cholinesterase inhibition (Filip *et al.* 1991; Madden *et al.* 1995; Goh *et al.* 2011; Soukup *et al.* 2013). We did not measure serum concentrations of drugs, they were evaluated indirectly according to the overall decrease of total cholinesterase activity. The measurement included fall in both acetylcholinesterase and butyrylcholinesterase activity. Nevertheless using the same method, different impact of tacrine and 7-MEOTA on gastric myoelectric activity is evident. Blood cholinesterase activity did not correlate with any EGG parameter either after tacrine or 7-MEOTA at any time. To minimise possible bias and to reduce the impact of interindividual variability of experimental pigs we calculated power ratio as a fraction of the areas of amplitudes after and before study drug administration. Thus our results could be considered as consistent.

Our original method of EGG enables to evaluate one-minute intervals of all recordings of gastric myoelectric activity. In this particular study, 30 basal and 150 study-recording intervals were assessed in all animals, so that detailed analysis was possible. Results of our previous porcine EGG studies were corresponding to human ones, e.g. effect of erythromycin (Douda *et*

al. 2014), atropine (Bures *et al.* 2015) or volume challenge (Tacheci *et al.* 2014). So that our current results on the myoelectric effect of tacrine and 7-MEOTA can be considered as relevant.

CONCLUSIONS

Tacrine and 7-MEOTA have different impacts on EGG. Tacrine decreased dominant frequency and induced long-lasting gastric arrhythmia. 7-MEOTA caused a short-term late increase of the EGG power in experimental pigs.

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