Neuroendocrinology

Neuroendocrinology , DOI: 10.1159/000522627

Received: August 31, 2021

Accepted: February 14, 2022

Published online: February 16, 2022

An In Vitro Study on the Contractility of Epileptic Myometrium and Effects of Antiepileptic Agents on Oxytocin-Induced Contractions of Myometrium Isolated from Absence Epileptic WAG/Rij Rats

Kurt A, Salihoglu AK, Ayar A

ISSN: 0028-3835 (Print), eISSN: 1423-0194 (Online)

https://www.karger.com/NEN

Neuroendocrinology

Disclaimer:

Accepted, unedited article not yet assigned to an issue. The statements, opinions and data contained in this publication are solely those of the individual authors and contributors and not of the publisher and the editor(s). The publisher and the editor(s) disclaim responsibility for any injury to persons or property resulting from any ideas, methods, instructions or products referred to the content.

Copyright:

© 2022 S. Karger AG, Basel

Accepted Manuscript

An *In Vitro* Study on the Contractility of Epileptic Myometrium and Effects of Antiepileptic Agents on Oxytocin-Induced Contractions of Myometrium Isolated from Absence Epileptic WAG/Rij Rats

Aysegul KURT, Arif Kamil SALIHOGLU & Ahmet AYAR

Department of Physiology, Faculty of Medicine, Karadeniz Technical University, Turkey

Running head: Antiepileptics Influence Uterine Contractions

Number of Tables: 2

Number of Figures: 2

Word count: 3181

Address for Correspondence

Prof. Dr. Ahmet AYAR, PhD, DVM

Karadeniz Technical University Medical Faculty,

Department of Physiology, TR61080 Trabzon, Turkey

E-mail: ayar@ktu.edu.tr

Tel: +90 462 377 77 07, +90 462 325 32 05

Mobile: +90 533 761 99 70

Accepted Manuscript

Abstract:

Background/Aims: To determine whether spontaneous and stimulated contractile activity of myometrium in epileptic rats is different from healthy ones and whether anti-epileptic drugs (AEDs) have any direct influence on myometrial contractility.

Methods: Myometrial strips from nonpregnant and pregnant adult epileptic WAG/Rij and Wistar rats were suspended in organ bath containing physiological salt solution (37°C and pH 7.4, aerated with 95% oxygen-5% CO_2), and isometric contractions were recorded. Effects of cumulative concentrations of selected AEDs including phenytoin, levetiracetam and valproic acid alone and in combination on oxytocin-induced contractions was examined.

Contractile parameters assessed included the area under curve (AUC), amplitude and frequency of contractions, evaluated by 10-minute periods. Data were analyzed using one-way ANOVA and Tukey HSD test.

Results: Spontaneous myometrial contractility and responses to oxytocin showed species difference. Compared with that of control Wistar rats, spontaneous contractions of myometrium from non-pregnant epileptic WAG/Rij rats was significantly higher and significantly lower in pregnant preparations. Upon stimulation with oxytocin

WAG/Rij myometrium showed significantly lower contractile response compared with preparations from healthy control Wistars ($p<0.01$). Phenytoin and valproate caused concentration-dependent significant attenuation ($P< 0.05$) of spontaneous and oxytocin-induced contractions of myometrium from WAG/Rij and Wistar rats, both non-pregnant and pregnant.

Conclusion: Myometrial smooth muscle from epileptic rats showed different spontaneous and oxytocin-induced contractility and AEDs showed contractile modulatory actions, phenytoin being the most and levetiracetam the least effective. Although *in vitro*, our findings may be of clinical implications with regard to obstetric complications in epileptics and use of AEDsduring pregnancy, and warrants further investigations.

Keywords: epilepsy, uterus, antiepileptic, contractility

Introduction:

Although epilepsy may pose increased risk of fertility problems □1□, it does not cause infertility. During pregnancy, majority of pregnant women have seizures that needs to be controlled by anti-epileptic drugs (AED) because of the potential consequences. So, there are concerns about risks of epilepsy and epilepsy medication during pregnancy. Seizures itself during pregnancy can cause adverse outcomes on both mother and fetus including placental abruption and preterm labor □1□.

Use of any drug potentially might influence uterine contractility, either stimulate or inhibit, and this may have maternal and foeto-neonatal complications ranging from relatively minor aggravations to serious emergencies including induction of abortion, delayed labor or atonic bleeding □2,3□. Some AEDs, such as phenytoin and phenobarbital presents serious concern. Valproate is contraindicated during pregnancy because of the increased risk of potential neurodevelopmental fetal abnormalities □4-6□.

Considering the risk and incidence of obstetrical complications, although not very high □4, 6,7□, in epileptic women at reproductive age and need of AED use during pregnancy □1,8□ the scope of this study involves testing two main hypothesizes: i) myometrial smooth muscle from epileptics might have different contractile properties and oxytocin sensitivity than healthy individuals, and ii) due to shared ion channel interaction between the uterine contractility and AED mechanism of action, AEDs might have influence on myometrial contractility, directly.

Methods

Animals and Tissue preparation

The protocols of the present study were reviewed and approved by the local Animal Research Ethics Committee

Tissue samples from adult female WAG/Rij rats (as a genetic model for absence epilepsy prone strain) and healthy Wistar albino rats (to serve as a control) were used in this study.

To obtain myometrial strips, non-pregnant and pregnant animals were decapitated, the uteri removed immediately and placed in a flask containing Krebs' solution of the following composition (in mmol/L): NaCl 118; KCl 4.7; $CaCl_2$ 2; $MgSO_4$ 1.2; KH2PO4 1.18; $NaHCO_3$ 15.8; EDTA 0.016; glucose 11.5. For modified Krebs solution, which was used for examining the mechanism of action of AEDs, NaCl was replaced with equimolar N -methyl- D -glucamine (NMDG) gluconate.

Tissue samples were rapidly transferred to the tissue bath containing Krebs solution and oxygenation supply. The excised uteri were carefully freed from the adjacent fat tissues and other extraneous structures and muscle strips, approximately 2.0 cm in length and 0.5 cm in width, were prepared in the direction of the longitudinal muscles for recording of isometric tension.

Accepted Manuscript

Recording of Isometric Contractions and Pharmacological Testing

Myometrium strips were suspended vertically in 20 ml organ baths filled with Krebs' solution and constantly bubbled with a mixture of 95% O2–5% CO2 to give pH 7.4 at 37°C. The lower end of the strip was fixed to a metal hook and the upper end was attached to an isometric force-displacement transducer (MAY; Commat, Ankara, Turkey); the transducer signals were amplified through an interface (MAY; Commat Lt) and data were captured on a personal computer.

The effects of cumulative concentrations of the selected antiepileptic agents including phenytoin, levetiracetam and valproate on the spontaneous and oxytocin-induced contractions of the myometrial strips were examined by 10-minute intervals. All agents were added to tissue bath cumulatively and remained in contact with the tissue thereafter. The pre-drug period (either spontaneous or oxytocin-induced contractile period) was recorded, so that in each strip the pre-drug period served as its own control. After the pharmacological protocol completed, possible mechanism contractile modifying effect of AEDs were assessed by application of high KCl (60 mM, to induce nonspecific membrane depolarization and asses involvement of this mechanism), Bay K8644 (a calcium ion channel agonist to asses involvement of calcium channel inhibition) or NMDG (substitution for extracellular non selective cations, asses involvement of non-selective cation channels in the obtained effect).

The effects of the selected AEDs on the oxytocin-induced contractions were quantified by changes in mean amplitude (initially calculated as mg), area under the contractile curve (AUC; the area under the tension trace for 10 min periods) and frequency (calculated as number of contractions/10 min) of the isometric tension recordings. All contractile activity parameters were averaged over 10 min intervals. Contractile activity (mean amplitude, frequency and AUC) during the control period was taken as 100%. Effects of each agent was analyzed and expressed as a percentage of control values (spontaneous or oxytocin-induced).

Drugs

The concentrations of AEDs tested was 10, 30 uM and 1 mM for phenytoin; 1, 3 and 10 mM for levetiracetam and 100, 300 uM, 1 mM and 3 mM for valproic acid, respectively. Each antiepileptic drug was dissolved in Krebs' solution and was added into the organ bath cumulatively, at concentrations indicated. Bay K8644 was obtained from Sigma and dissolved in dimethyl sulfoxide (DMSO).

Statistics

Data were analyzed statistically using SPSS for Windows (SPSS, Chicago, IL, USA). Graphs were prepared using Microcal Origin version 5.00 (Microcal Software, Northampton, MA, USA). Data were transformed by arcsine transformation to correct for the non-normal distribution of percentage data and, because uterine strip contractions were sampled over time and statistical significance was analyzed by one- or two-way analysis of variance (ANOVA), where appropriate. Post hoc pair-wise comparisons of means were performed by the Student–Newman–Keuls' method. $P < 0.05$ was considered significant.

Results:

Comparison of Contractility of Myometrium From Control Wistar and Epileptic WAG-Rij Rats

Under basal conditions, myometrial strips from WAG-Rij rats presented spontaneous contractile activity (stretch-induced) which were comparable with healthy controls in terms of contractile pattern (phasic cycles with comparable amplitudes). However, on quantification, there was significant difference between the spontaneous contractility of (measured in terms of mean AUC) myometrium from control Wistar and epileptic WAG-Rij rats, for both non-pregnant and pregnant animals. AUC of spontaneous contractions in control WAG-Rij rats was found to be significantly higher when compared to that of the control Wistar rats; whereas in myometrium strips from pregnant rats the mean AUC of contractions was significantly lower in WAG-Rij than control Wistars (Table 1). A summary of AUC of spontaneous and oxytocin induced contractility of myometria from healthy Wistar and epileptic WAG/Rij, both from non-pregnant and pregnant rats, and number of experiments and p values corresponding to these comparisons are presented in Table 1.

Next we tested the response of the myometrium from WAG-Rij rats to oxytocin, a well-known endogenous uterine contraction stimulant. The mean AUC of contractile response in isolated uterine strip from non-pregnant WAG-Rij rats was significantly higher (n=32) when compared to that of control Wistar animals (n=29); while

Accepted Manuscript

contractile response of pregnant myometrium from WAG-Rij animals was significantly lower (n=18) than that of the control Wistar rats (n=20, Table 1).

Effects of AEDs of Oxytocin-Induced Contractions of Myometrium from Control and Epileptic Rats

A full complement of comparisons among dose-dependent effects of each AEDs on contractile parameters, and dose-response curves for each effects are given in Table 2 & Fig 1.

Bath application of oxytocin stimulated contractions of myometrium from both non-pregnant control Wistar and epileptic WAG-Rij rats (no detailed statistical comparison is provided).

Cumulative application of both phenytoin and valproic acid caused concentration-dependent decrease in mean AUC of these oxytocin-induced contractions of control and epileptic rat myometrium (for the extend and statistical significance of effects of each concentrations of these agents refer to Table 1, Fig. 1, n=12). A representative original tracing showing stimulatory effects of oxytocin on frequency and amplitude of contractions and also the inhibitory effects of cumulatively applied different concentrations of phenytoin on myometrium isolated from pregnant WAG/Rij rats.

Conversely, the effect of levetiracetam on contractile parameters of myometrial strips showed dose-species and pregnant state-dependent variations, also variable effects on different contractile parameters was determined (for the extend and statistical significance of effects of each concentrations of these agents refer to Table 2, Fig. 1).

Some combinations of AED's were also tested on pregnant WAG-Rij myometrium. Combination of VPA (1 mM) with levetiracetam (3 mM) resulted virtually no change in normalized % mean amplitude (from 100% to 99.9±5%, P=0.9, n=7), modest increase in normalized % mean AUC (from 100% to 107.8±14%, P=0.3, n=7) and significant decrease in normalized % mean frequency (from 100% to 89.2±10%, P=0.9, n=7) of oxytocin-induced contractions of myometrium strips from non-pregnant WAG-Rij rats, respectively (Fig. 1).

Combination of VPA (1 mM) with phenytoin (30 μM) resulted decrease in normalized % mean amplitude (from 100% to 88.6±12%, P=0.05, n=7, which was just significant), significant decrease in normalized % mean AUC (from 100% to 68.4±17%, P=0.003, n=7) and highly significant decrease in normalized % mean frequency (from 100% to 63.7±12%, P=0.001, n=7) of oxytocin-induced contractions of myometrium strips from non-pregnant WAG-Rij rats, respectively (Fig. 2).

Modulation of Inhibitory Effects of Phenytoin on Myometrial Contractility by Calcium Channel Agonists Bay K864, replacement of Extracellular Na^+ with $NMDG^+$ and Membrane Depolarization by High K^+

DMSO, served as a solvent for BAYK 8644, had no significant effect on contractility (control AUC: 100±0 % vs, 92±16 % (n=6, P>0.95) of myometrium from non-pregnant Wistar rats.

Phenytoin significantly inhibited the AUC of oxytocin-induced contraction of non-pregnant Wistar rat (oxytocin: 100±00%, phenytoin: 47±9%, n=3, P<0.01), and subsequently applied Bay K8644 did not significantly alter the contractility in comparison with the preceding periods' mean AUC of contractions (BAY K8644: 51±13%, n=3, P>0.05 vs. phenytoin, 47±9%, P<0.03 vs. oxytocin).

When extracellular Na^+ was replaced with $NMDG^+$ oxytocin still stimulated, though not significantly, contractility in non-pregnant WAG-Rij uterus (mean AUC of spontaneous contractions: 100±00 %, oxytocin: 145±30%, n=3, P<0.14). Subsequently applied phenytoin significantly inhibited the AUC of these contractions (oxytocin: 100±00%, phenytoin: 39±10%, n=3, P<0.01), and subsequently applied BAY K8644 did not significantly alter the contractility in comparison with the preceding periods' mean AUC (BAY K8644: 37±23%, n=3, P=0.05 vs. phenytoin, P<0.03 *vs.* oxytocin).

Discussion

In this *in vitro* functional study, we show that epileptic uterus may be different than healthy uterus in terms of spontaneous contractility and oxytocin sensitivity. We also present experimental data that the selected AEDs have modulatory actions on uterus contractility. The selected AEDs showed similar actions on the contractions of isolated myometrial strip from epileptic WAG/Rij and healthy Wistar rats.

Accepted Manuscript

Spontaneous contractility (monitored by amplitude, AUC and frequency of contractions) of myometrial strips from WAG/Rij rats was higher for non-pregnant but lower for pregnant rats than the control Wistars (Table 1). We have no explanation about the cause and possible mechanism of this, but this information might have implication with the relatively impaired reproductive capacity of epileptic women [1]. There is evidence including from animal seizure models that altered expression of ion channels are involved in the pathology of epilepsy [9] and these may be underlining the difference we determined in contractility of WAG/Rij epileptic rat uterus. In terms of functionality, the spontaneous (stretch-induced) contractions of non-pregnant uterus have putative role in rapid sperm transport [10]. So these findings are compatible with intact fertile capacity of epileptic women [1], despite there is increased risk of fertility problems associated with epilepsy [11]. Furthermore it can be considered that, contractile impairment, when no AED was used, might not contribute the fertility problems associated with epilepsy. The spontaneous contractility of uterus suggested to show variations depending on the stage of estrus cycle, fainting after ovulation to facilitate implantation by optimizing the contact between the blastocyst and the endometrium [12]. But, we did not assess different gestational stage-based comparisons between the epileptic WAG/Rij and healthy control myometrial strips. Furthermore, these findings might have implications with regard to the catamenial epilepsy, as higher level of spontaneous contractility among non-pregnant epileptic WAG/Rij myometrium might also have relation with dysmenorrhea related abdominal pain. Indeed it is already suggested that catamenial epilepsy affects a considerable portion (up to 70%) of women with epilepsy [13,14]. There are also a couple of case reports indicating co-morbidities of epilepsy and abdominal pain due to dysmenorrhea [15,16].

With regard to the contractile response to oxytocin, myometrial strips from non-pregnant WAG/Rij rats were significantly more responsive to oxytocin than those obtained from non-pregnant Wistar rats (Table 2). Although no mechanistic investigation was performed, this is probably due to higher level of oxytocin receptor presence. And this finding further support the implication of role of these contractions and higher rate of dysmenorrhea related abdominal pain [17] in epileptic women.

Oxytocin also significantly stimulated contractility of pregnant WAG/Rij myometrium. However, myometrial strips from pregnant WAG/Rij rats were significantly less responsive to oxytocin than Wistar tissue. It is clear that pregnancy is another factor modulating the contractility of myometrial smooth muscle from epileptic WAG/Rij rats. We have no evidence about what mechanism underlies the difference and what would be the functionality of this difference. Because the pregnant epileptic WAG/Rij myometrium significantly stimulated by oxytocin, this should be enough to mediate vaginal birth. But the finding of weaker oxytocin-induced myometrial contractions than healthy controls might implicate relation with the reported higher rates of cesarean section and heavy postpartum hemorrhage after delivery among epileptic women than healthy women [7]. Yet, the impact of AEDs, which we also determined in this study, might also contribute these increased odds for need of cesarean section.

Since most AEDs inhibit epileptic seizures by acting on ion channels [18], mainly voltage-dependent sodium and calcium channels, it is plausible to suspect that AEDs may have considerable contractile modulatory effects on smooth muscle including myometrium, thereby has side effect potential to cause abortion, uterine dystocia or increased bleeding in epileptic pregnant and non-pregnant women. Here, we investigated possible contractile modulatory effects of AEDs including members from the first generation-older agent anticonvulsants (phenytoin and valproic acid) and newer agents (levetiracetam) alone and in some combination ("valproic acid + phenytoin" and "valproic acid phenytoin") on the oxytocin-induced contractility of isolated myometrial strips of WAG/Rij and control Wistar rats. Phenytoin, levetiracetam and valproate were selected, because they are nowadays the most widely used AEDs in the developed countries.

The magnitude of inhibition provided by levetiracetam on AUC of oxytocin-induced contractions was not significantly different between WAG/Rij and Wistar myometrium tissues (Table 2), both non-pregnant and pregnant. However, the extend of the inhibitory effect of levetiracetam on frequency of oxytocin-induced contractions was significantly greater in pregnant myometrial strips from WAG/Rij rats compared with control Wistar (Table 2). Being more effective on pregnant myometrium might have clinical implications because this agent is in clinical use in controlling seizures during pregnancy and being supposed to be relatively free of teratogenic side effects [19].

There was no significant difference between the extent of the effects of levetiracetam on mean amplitude and AUC of oxytocin-induced contractions of myometrial strips isolated from WAG/Rij and Wistar rats, pregnant or non-pregnant.

Study Limitations and Clinical Relevance Potential of Our Findings

First of all, this is an *in vitro* animal study and investigating acute effects of AEDs on myometrial contractions. It would be better to use the myometrial strips after chronically administered the AEDs to rats and observe the comparisons between the pretreated group and the non-pretreated group. But *in vitro* studies still have certain value in evidence hierarchy and findings of this study have a potential value for rationale of further investigations in human myometrium and clinical studies. In this regard, using myometrial strips from genetic epileptic animals and using both non-pregnant and pregnant rat tissue gives some power and relevance to this study.

Secondly, the doses of some epileptics are rather higher than clinically relevant doses. This aspect still provides some relevant information since doses of these agents may need to be doubled or tripled during pregnancy to maintain steady-state plasma drug concentrations at their pre-pregnancy values. Given these considerations, the translational value of our study in humans is rather limited.

With regard to the concentrations of AEDs we calculated the tested concentrations from their routine clinical use and tried to test concentration ranges that includes clinically relevant concentrations, except for levetiracetam □20□. The magnitude of the inhibitory effect was in the order of phenytoin > valproic acid > levetiracetam. This is further to the fact that levetiracetam did not have any significant effect at clinically relevant concentrations on contractile parameters of neither WAG/Rij nor Wistar myometrium. Combining valproic acid with levetiracetam provided minimal effect on contractility while combining valproic acid with phenytoin did not prevent its significant impact on contractility. This results might have clinical implication for use of levetiracetam during pregnancy as it is currently widely used during pregnancy due to evidence of effectiveness in control of seizures and safety □21,22□.

As a proof of concept to demonstrate contractile modulatory effect of AEDs on myometrial tissue *in vitro*, our study did not examine and cannot resolve the mediating mechanism(s) of this effect. To explore potential mechanism of action of the AEDs we utilized some protocols, after testing the final concentration of each agent. Among tested AEDs, phenytoin and valproic acid is suggested to inhibit voltage-gated membrane sodium channels □18□.

With regard to the effects of levetiracetam on contractile parameters of oxytocin-induced contractions of WAG/Rij and Wistar rat myometrium, its effect was lowest when clinical relevant doses considered and this effect is might be due to its unique mechanism of action, not involving membrane ion channels. Perfusion with NMDG containing solution, extracellular sodium being replaced with NMDG, significantly restored the phenylephrine-induced inhibition suggesting involvement of sodium channels in this effect. The calcium channel opener BAY K 8644 and challenge with high KCl (60 mM) mM) did not significantly restore the phenytoin-induced inhibition of contractility. This presents an evidence that interaction with membrane voltage-gated calcium channels did not play a significant role in this action. When interpreting our results regarding mechanism of action it should be noted that this protocols were applied after the highest doses of each agent and at highest doses tested they might have produced some non-specific actions. It is likely that AEDs have direct effects on smooth muscle, because also have some preliminary data on other tissues including detrusor and lower esophageal sphincter.

The epilepsies can be divided into two major groups: ‘symptomatic’: an acquired or inborn structural or metabolic defect of the brain can be identified as the underlying cause of the disease and ‘idiopathic’: genetically determined and characterized by the lack of structural or other predisposing causes. Changes of ion channel expression and/or function, which may contribute to all forms of epilepsy, can be the cause of seizure activity □23□. Channelopathy may not be solely localized to structures primarily related to epilepsy but it may involve in other tissues including uterine smooth muscle. Hence, we hypothesized whether spontaneous contractility and oxytocin sensitivity of epileptic WAg/Rij uterus is different from healthy control rats. This represents physiological relevance while the examination of effects of AEDS represents pharmacological relevance of our study.In conclusion, results from this study show that under basal conditions myometrial smooth muscle from epileptic rats showed different spontaneous contractility than control rats and upon stimulation with oxytocin, strips from WAG-Rij rats showed a lower sensitivity. Anti-epileptics showed contractile modulatory actions in the isolated

Accepted Manuscript

myometrial strip from both WAG/Rij and Wistar rats, phenytoin being the most and levetiracetam the least effective.

Statements

Statement of Ethics: All procedures performed in studies involving animals

were in accordance with the ethical standards of the Institution at which the study was conducted. The protocols of the present study were reviewed and approved by the Karadeniz Technical University Animal Research Ethics Committee (2018-34).

Conflict of Interest Statement: In regards to author A. Ayar being an Editorial Board member of the Journal. The authors declare no other conflict of interest.

Funding Sources: No grant or any financial support was received for the study.

Author Contribution

All three authors have contributed to experiments and data analysis, AA designed the study and AA and AGK drafted the manuscript.

Data Availability Statement:

All data generated during this study are included in this manuscript. Further enquiries can be directed to the corresponding author.

Presentation at a meeting:

*Preliminary form of this data is presented in abstract form (oral communication):

Kurt A, Salihoglu AK, Ayar, A. The antiepileptic agents phenytoin and valproate inhibits oxytocin-induced contractions of myometrium isolated from absence epileptic WAG/Rij rats. Physiology 2019 (Aberdeen, UK) (2019) Proc Physiol Soc 43, C126

References:

□1□ Walker SP, Permezel M, Berkovic SF. The management of epilepsy in pregnancy. BJOG. 2009;116: 758-67.

□2□ Siricilla S, Iwueke CC, Herington JL. Drug discovery strategies for the identification of novel regulators of uterine contractility. Curr Opin Physiol. 2020; 13: 71-86.

□3□-Pennell PB. Use of Antiepileptic drugs during pregnancy: Evolving concepts. Neurotherapeutics. 2016; 13: 811-820.

□4□-Mawer G. Core outcomes for studies of pregnancy with epilepsy. BJOG 2017; 124:668.

□5□-Tomson T, Battino D, Perucca E. Teratogenicity of antiepileptic drugs. Curr Opin Neurol. 2019; 32: 246-252.

□6□-Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Perucca E, Sabers A, Thomas SV, Vajda F; EURAP Study Group. Comparative risk of major congenital malformations with eight different antiepileptic drugs: a prospective cohort study of the EURAP registry. Lancet Neurol. 2018; 17:530-538.

□7□-Borthen I, Eide MG, Veiby G, Daltveit AK, Gilhus NE. Complications during pregnancy in women with epilepsy: population-based cohort study. BJOG. 2009; 116:1736-42.

Accepted Manuscript

□8□-Viale L, Allotey J, Cheong-See F, Arroyo-Manzano D, Mccorry D, Bagary M, Mignini L, Khan KS, Zamora J, Thangaratinam S; EBM CONNECT Collaboration. Epilepsy in pregnancy and reproductive outcomes: a systematic review and meta-analysis. Lancet. 2015; 386: 1845-52.

□9□-Kaplan DI, Isom LL, Petrou S. Role of sodium channels in epilepsy. Cold Spring Harb Perspect Med 2016: a022814.

□10□-van Gestel I, IJland MM, Hoogland HJ, Evers JL. Endometrial wave-like activity in the non-pregnant uterus. Hum Reprod Update. 2003; 9: 131-8.

□11□-Pennell PB, French JA, Harden CL, Davis A, Bagiella E, Andreopoulos E, Lau C, Llewellyn N, Barnard S, Allien S. Fertility and birth outcomes in women with epilepsy seeking pregnancy. JAMA Neurol. 2018; 75: 962-969.

□12□-Fanchin R, Ayoubi JM, Righini C, Olivennes F, Schönauer LM, Frydman R. Uterine contractility decreases at the time of blastocyst transfers. Hum Reprod. 2001; 16:1115-9.

□13□-Herzog AG, Harden CL, Liporace J, Pennell P, Schomer DL, Sperling M, Fowler K, Nikolov B, Shuman S, Newman M. Frequency of catamenial seizure exacerbation in women with localization-related epilepsy. Ann Neurol. 2004; 56: 431-434.

□14□- Schipper HM. The impact of gonadal hormones on the expression of human neurological disorders. Neuroendocrinology. 2016;103(5):417-31.

□15□- Marchitto N, Ceratti U, Dalmaso S, Raimondi G. A case of refractory epilepsy and related pain due to dysmenorrhea solved with loading dose of lacosamide: clinical and neurophysiological correlates. Acta Biomed. 2017; 88: 334-336.

□16□-Zdraveska N, Kostovski A. Epilepsy presenting only with severe abdominal pain. J Pediatr Neurosci. 2010; 5: 169-70.

□17□-Guo SW, Mao X, Ma Q, Liu X. Dysmenorrhea and its severity are associated with increased uterine contractility and overexpression of oxytocin receptor (OTR) in women with symptomatic adenomyosis. Fertil Steril. 2013; 99:231-240.

□18□-Macdonald RL, Kelly KM. Antiepileptic drug mechanisms of action. Epilepsia. 1995; 36: S2-12.

□19□-Longo B, Forinash AB, Murphy JA. Levetiracetam use in pregnancy. Ann Pharmacother. 2009; 43:1692-5.

□20□-Patsalos PN, Berry DJ, Bourgeois BF, Cloyd JC, Glauser TA, Johannessen SI, Leppik IE, Tomson T, Perucca E. Antiepileptic drugs--best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia. 2008; 49: 1239-76.

□21□-Ba Battino D, Tomson T, Bonizzoni E, Craig J, Lindhout D, Sabers A, Perucca E, Vajda F; EURAP Study Group. Seizure control and treatment changes in pregnancy: observations from the EURAP epilepsy pregnancy registry. Epilepsia. 2013; 54: 1621-7.

□22□-Putta S, Pennell PB. Management of epilepsy during pregnancy: evidence-based strategies. Future Neurol. 2015; 10:161-176.

□23□-Mulley JC, Scheffer IE, Petrou S, Berkovic SF. Channelopathies as a genetic cause of epilepsy. Curr Opin Neurol. 2003;16:171-6.

Figure Legend

Fig 1. Dose-dependent effects of levetiracetam, phenytoin and valproic acid on oxytocin-induced contractions of myometrium strips isolated from non-pregnant and pregnant healthy Wistar and epileptic WAG-Rij rats.

After spontaneous contractions were stimulated by oxytocin, was then measured by adding increasing cumulative concentrations of antiepileptic agents at 10 minute intervals. Effects of LEV (non pregnant: (**a**), pregnant:(**b**) and PHT (non pregnant: (**c**), pregnant:(**d**)) was tested on both non-pregnant and pregnant myometrium, while VPA (**e**) was tested in only non-pregnant myometrium strips. Data are expressed as the mean±SD of experiments using

Accepted Manuscript

myometrial samples from 50 different animals for each. AUC of control (oxytocin-induced contraction) period was assigned as 100%, and comparisons between the control and post-treatment period was made within and between the antiepileptic agents, also in a dose-dependent manner. Bold values (*[a,b,c]) indicates significant difference (*: P<0.05) vs. corresponding values for the control period, in the same row (* [a]: p<0.05, * [b]: p<0.01, * [c]: p≤0.001).

LEV: levetiracetam (1, 3 and 10 mM), PHT: phenytoin (10, 30 uM and 1 mM) and VPA: valproic acid (100, 300 uM, 1 mM and 3 mM)

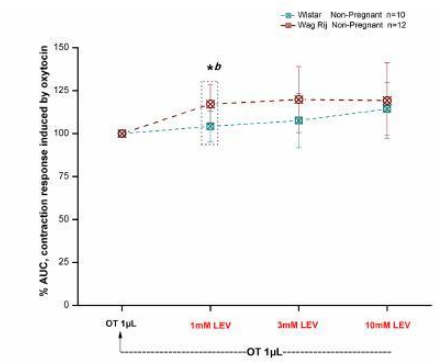
Figure 2. Effects of phenytoin on oxytocin-induced myometrial contractions of pregnant WAG/Rij rats

Typical original trace showing the cumulative effects of different concentration of phenytoin (10 µM, 30 µM and 30 µM) on oxytocin-stimulated contractile activity. Oxytocin and gadoterate meglumine was added to tissue bath at points as indicated by arrows and were

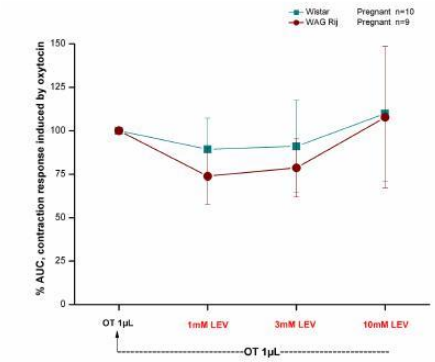
continuously present throughout the experiment.

Accepted Manuscript

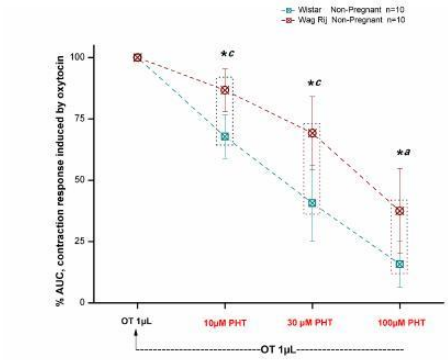
a


Wistar Non-Pregnant n=10
Wag Rij Non-Pregnant n=12
% AUC, contraction response induced by oxytocin
*b
OT 1µL
1mM LEV
3mM LEV
10mM LEV
OT 1µL

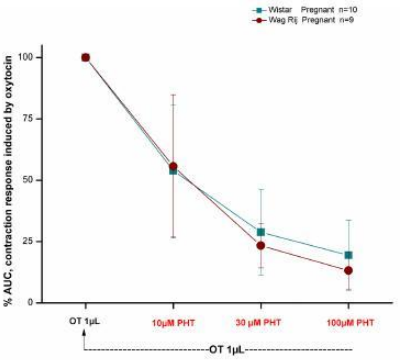
b


Wistar Pregnant n=10
WAG Rij Pregnant n=9
% AUC, contraction response induced by oxytocin
OT 1µL
1mM LEV
3mM LEV
10mM LEV
OT 1µL

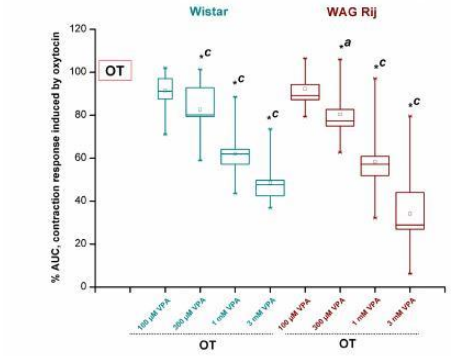
c

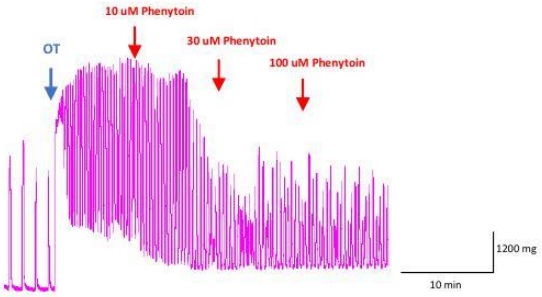

Wistar Non-Pregnant n=10
Wag Rij Non-Pregnant n=10
% AUC, contraction response induced by oxytocin
*c
*c
*a
OT 1µL
10µM PHT
30 µM PHT
100µM PHT
OT 1µL

d


Wistar Pregnant n=10
Wag Rij Pregnant n=9
% AUC, contraction response induced by oxytocin
OT 1µL
10µM PHT
30 µM PHT
100µM PHT
OT 1µL

e


Wistar
WAG Rij
OT
% AUC, contraction response induced by oxytocin
*c
*c
*c
*a
*c
*c
100 µM VPA
300 µM VPA
1 mM VPA
3 mM VPA
OT
OT


10 uM Phenytoin
OT
30 uM Phenytoin
100 uM Phenytoin
1200 mg
10 min

Accepted Manuscript

Table 1. Comparison of spontaneous and oxytocin-induced contractions of epileptic WAG/Rij and healthy control rats

			N	*Mean*	*Std. Deviation*	*Std. Error*	*P Value*
Non-Pregnant	***WİSTAR***	Spontaneous	30	100	100	100	**0,001**
		Oxytocin	30	289,7	342,2	342,2	**0,001**
Pregnant		Spontaneous	20	249,8	314,5	385,2	**0,001**
		Oxytocin	20	587,3	1036,9	1269,9	**0,001**
Non-Pregnant	***WAG/Rij***	Spontaneous	32	140,1	125,5	121,6	**0,001**
		Oxytocin	32	395,7	363,1	351,6	**0,001**
Pregnant		Spontaneous	18	179,0	230,7	297,9	**0,001**
		Oxytocin	18	413,6	410,4	529,8	**0,001**

Data are normalized with respect to the spontaneous contractions of Wistar rat myometrium

Accepted Manuscript

Accepted Manuscript

Table 2. Dose-dependent effects of antiepileptic agents on oxytocin-induced contractions of myometrium strips isolated from non-pregnant and pregnant healthy Wistar and epileptic WAG-Rij rats

Species/Status of Muscle Donor		Agent	Concentration	Mean Amplitude ± SD (%)	Mean Frequency ± SD (%)	*n*
		Oxytocin		100±0.0	100±0.0	100
Wistar	Non-Pregnant	Phenytoin	10 µM	**89.4±9.7*** [b]	**83.7±9.7*** [c]	10
			30 µM	**72.1±14.9*** [c]	**51.9±24.1*** [c]	
			100 µM	**27.1±29.5*** [b]	**17.7±23.5*** [b]	
	Pregnant		10 µM	61.7±27.7	**67.7±35.5*** [a]	10
			30 µM	**28.4±30.3*** [b]	**32.3±51.8*** [c]	
			100 µM	**8.4±14.3*** [c]	**14.9±29.6*** [b]	
WAG-Rij	Non-Pregnant		10 µM	96.8±6.8	**85.9±11.8*** [b]	10
			30 µM	**84.9±11.2*** [b]	**72.5±14.2*** [c]	
			100 µM	**69.5±14.9*** [c]	**40.5±30.8*** [c]	
	Pregnant		10 µM	**70.4±13.7*** [c]	66.8±36* [a]	9
			30 µM	**31.7±25.7*** [c]	39.7±28.3* [c]	
			100 µM	**8.2±18.9*** [b]	12.9±23.9* [b]	
Wistar	Non-Pregnant	Levetiracetam	1 mM	**106±4.9*** [b]	97.6±5.9	10
			3 mM	105.9±9.7	97±8.0	
			10 mM	105.7±15.1	99.4±11.4	
	Pregnant		1 mM	92±12.1	96.3±19.7	10
			3 mM	92.3±18.5	**90.8±12.3*** [a]	
			10 mM	98.9±15.5	95±14.5	
WAG-Rij	Non-Pregnant		1 mM	**105.7±5.3*** [b]	**86.2±13.8*** [b]	12
			3 mM	**109.3±10.28*** [a]	91.3±15.9	
			10 mM	107.5±12.1	**77.8±29.3*** [a]	
	Pregnant		1 mM	**91.7±7.9*** [a]	92.9±12.9	9
			3 mM	96.2±6.7	100±26.8	
			10 mM	**92.1±6.9*** [b]	**115.7±23.3**	
Wistar	Non-Pregnant	Valproic Acid	100 µM	93.9±9.9	**90.7±7.9*** [b]	10
			300 µM	91.7±15.6	**83.2±7.4*** [c]	
			1 mM	**84±16.3*** [a]	**70.4±8.6*** [c]	
			3 mM	**66.9±17.7*** [c]	**56.9±11.6*** [c]	
WAG-Rij			100 µM	97.9±3.3	**84.4±10.9*** [c]	10
			300 µM	**94.9±5.5*** [a]	**71.9±12.1*** [c]	
			1 mM	**86±9.4*** [c]	**57.6±15.9*** [c]	
			3 mM	**73.2±27.7*** [b]	**32.2±7.8*** [c]	

Data are expressed as the mean±SD of experiments using myometrial samples from indicated number for each.
The mean amplitude and frequency of control (oxytocin-induced contraction) period was assigned as 100%, and comparisons between the control and post-treatment period was made within and between the antiepileptic agents, also in a dose-dependent manner.
Bold values indicates significant difference (*: $P<0.05$) vs. corresponding values for the control period, in the same row (* [a]: $p<0.05$, * [b]: $p<0.01$, * [c]: $p\leq0.001$).

Accepted Manuscript