Ex-vivo modulation of spontaneous uterine contractility by some sodium ion channel blockers

Bafor Enitome Evi[*1], Adekunle Idowu Florence[1], Omoruyi Osemelomen.[1], Okosun Isibhakhomhen Victoria[1], Ozolua Raymond Iduojemu[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria

*Reproductive Health/Ethnopharmacology Research Group (RHERG), Department of Pharmacology & Toxicology, University of Benin, Nigeria

ABSTRACT

Local anaesthetics of the amide class which act by sodium ion (Na^+) channel blockade had been reported to stimulate uterine contractility rather than cause inhibition. This study therefore sets out to investigate the effect of selected Na^+ channel blockers that do not belong to the class of local anaesthetics, on uterine contractility. This was necessary in order to ascertain if the effect previously observed with the amide anaesthetics were a function of the class of anaesthetics, a function of Na^+ channel blockade or other interactions. Three Na^+ channel blocking drugs and one amide anaesthetic drug were used for the investigation. These included: lidocaine (0.0002 - 2.222 µg/ml), quinine (0.003 - 1.332 µg/ml), chloroquine (0.64 -710 ng/ml) and phenytoin (0.001 - 1.11 µg/ml). These drugs were added cumulatively to the isolated mouse uterus which was mounted in a 10 ml organ bath filled with continuously aerated physiological solution and set at a temperature of 37ºC. The effect of these drugs on the amplitude and frequency of spontaneous uterine contractions were determined. Lidocaine, quinine and chloroquine were observed to concentration-dependently increase the amplitude and frequency of uterine spontaneous contractions ($p < 0.05$). However, phenytoin was observed to decrease both the amplitude and frequency of uterine spontaneous contractions ($p < 0.05$). The stimulation contradicts the effect of Na^+-channel blockade on smooth muscle contractility and therefore suggests other mechanism(s) of activity apart from Na^+-channel blockade or a new role for Na^+-channel blockade on uterine smooth muscles. This study has shown that besides the amide anaesthetics, other Na^+-channel blocking drugs produce stimulation of uterine contractility.

Key words: Lidocaine, Quinine, Chloroquine, Phenytoin, Sodium channels, Uterus

INTRODUCTION

Action potentials in excitable cells are dependent on the activity of voltage gated Na^+- channels (VGSCs) (Yu and Catterall, 2003). VGSCs are well studied in nerves, skeletal muscles and the heart but less so in smooth muscles (Seda et al., 2007). Studies so far have shown that changes in the expression (Yu and Catterall, 2003) and function of the VGSCs play significant roles in several pathologies (Antzelevitch et al., 2003; Clare et al., 2000). Ion channels can be described as macromolecular protein pores located in plasma membranes which function to permit passage of certain ions in and out of the cell. As such they play important roles in the transmission of electrical signals in nerves, synapses and muscles (Chanrachakul, 2006). VGSCs play a role in the regulation of cellular excitability (Zuliani et al., 2009) and exist as heteromeric transmembrane protein complexes made up of a pore-forming α-subunit of about 220-260 KDa in size and about ten of these α-isoforms have so far been identified (Zuliani et al., 2009). Classification of VGSCs is usually according to their sensitivity to the puffer-fish toxin called tetrodotoxin (TTX). Hence, they are classified as TTX – sensitive sodium channels ($Na_v1.1$ – 1.4, 1.6, and 1.7) and TTX-resistant sodium channels ($Na_v1.5$, 1.8 and 1.9) (Goldin, 2001). The pore of the channel is lined by the S6 segment itself and it is where most of the binding sites for local anaesthetics and anticonvulsant drugs are located (Zuliani et al., 2009). Sodium channel blockers exhibit a wide range of chemical moieties but a common functional group is the presence of a phenyl ring that is connected to a basic nitrogen, with some combination of aromatic and aliphatic groups (Nardi et al., 2012; Zuliani et al., 2009). Studies have shown that the most medically relevant drugs are small organic molecules capable of binding inside the pore to obstruct ion flow by either direct blockade of the ion conduction pathway or by stabilizing a non-conductive channel conformation (Hille, 2001). Blockade by this mechanism would invariably result in a reduction in the propagation of signals clinically seen as a reduction in seizures, pain and arrhythmias (Martin and Corry, 2014).

*Corresponding author: **Email**: enitome.bafor@uniben.edu **Mobile Number**: +2348099187555

Abnormal expression of sodium channels have been reported to be responsible for a range of diseases related to nerve and muscular function including neuropathic pain, cardiac arrhythmia and epilepsy (Ashcroft, 2006; Waxman, 2007). Therefore drugs that modify the passage of Na^+ particularly via blockade may be clinically useful in alleviating these disease conditions (Martin and Corry, 2014). The observation of multiple modes of drug block led to the suggestion that there are two access routes for drugs to enter the channel: through the activation gate from the cytosol or directly from the lipid bilayer through a 'hydrophobic route' (Hille, 1977). In humans, nine different types of VGSCs, have been described as earlier mentioned which are preferentially expressed in different tissues (Catterall, 2012). Most sodium channel blocking drugs target all subtypes (Zuliani et al., 2009). The presence of a common chemical moiety in sodium channel inhibitors also raises the question on how drugs with similar structures and binding sites, such as local anaesthetics and anticonvulsants, have differing therapeutic effects (Lipkind and Fozzard, 2010). VGSCs have been shown to be present in rat myometrial tissue (Inoue and Sperelakis, 1991; Martin et al., 1990; Seda et al., 2007; Yoshino et al., 1997; Young and Herndon-Smith, 1991). Activation, (and not blockade), of VGSCs in the myometrium have been reported to represent a highly efficient mechanism necessary for generating uterine motility in the rat uterus (Seda et al., 2007). VGSCs have been found in late stages of pregnancy and have been reported to play a role in labour (Inoue and Sperelakis, 1991; Sperelakis et al., 1992). Certain subunits of VGSC (2a1, 3a, 4a, 5a, 8a and 9a) have also been reported to mediate VGSC-induced uterine contractions (Seda et al., 2007). These contractions appear to occur with a simultaneous increase in $[Ca^{2+}]_i$ (Seda et al., 2007). It had also been reported that activation of VGSCs causes contractions through the influx of Ca^{2+} which is due to the opening of L-type Ca^{2+} channels (Seda et al., 2007). Compounds with effect on VGSCs are supposed to be easily differentiated from those that act on Ca^{2+} channels (Seda et al., 2007). Based on these research, VGSC blockers should inhibit uterine contractions. It was however observed from a previous research (Bafor et al., 2015) that lidocaine a VGSC blocker stimulated a sustained increase in amplitude and frequency of uterine contractions. This result is contradictory to what is previously known about VGSC blockers. This study is therefore aimed at investigating the effect of other sodium channel blockers on myometrial contractility in order to find out if sodium-channel blockers in general have a stimulatory effect on the myometrium and if activity observed has occurred by other mechanisms besides Na^+-channel blockade.

MATERIALS AND METHOD

Animals

Mature virgin female mice weighing between 20.0-30.0 g were obtained from the Animal House, Faculty of Pharmacy, University of Benin, Edo state, Nigeria. Ethical clearance was obtained from the animal use ethical Committee of the Faculty of Pharmacy, University of Benin, Nigeria, and experiments were in line with standards of the Public Health Service policy on humane care and use of Laboratory Animals 2002. The animals were maintained on standard diet of animal pellets (Premier Feed Mills, Lagos state, Nigeria) and clean tap water.

Drugs and reagents

Chloroform, methanol, and phytochemical analysis reagents were obtained from BDH chemicals, UK, acetylcholine (Sigma Aldrich, UK), castor oil (Bell, Sons & Co. (Druggists) Ltd., Merseyside, UK), activated charcoal (General Carbon Co., NJ USA), Loperamide HCl (Shine Pharmaceuticals, Gujarat, India). Salts for the physiological solution were obtained from BDH chemicals, England, UK.

Contractility Studies

Tissue preparation

Twenty four hours prior to the day of experiments, each mouse was administered diethylstilbesterol (1 mg/kg) orally. The mice were then humanely killed by cervical dislocation and the uterine horns were immediately excised and immediately placed into a Petri dish containing aerated physiological salt solution. The uterine tissues were cleaned of connective tissues and one uterine horn was transected medially in half and lengths of approximately 1-2 mm each were obtained. The uterine segment was then mounted in a warmed 10 ml organ bath maintained at 37°C and containing aerated physiological salt solution of the following composition in M: NaCl 154.00, $NaHCO_3$ 5.95, D-glucose 2.78, KCl 5.63, and $CaCl_2 \cdot 2H_2O$ 2.05.

Experimental protocol

The mounted tissues were equilibrated under resting tension of 0.5 g for 30 min. The force and frequency of uterine contractions in the longitudinal muscle layers were measured using a 7003E-isometric force transducer (Ugo Basile, Varise, Italy) connected to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (Ugo Basile, Varese, Italy). The channel recorder was previously set at a sensitivity of 1.00 chart speed of 5 mm/min, while the transducer was set to record a tension of 1.00 g so as to establish a relationship between the force applied to the transducer and the gauge deflection.

Lidocaine, Chloroquinine, Quinine and Phenytoin on Spontaneous Contractions

The direct effect of successive concentrations of lidocaine, chloroquine, quinine or phenytoin on uterine smooth muscle contractility were investigated. Concentration-response relationships for each drug were obtained using the following concentrations 0.002-0.14 ng/ml respectively. A contact time of 3 min was allowed following each concentration of drug administered. After each set of administration, a wash-out period of 10 min was allowed before the next drug administration.

Statistical Analysis

The mean frequency and amplitude were calculated from contractions occurring at the last 5 min of the phasic contractions using the GraphPad Prism 6.0, (GraphPad software Inc, San Diego, CA, USA). Results were obtained as percentages of control applications (control=100%). In some experiments, changes in force amplitude were expressed with respect to basal (resting) force level (100%) and the peak force (91%) in control conditions. All data shown were expressed as mean ± standard error of mean (SEM) and 'n' represents the number of samples each from a different animal. Significance was evaluated using appropriate t-tests, and where necessary, one way analysis of variance with Dunnett's post hoc and P values ≤ 0.05 was taken to be significant.

In datasets with sufficient data points, mean log concentration-response curves were analyzed by fitting data to a four-parameter logistic equation, using non-linear regression with GraphPad Prism 6.0 (GraphPad software, San Diego, CA, USA) to determine the pEC_{50} values (Y = Bottom + (Top – Bottom)/(1+10^((LogEC50-X)*HillSlope)). Where Y = response which starts at the Bottom and goes to the Top in sigmoid shape, X = logarithm of concentration and EC_{50} is the concentration that produces half the maximal responses. Average potencies for frequency and amplitude of contractions were calculated and compared after curve fitting where possible. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparison tests. P ≤ 0.05 indicated statistical significance in all cases.

RESULTS

Effect of Lidocaine on Spontaneous Uterine Contractility

Lidocaine, a sodium channel blocker was observed to increase the amplitude ($p < 0.05$) and significantly increase the frequency ($p < 0.01$) of the isolated uterine smooth muscle (Fig. 1).

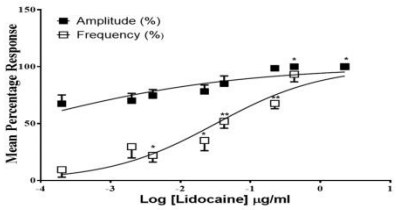

Figure 1. Effect of lidocaine (0.0002 – 2.222 µg/ml), a sodium channel blocker on the amplitude (■) and frequency (□) of the uterine smooth muscle. Shown are concentration-response curves for lidocaine (n =5). Data points represent mean ± SEM. Error bars not seen lie within the dimensions of the symbols. * p< 0.05; ** p < 0.01 compared to control.

Effect of Chloroquine on Spontaneous Uterine Contractility

Chloroquine, also a sodium channel blocker was observed to stimulate an increase in the frequency of the isolated uterine smooth muscle (Fig. 2). However, though a slight increase in amplitude was observed, it was not statistically significant (Fig. 2).

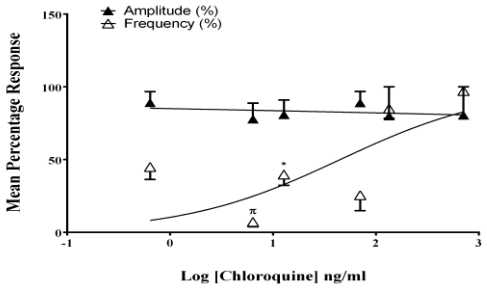

Figure 2. Effect of chloroquine (0.64 - 710.4 ng/ml), a sodium channel blocker on the amplitude (▲) and frequency (△) of the uterine smooth muscle. Shown are concentration-response curves for chloroquine (n =5). Data points represent mean ± SEM. Error bars not seen lie within the dimensions of the symbols. $^{\pi}$ p< 0.05 of inhibitory activity compared to control; * p < 0.05 of stimulatory activity compared to control.

Effect of Quinine on Spontaneous Uterine Contractility

Quinine was observed to stimulate an increase in the amplitude and frequency of the isolated uterine smooth muscle (Fig. 3).

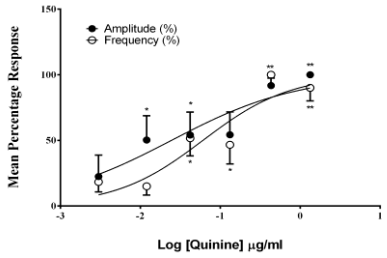

Figure 3. Effect of quinine (0.003 – 1.332 µg/ml),a sodium channel blocker on the amplitude (●) and frequency (○) of the uterine smooth muscle. Shown are concentration-response curves for quinine (n = 4 - 5). Data points represent mean ± SEM. Error bars not seen lie within the dimensions of the symbols. * p< 0.05; ** p < 0.01 compared to control.

Effect of Phenytoin on Spontaneous Uterine Contractility

Phenytoin was observed in this study to inhibit the amplitude and frequency of spontaneous uterine contractions (Fig. 4).

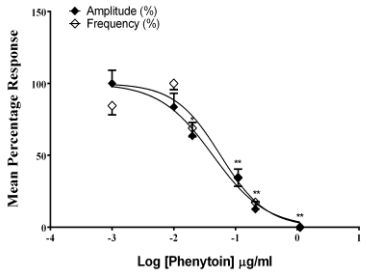

Figure 4. Effect of phenytoin (0.001 – 1.11 µg/ml), a sodium channel blocker on the amplitude (◆) and frequency (◇) of the uterine smooth muscle. Shown are concentration-response curves for quinine (n = 4 - 5). Data points represent mean ± SEM. Error bars not seen lie within the dimensions of the symbols. * p< 0.05; ** p < 0.01 compared to control.

DISCUSSION

Early electrophysiological studies have demonstrated the presence of voltage-dependent Na^+ currents in cultured and freshly isolated smooth muscle cells from human and rat myometrium (Inoue and Sperelakis, 1991; Young and Herndon-Smith, 1991). It has been suggested that VGSCs are functional in the late stages of pregnancy and play a role in the initiation of labour (Inoue and Sperelakis, 1991; Sperelakis et al., 1992). Yoshino et al. (Yoshino et al., 1997) have described the presence of Na^+ currents in about 50% of smooth muscle cells isolated from the myometrium of non-pregnant rats. So indeed Na^+ channels exist in the myometrium and contribute to the regulation of both pregnant and non-pregnant states.

This study has shown that indeed some Na^+ channel blockers cause uterine stimulation as observed with the drugs lidocaine, quinine and chloroquine. These drugs significantly increased both the amplitude and frequency of uterine contractions at the concentrations used in this study. However, inhibition was observed with phenytoin in this study which is also a Na^+ channel blocker. The inhibition by phenytoin was similar to results obtained by Seda and colleagues who described the stimulatory effects of the Na^+ channel activators (Seda et al., 2007).

Possible explanations for the stimulatory activity observed with lidocaine, quinine and chloroquine maybe the binding of these drugs to alternative subunits of the Na^+ channel which may possess stimulatory activities and this may be related to the chemical structural differences between the different Na^+ channel blockers. Another possible reason is the interaction of these drugs with Ca^{2+} channels. However, this remains to be absolutely verified. Further studies are therefore recommended to investigate the mechanisms involved.

CONCLUSION

This study has shown that some Na^+ channel blockers such as lidocaine, quinine and chloroquine stimulate uterine contractility while others like phenytoin cause inhibition of uterine contractility. This study has therefore shown an interesting twist to what is previously known about Na^+ channel blockers. It is therefore advised that these drugs are used with care on pregnant women. This study has also opened new research areas such as the investigation of possible connection with Na^+ channels and uterine stimulation and may also improve our knowledge on myometrial regulation.

ACKNOWLEDGEMENT

The authors would like to acknowledge Pharm. Fabian Amaechina for his assistance in procuring some of the drugs utilized in this study.

Conflict of Interest Statement

The authors declare no conflict of interest.

REFERENCES

Antzelevitch, C., Brugada, P., Brugada, J., Brugada, R., Towbin, J.A., Nademanee, K., (2003). Brugada syndrome: 1992-2002: A historical perspective. *J. Am. Coll. Cardiol*. 41:1665–1671. doi:10.1016/S0735-1097(03)00310-3

Ashcroft, F.M., (2006). From molecule to malady. *Nature* 440:440–447. doi:10.1038/nature04707

Bafor, E.E., Obarisiagbon, P.A., Itamaomon, J.L., (2015). Investigation of The Myometrial Stimulatory Effect of Amide Anaesthetics. *J. Pharm. Allied Sci.* 12: 2191–2209.

Catterall, W.A., (2012). Voltage-gated sodium channels at 60: structure, function and pathophysiology. *J. Physiol.* 590: 2577–2589. doi:10.1113/jphysiol.2011.224204

Chanrachakul, B., (2006). Ion channels: new targets for the next generation of tocolytics agents. *J. Med. Assoc. Thai*. 89: Suppl 4.

Clare, J.J., Tate, S.N., Nobbs, M., Romanos, M.A., (2000). Voltage-gated sodium channels as therapeutic targets. *Drug Discov.Today*. 5(11):506-520. doi:10.1016/S1359-6446(00)01570-1

Goldin, A.L., (2001). Resurgence of sodium channel research. *Annu. Rev. Physiol.* 63:871–894. doi:10.1146/annurev.physiol.63.1.871\r63/1/871 [pii]

Hille, B., (2001). Ionic channels of excitable membranes, 3rd ed. Sinauer Associates Inc., MA, Sunderland.

Hille, B., (1977). Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* 69: 497–515. doi:10.1085/jgp.69.4.497

Inoue, Y., Sperelakis, N., (1991). Gestational change in Na+ and Ca2+ channel current densities in rat myometrial smooth muscle cells. *Am J Physiol* 260: C658-663.

Lipkind, G.M., Fozzard, H.A., (2010). Molecular model of anticonvulsant drug binding to the voltage-gated sodium channel inner pore. *Mol. Pharmacol.* 78: 631–638. doi:10.1124/mol.110.064683

Martin, C., Arnaudeau, S., Jmari, K., Rakotoarisoa, L., Sayet, I., Dacquet, C., Mironneau, C., Mironneau, J., (1990). Identification and properties of voltage-sensitive sodium channels in smooth muscle cells from pregnant rat myometrium. *Mol. Pharmacol*. 38: 667–673.

Martin, L.J., Corry, B., (2014). Locating the Route of Entry and Binding Sites of Benzocaine and Phenytoin in a Bacterial Voltage Gated Sodium Channel. *PLoS Comput. Biol.* 10 (7):e1003688. doi:10.1371/journal.pcbi.1003688

Nardi, A., Damann, N., Hertrampf, T., Kless, A., (2012). Advances in Targeting Voltage-Gated Sodium Channels with Small Molecules. *Chem. Med. Chem*. 7(10):1712-1740. doi:10.1002/cmdc.201200298

Seda, M., Pinto, F.M., Wray, S., Cintado, C.G., Noheda, P., Buschmann, H., Candenas, L., (2007). Functional and molecular characterization of voltage-gated sodium channels in uteri from nonpregnant rats. *Biol. Reprod*. 77: 855–863. doi:10.1095/biolreprod.107.063016

Sperelakis, N., Inoue, Y., Ohya, Y., (1992). Fast Na+ channels and slow Ca2+ current in smooth muscle from pregnant rat uterus. *Mol. Cell. Biochem.* 114: 79–89. doi:10.1007/BF00240301

Waxman, S.G., (2007). Channel, neuronal and clinical function in sodium channelopathies: from genotype to phenotype. *Nat. Neurosci*. 10: 405–409. doi:10.1038/nn1857

Yoshino, M., Wang, S.Y., Kao, C.Y., (1997). Sodium and calcium inward currents in freshly dissociated smooth myocytes of rat uterus. *J. Gen. Physiol.* 110: 565–577. doi:10.1085/jgp.110.5.565

Young, R.C., Herndon-Smith, L., (1991). Characterization of sodium channels in cultured human uterine smooth muscle cells. *Am. J. Obstet. Gynecol*. 164: 175–181.

Yu, F.H., Catterall, W.A., (2003). Overview of the voltage-gated sodium channel family. *Genome Biol*. 4(3): 207. doi:10.1186/gb-2003-4-3-207

Zuliani, V., Patel, M.K., Fantini, M., Rivara, M., (2009). Recent advances in the medicinal chemistry of sodium channel blockers and their therapeutic potential. *Curr. Top. Med. Chem*. 9: 396–415.