Novelty Journals

International Journal of Novel Research in Life Sciences
Vol. 1, Issue 2, pp: (1-5), Month: November-December 2014, Available at: www.noveltyjournals.com

The Effect of Some Local Anesthetic Agents on the Surface Electrical Properties of Erythrocytes: In Vitro Study

Husni S. Farah

Department of Medical Biochemistry, College of medicine, Taif University, Kingdom of Saudi Arabia (KSA)

Abstract: **In vitro effect of three local anesthetic agents (Procaine, Tetracaine , Lidocaine) on the surface electrical properties of erythrocytes was investigated. The surface electrical properties were determined by the electro-rotation technique. It was found that the surface conductance, Ks, was the most sensitive parameter, while the surface electrical of capacity of RBC (CM) refractory to treatment with the local anesthetic tested. The values for Ks decreased with the increase in the concentration of the local anesthetic agents giving concentration-response relationships. The values of EC50 were found to be in the following order: Tetracaine (59.57 nM) >Lidocaine (47.52 nM) > Procaine (23.62 nM). The values were proportional with the lipid / water partition coefficient of the respective anesthetic agent.**

Keywords: **local anesthetics, procaine, tetracaine, lidocaine, erythrocytes, surface electricity**

1. INTRODUCTION

Local anesthetic agents reversibly block conduction along nerve axon and other excitable membranes that utilize the sodium channels as the primary means of action potential generation (Johnson, 2004)). Local anesthetic drugs prevent or suppress initiation and propagation of nerve impulses by modulating membrane permeability to ions , particularly sodium ion influx, which is necessary for generating action potential (Brayfield, 2014). However, the possible effect on the electrical properties on non- excitable cellular membranes did not meet much attention. A wide variety of drugs and axons exert their biological activity by interfering with distribution of electric changes across biological membrane or with mobility and propagation of electric impulses (Johnson et al., 2004 ; Bear et al., 2001). Alteration of surface electrical properties by local anesthetic agents, would be expected. Identification of the affected parameters is of importance in understanding their mechanism of action.

The present study was undertaken to investigate the effect of some local anesthetic agents on the surface electrical properties of non-excitable biological membrane. The electrical parameters were assessed by electro-rotation technique (Han et al., 2013; Dalton et al., 2004 ; Cen et al., 2004), a simple and low-cost technique with good potential which has been used successfully in earlier work (Liu et al., 2013).

2. MATERIALS AND METHODS

Freshly withdrawn blood was used throughout this study. Stored samples were not used in order to avoid variation in the electrical properties of erythrocytes membrane brought about by storage (Yao-Xiong Huang et al., 2011). Blood was always withdrawn from the same person because the effects of blood groups and other genetically determined factors, if any, have not been determined yet (ManaSezdi and Yektaulgen, 2006). Different concentrations of 3 local anesthetic agents (Procaine ,Tetracaine , Lidocaine) were prepared in the concentration range of 50 – 500 nM in 330 milliosmol

medium. For the electro-rotation technique experiment, microamounts of the blood was pipetted into a test tube containing the desired concentration of the local anesthetic. The medium conductivity was then adjusted, by the Herpes buffer. Ten micro-liters of this medium was pipetted into the vicinity of the rotation chamber and the true conductivity was obtained by applyingthe relation (Arnold and Zimmerman, 1989)

$$\sigma = \sigma' (1 - \Delta T(0.02))$$

Where ΔT is the temperature difference between the solution in air and the rotation chamber, and σ' represents the apparent conductivity (the conductivity of medium in air). The rotation apparatus that was used in this study depends on only cells that rotate at the centre of the chamber, the characteristic frequency (Fc) was measured for each cell using the contra-field rotation technique (Arnold and Zimmerman, 1989). The radius of each cell was measured using a micrometer attached to ocular piece of the interval microscope used.

The surface electrical capacitance of erythrocyte membrane per unit area (CM) and the surface electrical conductance per unit area (KS) were calculated by applying the relation (Xun et al., 1990):

$$Fc = KS / (\pi.a.CM) + a.CM/(2\pi.CM) + \sigma/(\pi.CM)$$

Where Fc is normalized against the radius of the cell in order to avoid the effect of size-heterogeneity found in a typical cell population.Between 20 and 30 cells were tested for each concentration used.

3. RESULTS

A clear tendency for decreasing KS values with the increasing in the concentration of local anesthetic agents is quite obvious (Figure 1). The decline may be described as having an initial rapid decrease at lower concentrations, which tend to become less steep as the concentration surpass the 100 – 200 nM range (Figure 1). When the percentage changes (decrease) from control value were plotted against the local anesthetic agents concentration, on logarithmic scale, straight lines of typical concentration – response relation were obtained (Figure 2). For these lines, EC50 (the concentration which causes 50% reduction in KS value) values for the three local anesthetics used were calculated and were found to be in the following order : Tetracaine (59.57nM) > Lidocaine (47.52 nM) > Procaine (23.62nM).

Attempts to correlate the EC50 values with the physico – chemical properties of the different compounds used were not successful except when they were correlated with lipid/water partition coefficients. When these EC50 values were plotted against lipid solubility estimates (Bannet and Brown, 2003), a straight line was obtained (Figure 3). The influence on the values of CM of changing the concentration of the different anesthetics showed no statically difference (Table 1). All the values were more or less scattered around the same mean. Differences in CM values at zero concentration probably represent day-to-day variations in experimental conditions.

4. DISCUSSION

Biochemical, biophysical and molecular biological investigations have led to a rapid expansion of knowledge about the mechanism of action of local anesthetics. A variety of physico – chemical models have been proposed to explain how local anesthetic achieve nerve conduction blockade. The successful use of erythrocyte membrane as a model local anesthetics was demonstrated in the present study. Changes in surface conductance (KS) could be clearly seen.

A standardized measure of the activity of each to demonstrate alteration of surface electrical properties by local anesthetic molecules tested , in the form of the concentration which cause 50% reduction in KS value (EC50) had very strong inverse correlation with the lipid/water partition coefficient of the respective compounds. The more the lipid solubility, the smaller was the molar concentration to produce 50% reduction in KS value. It has been proposed that the interaction of relatively lipophilic local anesthetic molecule with membranes causes a conformational change in the organization of membrane lipids, resulting in membrane expansion. This could supposedly be achieved by unfolding of membrane. It is also possible that the lipophilic portion of the local anesthetic molecule binds to non-specific ubiquitous sites within the

membrane lipids, leaving the protonated positively charged amine terminal of the molecule on the external surface of the membrane (Bowdle et al., 1994).

Contrary to clear changes in KS, the calculated CM values were refractory to change in response to treatment of erythrocytes with the local anesthetic tested. The refractoriness of the specific membrane capacitance to change after treatment with the local anesthetic procaine and tetracaine was observed clearly in voltage relaxation experiment on the algal cell membrane of valonia utricularis using intra cellular electrode (Buchner et al.,1986).

It is clear from the present work that the surface electrical conductance of the erythrocyte membrane which represents a non-excitable lipid bilayer, is affected by local anesthetics. The role of such phenomenon in altering propagation of impulses along a nerve axon or an excitable cellular membrane; away from the action on sodium pump, needs further investigation.

REFERENCES

[1] Arnold WM, Zimmermann U (1989). Pro. 1st International conference on low cost experiment in biophysics, Cairo Univ., 18 -20 December .1 -10.

[2] Bear MF, Cnners BW , Paradise MA (2001). Neuroscience: Exploring the brain. Baltimore: Lippincott. 131 – 142.

[3] Bennet PN, Brown MJ (2003). Clinical Pharmacology. Edinburgh. Churchill living stone .224 -265.

[4] Bowdle TA, Akira H, Kharasch ED (1994). The pharmacologic basis of anesthesiology: Basic science and practical application .Edinbugh: Churchill Livingstone. 76 - 102.

[5] Brayfield A . (2014). Martindale : The complete drug reference . 38th ed. The pharmaceutical press

[6] Buchner KH, Walter L, Zimmermann U (1986). Influence of anesthetic on the movement of mobile charges in the algal cell membrane of valoniautricularis. Biophys.Acta; vol. 903. 241 – 250.

[7] Cen EG , Dalton C, Li Y, Adamia S, Pilarski LM, Kaler KV (2004). A combined dielectrophoresis, travelling wave dielectrophoresis and electrorotation microchip for the manipulation and characterization of human malignant cells. J. Microbial. Methods; vol. 58 (3).387-393.

[8] Dalton C, Goater AD, Burt JP, Smith HV (2004). Analysis of parasites by electrorotation.J.Appl. Microbiol.; Vol.96 (1).24-30.

[9] Han SI, Jod YD, Han KH (2013). An electro rotation technique for measuring the dielectric properties of cell with simultaneous use of negative guadrupolerdielectrophoresis and electrorotation. Analyst; vol.7 (138). 1529 – 1537.

[10] Johnson ME, Uhl CB, Spittler KH, Wang H, Gores GJ (2004). Mitochondrial injury and caspase activation by the local anesthetic lidocaine.Anesthesiology; vol. 101 (5). 184 – 190.

[11] Kandel E, Schwartz J, Jessel T (2002). Principles of neural science.4th ed. McGraw-Hill. N.Y. 141-156.

[12] Liu L, Tang JC, Zhang F, Wang X, Xu BJ (2013). The determination of the viability of schistosomal egg by a novel technique: Electrorotation. Tropical Biomedicine; vol.30 (3).367-374.

[13] Mana S (2006). Effect of physiological parameters on the electrical properties of blood bank erythrocytes suspension.Proceeding of 28th IEEE, EMBS. Annual International Conference .N.Y. USA.18-27.

[14] Yao X, Zheng JW, Jitendra M, Bao TH, Xin L (2011). Human red blood cell aging: Correlative changes in surface charge and cell properties .J. cellul.Mol.Medicine; vol. 15 (12). 2634-2642.

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APPENDIX – A

List Of Figures:

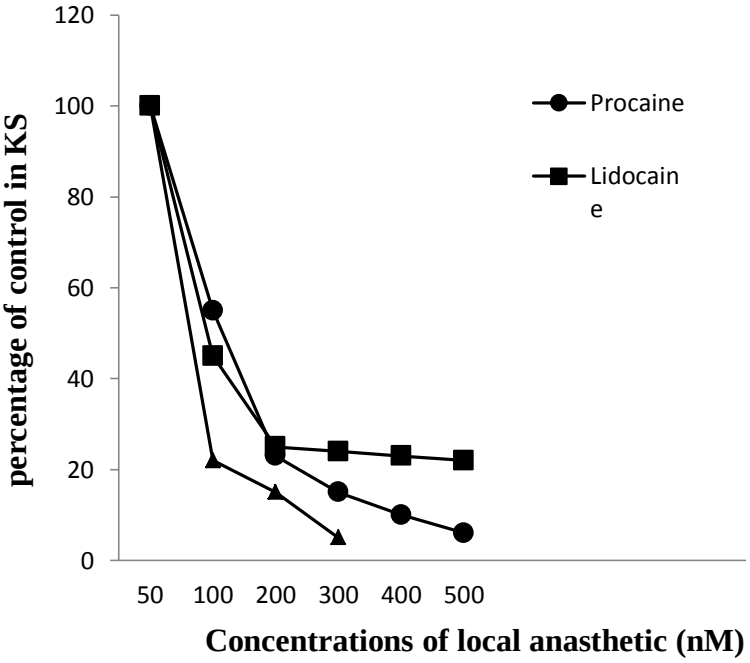

Figure: 1. Changes in concentration of the local anasthetics with KS.

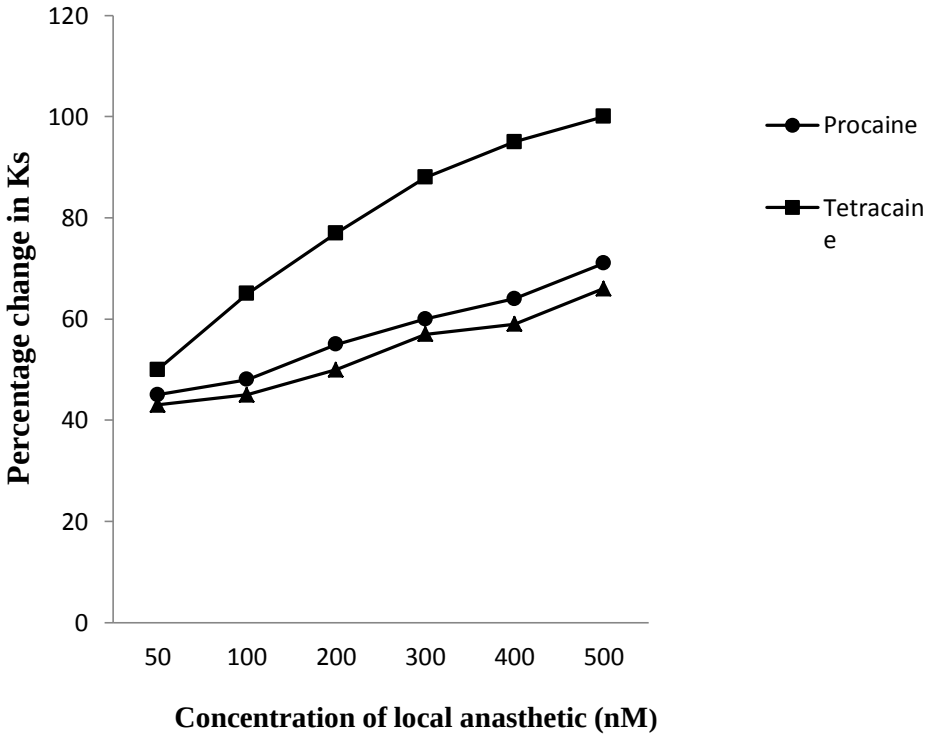

Figure: 2. Percentage changes from control value against drug concentration.

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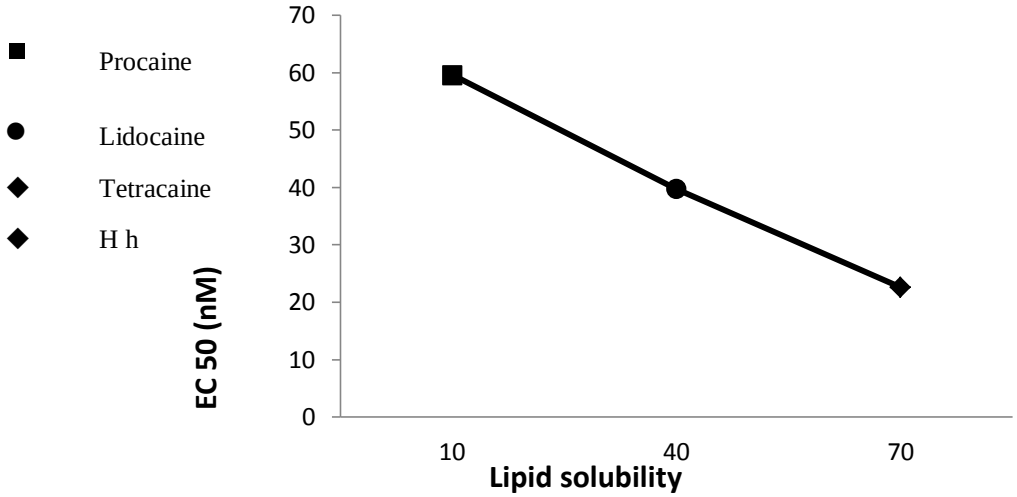

Figure: 3. Lipid solubility estimates against EC50 values.

APPENDIX – B

List Of Tables:

Table: 1.Variation of the membrane capacitance with the concentrations of anesthetic agent.

Concentration(nM)	Local anesthetic		
	Lidocaine	Procaine	Tetracaine
0	0.634	0.741	0.719
50	0.592	0.661	0.678
100	0.595	0.621	0.617
200	0.616	0.626	0.654
300	0.655	0.601	0.646
400	0.620	0.628	0.640
500	0.598	0.614	0.655