Research Journal of Pharmacology 4 (1): 1-4, 2010
ISSN: 1815-9362

Comparative *in vitro* Study of Relative Anesthetic Potency of Ropivacaine and Lidocaine

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Abstract: The purpose of this experimental study was the accurate evaluation of relative anesthetic potency of lidocaine and ropivacaine electrophysiologically *in vitro*. Total 18 sciatic nerves from male Wistar rats weighing between 250-300 g were used. The sciatic nerves were dissected from the spinal cord to the knee, immersed in a standard saline solution and mounted across a three-chambered recording bath. The 18 nerves were divided in 2 groups. In group A 300 μL of ropivacaine 0.2% and in group B 300 μL of lidocaine 0.2% were applied. Each local anesthetic remained in the perfusion chamber until the reduction of the compound action potential reach the 50% of the originally measured value (IT_{50}) and this reduction was accepted as establishment of anesthesia. Ropivacaine 0.2% was proved to be of statistically less potency regarding the establishment of anesthesia compared to lidocaine 0.2% ($p = 0.000497$) in the pH of 7.4. The IT_{50} for the nerves, which came in direct contact with ropivacaine 0.2% was estimated to be 25.28±7.61 min ($n = 9$). As for lidocaine 0.2% the IT50 was estimated to be 11.78±4.18.

Key words: Lidocaine, ropivacaine, comparative study, *in vitro*, sciatic nerve, compound action potentials

INTRODUCTION

Local anesthetics are drugs that block reversibly nerve conduction, when they are applied locally to nerve tissue at appropriate concentrations. They are classified in two major groups.

Esters (cocaine, benzocaine, procaine, chloroprocaine) Amides (lidocaine, mepivacaine, bupivacaine, prilocaine, etidocaine, articaine) (Malamed, 1980).

Onset of action, potency and duration of action are determined by the specific local anesthetic's pKa, lipid solubility, protein binding, tissue pH and vasodilatory effects. pKa is the primary factor that determines onset of action. Regarding potency, local anesthetics with high partition coefficients that increase lipophilic properties easily pass into the lipid nerve membrane. Duration of action is determined from the addition of epinephrine to some local anesthetic solutions that prolong duration of action by causing vasoconstriction and decreasing systemic absorption.

Also the degree of protein binding primarily determines duration of action with high protein binding increasing the duration (Wildmith, 1986). Ropivacaine is a relatively new long-acting local anesthetic introduced in the market in the last few years, that have been developed after reports of simultaneous seizure and cardiac arrest after accidental intravascular injection of bupivacaine (Leone *et al*., 2008). Ropivacaine is an amide local anesthetic with a chemical structure, pKa and protein binding that resemble bupivacaine (Concepcion *et al*., 1990; Moller and Covino, 1990). Initial studies in humans indicate that the potency and duration of action of ropivacaine are similar to those of bupivacaine (Brown *et al*., 1990).

In contrast to bupivacaine, ropivacaine produces less motor blockade and has a greater tendency to block A-delta and C fibers (Feldman and Covino, 1988).

These characteristics of ropivacaine may be advantageous for those experiencing acute and chronic pain. In humans, 20 mL of 0.5% ropivacaine or bupivacaine placed in the epidural space produces similar sensory and motor blocking characteristics, with the exception that bupivacaine produces a blockade of slightly longer duration (Brown *et al*., 1990). Ropivacaine dissociates from sodium channels more rapidly than bupivacaine, produces less accumulation of sodium channel blockade at physiologic heart rates and is less cardiotoxic than bupivacaine (Reiz *et al*., 1989; Moller and Covino, 1990; Feldman *et al*., 1989; Arlock, 1988).

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This drug is unique among local anesthetics because it is prepared as the S-isomer rather than as a racemic mixture. Previous studies involving the isomers of local anesthetics suggest that cardiac toxicity of the S-isomer may be less than that of racemic preparations. Indeed, ropivacaine's lipid solubility and depressant effect on cardiac excitation and conduction are intermediate between lidocaine and bupivacaine (Moller and Covino, 1990; Scott *et al*., 1989). Thus, even though the risk of cardiovascular toxicity has not been completely eliminated, it appears that ropivacaine offers clear advantages over bupivacaine and will likely replace bupivacaine in clinical practice once it is released for clinical use (Finucane, 1990).

There is an absence of studies evaluating relative potencies between lidocaine the gold standard for comparative purposes and ropivacaine at *in vitro* experiments and in equal concentrations. The purpose of this experimental study was the accurate evaluation of relative anesthetic potency of lidocaine and ropivacaine electrophysiologically in the sciatic nerve of the rat *in vitro*.

MATERIALS AND METHODS

Total 18 sciatic nerves from male Wistar rats weighing between 250 and 300 g were used. The animals were killed with deep anesthesia using sodium pentothione (100 mg kg^{-1} bodyweight i.p.). All experimental procedures were conducted in accordance with the protocols outlined by Aristotle University of Thessaloniki, Greece, regarding the recommended standard practices for Biological Investigations. The sciatic nerves of the rat were dissected from the spinal cord to the knee, immersed in a standard saline solution and mounted across a three-chambered recording bath, made of Plexiglas. The recording bath consists of three chambers. The first is the recording chamber, where the active electrode of an AC differentiate amplifier and the distal part of the nerve were immersed. The second is the perfusion chamber, where the 70% of the nerve was immersed and the grounds of recording and stimulating electrodes were immersed. The third is the stimulating chamber, where the proximal part of the nerve and the active stimulating electrode were immersed. For the electrophysiological experiments the three parts of the nerve in each of the chambers were electrically insulated with impression silicone paste Xanthopren (Heraeus Kulzer, Germany). When the impression paste dried, the three chambers were filled with an oxygenated physiological solution, enough to cover each of the three parts of the nerve, the composition of the saline being (in mM): 111 NaCl, 2.41 KCl, 10 HEPES, 2 $CaCl_2$ and 1.0 glycose (pH 7.4) in a temperature constantly maintained at 24°C. As an indication of the study physiological function of the nerve fibers in the isolated sciatic nerve, the Compound Action Potential (CAP) was generated and recorded using standard electrophysiology equipment. Samples of the evoked CAP were digitized and stored in a computer every min throughout the experiment. The amplitude was measured with a special software. The data were expressed as a percentages of the amplitude of the compound action potential after 1 h equilibration of the nerve in the saline, which served as a control. The percentage values were expressed as a mean±SD. The unpaired t-test was used to compare the values between the nerves after the application of the examined local anesthetics.

The 18 nerves were divided in 2 groups (A, B), A for ropivacaine and B for lidocaine. In each group of 9 nerves, a separate local anesthetic was applied. In group A 300 μL of ropivacaine 0.2% and in group B 300 μL of lidocaine 0.2% were applied. Each local anesthetic remained in the perfusion chamber until the reduction of the CAP reach the 50% (IT_{50}) of the originally measured value and this reduction was accepted as establishment of anesthesia. The recording of the compound action potentials was every minute, 30 min before the application of the anesthetics and after.

RESULTS AND DISCUSSION

The study of the electrophysiological properties of the nerve tissue from the rat permits the continuous and accurate monitoring of the effects of local anesthetics, when they are in close and direct contact with the nerve tissue. The advantage of the nerve of the rat is close to man in terms of physiology and biochemistry and in consequence is an attractive model for safety evaluation and biomedical research. This recording system has been used in a variety of *in vitro* neurotoxicological studies (Geronikaki *et al*., 2009; Moschou *et al*., 2008; Andreou *et al*., 2007).

In the experiments, the nerve was stimulated with supramaximal stimuli in order to activate all the axons and to obtain the maximum value of the CAP. The main advantage of the three-chambered bath, under normal conditions is that the amplitude of the evoked maximum CAP remains stable for a relatively long period of time, which is 20-22 h for the nerve of the rat.

Local anesthetics bind directly to the intracellular voltage-dependent sodium channels. Lipid solubility appears to be the primary determinant of intrinsic anesthetic potency. Chemical compounds, which are highly lipophilic tend to penetrate the nerve membrane more easily, so the fewer molecules are required for conduction blockade resulting in enhanced potency (Leone *et al*., 2008). After the application of the examined

local anesthetics there was a gradual decrease of the amplitude of the CAP and the time required for the CAP to reach 50% of the original before the application value was called IT_{50} and was measured in min. Ropivacaine is a relatively recent addition to the local anesthetic armamentarium.

There is an absence of studies evaluating relative potencies between lidocaine the gold standard for comparative purposes and ropivacaine, in clinical trials and *in vitro* experiments and in equal concentrations. Spinal block produced with 10 mg ropivacaine 5 mg mL^{-1} was as effective as that produced by 50 mg of lidocaine 10 mg mL^{-1} (Fanelli *et al*., 2009). Another study that compared the effectiveness of ropivacaine and lidocaine as paracervical for elective abortion showed that intraoperative pain was significantly lower in the ropivacaine group (Agostini *et al*., 2008) but lidocaine with epinephrine was more successful than ropivacvaine solutions in obtaining pulpal anesthesia as an intraligamentary anesthetic (Meechan, 2002). According to Ernberg and Kopp (2002), ropivacaine could be accepted as drug of choice in oral surgery because its very long duration of both pulpal and soft tissue anesthesia may be favorable in reducing postoperative pain.

El-Sharrawy and Yagiela (2006) studying different concentrations of ropivacaine blocking the inferior alveolar nerve concluded that the concentrations of 0.75 and 0.5% were effective for intraoral nerve blockade, with both a rapid onset and a prolonged duration of pain control. Other authors refer a relative slow onset for infiltration anesthesia and mandibular nerve block (Ernberg and Kopp, 2002; Oliveira *et al*., 2006).

In the study, ropivacaine 0.2% was proved to be of statistically less potency regarding the establishment of anesthesia compared to lidocaine 0.2% ($p<0.005$) in the pH of 7.4. The IT_{50} for the nerves, which came in direct contact with ropivacaine 0.2% was estimated to be 25.28±7.61 min (n = 9). As for lidocaine 0.2%, the IT_{50} was estimated to be 11.78±4.18. Statistical analysis of the IT_{50} valus of ropivacaine and lidocaine indicates that there was statistical significant difference in their action ($p = 0.000497$) (Table 1). This can be possibly explained from its higher pKa value.

Table 1: Depression of CAPs 50% (IT_{50}) establishment of anesthesia (time in min)

Anesthetic	1	2	3	4	5	6	7	8	9	X±SD
Ropivacaine (0.2%)	36	36.5	27	25.5	16	18	16.5	27	25	25.28±7.61
Lidocaine (0.2%)	8	6.0	13	14.0	9	19	9.0	16	12	11.78±4.18
t-test										
p = 0.000497										
p<0.005 S.S										

CONCLUSION

Total 8.1 from lidocaine 7.9, which means that a smaller percentage of molecules of free base can penetrate nerve membrane for conduction blockade in the environment of pH of 7.4, resulting in reduced potency. The absence of similar *in vitro* studies make obvious the need of a more extensive comparative experimental research of the anesthetic properties of ropivacaine.

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