An *in vitro* Experimental Study of the Action of Local Anaesthetics on Myocardium

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Purpose: The purpose of this study was to evaluate the effect of two local anaesthetics, mepivacaine and lidocaine with epinephrine, on the isolated heart of the frog *in vitro*, using basic electrophysiological methods.

Materials and Methods: The atria of the heart of 20 frogs were removed and the force and frequency of heart contraction was measured using appropriate data acquisition software. The isolated heart was first bathed in a control saline for 20 min. The control saline was then replaced with saline containing the compound under investigation and after 30 min. incubation the preparation was washed with saline for 20 min. Ten different experiments were conducted for each of the two applied local anaesthetics, mepivacaine and lidocaine with epinephrine 1:80000 having a concentration of 0.81 mM.

Results: Regarding mepivacaine, force and frequency measurements presented a negative inotropic action, while lidocaine with epinephrine revealed a positive one. After the preparation was washed with the control saline, both anaesthetics revealed similar recovery patterns.

Conclusions: In our experiment, the force of heart contraction induced by lidocaine was increased by about 40%, suggesting that lidocaine with epinephrine is more beneficial for the healthy myocardium.

Key words: mepivacaine, lidocaine, epinephrine, myocardium, heart contraction, heart rate

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INTRODUCTION

Local anaesthetic agents can produce profound effects in the cardiovascular system. The systemic administration of these agents can exert a direct action both on cardiac muscle and on peripheral vascular smooth muscle. In general, the cardiovascular system appears to be more resistant to the effects of local anaesthetic agents than the central nervous system (Scott, 1981).

Electrophysiological studies *in vivo* in both dogs and humans essentially reflect the *in vitro* findings observed in isolated cardiac tissue (Lieberman et al, 1968; Sugimoto et al, 1969). Studies also on the isolated whole rabbit heart have essentially confirmed the results observed on isolated atria (Block and Covino, 1982). Using a preparation similar to ours the isolated atria of either frog or rat have already been used for cardiotoxicological studies (Berlin et al, 1984; Monti et al, 1986; Schwartz et al, 1999). Local anaesthetic agents have profound effects on the mechanical activity of cardiac tissue. Detailed investigations on isolated cardiac tissues, isolated whole hearts, and intact animals have been carried out to elucidate the inotropic action of local anaesthetic drugs. Studies on isolated atria from

guinea pigs have shown that all local anaesthetics essentially exert a dose-dependent negative inotropic action (Feldman et al, 1982). The ability of local anaesthetic agents to affect the contractility of cardiac muscle is proportional to their ability to suppress conduction in peripheral nerves.

The purpose of this study was the *in vitro* comparative evaluation of the inotropic and chronotropic effects of two local anaesthetics widely used in clinical dentistry, namely mepivacaine and a solution of lidocaine with epinephrine, on the isolated frog atria. Frogs are considered as excellent animals for studies since their physiology is similar to man's. Moreover, a recent study confirms the presence of α_1-adrenoreceptors in the heart of amphibians and obtains values compared to those reported for the mammalian heart (Lazou et al, 2002).

MATERIALS AND METHODS

Twenty frogs (*Rana ridibunda*) of either sex, weighing between 140 and 160 g were purchased from local suppliers and kept in tanks with a temperature of 18±2 °C in alternating 12 h light and 12 h dark environment. All procedures were conducted in accordance with the protocols outlined by Aristotle University of Thessaloniki regarding the recommended standard practices for Biological Investigations. To isolate the atria of the heart of the frog, the whole ventricle was removed and the walls of the atria were dissected perpendicularly. Thus, the isolated left and right atria formed a strip. The strip was placed in the recording chamber in a small Petri dish half-filled with Sylgard™ resin, and bathed in an oxygenated physiological solution of the following concentration (in mM): 111 NaCl, 2.41 KCl, 5 HEPES, 2 $CaCl_2$ and 5.5 glucose, pH=7.2. The preparation was kept at room temperature. To monitor the force generated by the spontaneous contraction of the atria, the one end of the strip, the left atrium was pinned to the bottom of the chamber and the other end was attached to the arm of a force-displacement transducer (Grass Instrument Company, FT-03C). Using the micromanipulator, to which the transducer was fixed, the atria were stretched sufficiently by applying a force of 0.120-0.160 g to permit the monitoring of the force generated by their contraction. The saline solution, having a temperature of 23±2 °C, was dripped continuously into the recording chamber via an intravenous set, with a flow rate of 2-2.5 ml/min., and was removed via a suction system. The saline was stored in a 500 ml flask where it was oxygenated continuously with O_2 (100% oxygen). Under these conditions the isolated atria were able to maintain spontaneous contraction for more than 20 h. A similar experimental set-up has been used for the investigation of the effect of herbicides on the isolated heart of insect and amphibian (Papaefthymiou et al, 2002).

To investigate the action of mepivacaine and lidocaine with epinephrine 1:80000, the isolated heart of each frog was first bathed in control saline for 20 min., which was then replaced by saline where the local anaesthetic under investigation was diluted at a concentration of 0.81 mM. After 30 min. of incubation in the presence of the compound, the preparation was washed with saline for another 30 min. Ten experiments were conducted for each compound tested using the protocol described above.

In 12 additional experiments frog atria were also exposed to epinephrine alone in two different concentrations of 60 nM (n=6) and 10 nM (n=6).

From the heart contractions, which were monitored continuously during the experiment using appropriate data acquisition software, two main parameters were measured every 30 s: 1) the maximum force (F) of heart contraction; and 2) the frequency (f) of heart contractions, the latter being estimated from the time interval (T) between two contractions ($f=T^{-1}$). In the experiments described here, the relative percentage of force and frequency were plotted *vs* time.

RESULTS

The spontaneous contraction force of the isolated atria bathed in control saline and in saline containing the two different local anaesthetics used in our study is shown in Fig. 1.

The exposure of the atria to lidocaine with epinephrine 1:80000 causes an immediate increase in the force of heart contraction. The maximum effect is an increase of 38.5% relative to the control solution, observed 4.5 min. after the application of the compound. By contrast, exposure of the atria to mepivacaine causes an immediate decrease in the force of the heart contraction. The maximum effect is a decrease of 86% relative to the control, observed 30 min. from the application of the compound, just before the washing of the preparation with control saline. After the preparation was washed with the control saline there was no statistically significant difference between the two compounds in the recovery of the force of heart contraction.

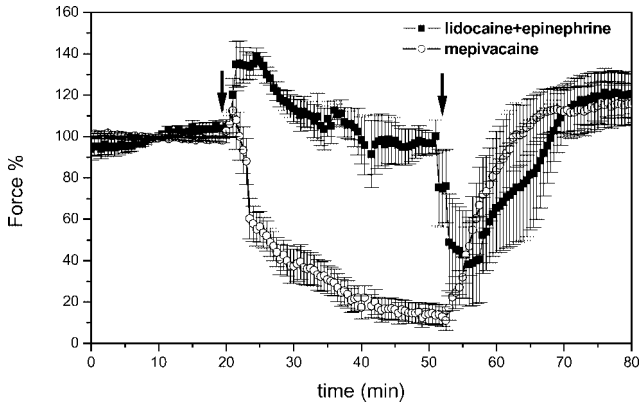

Fig. 1 Effect of local anaesthetics on the relative force of frog heart contractions.

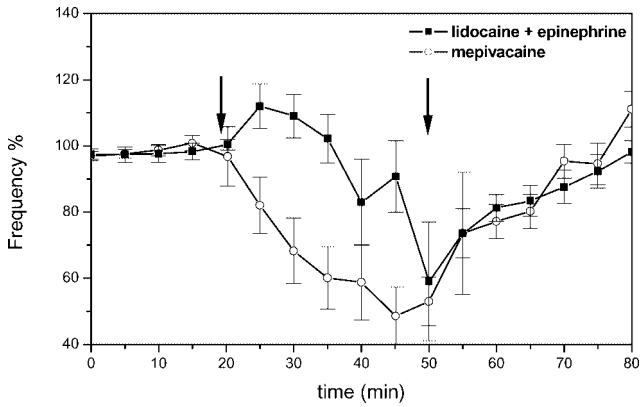

Fig. 2 Effect of local anaesthetics on the relative frequency of frog heart contractions.

Regarding the frequency of heart contractions, the lidocaine with epinephrine solution initially increases the heart rate by 10% for 3 min. after application, followed by a slow decrease parallel with that of mepivacaine (Fig. 2) until their removal from the saline and the end of their application. After the preparation was washed with the control saline there was no statistically significant difference between the recovery behaviours regarding the frequency of heart contractions.

Epinephrine alone causes positive inotropic and chronotropic action in the heart of the frog. When the atria were incubated in 60nM of epinephrine, there was a rapid increase in the power of contraction to about 30% of the control values (Fig. 3, curve with dark squares), and about a 10% increase in the frequency of the atria (frequency data not shown in the interests of diagrammatic clarity). Even during incubation at a lower epinephrine concentration of 10 nM, there was a 14±2% increase in the force contraction

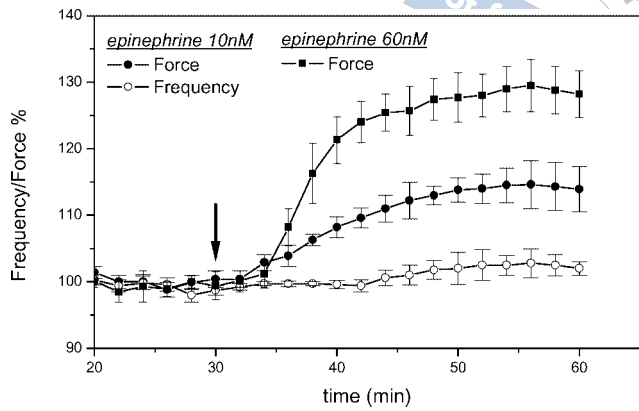

Fig. 3 Effect of epinephrine on the relative force and frequency of frog heart contractions.

(Fig. 3, dark circles), but there was no significant increase ($p=0.349$) in the frequency (Fig. 3, curve with open circles).

DISCUSSION

The results of our study confirm that local anaesthetic agents exert profound effects on the mechanical activity of cardiac muscle. Studies on isolated guinea pig atria and isolated whole rabbit hearts have also shown that all local anaesthetics exert a dose-dependent negative inotropic action (Block and Covino, 1982; Feldman et al, 1982). It is known that a sequence of cardiovascular events usually occurs following the systematic administration of local anaesthetic agents and part of the cardiac toxicity that results from high plasma concentrations of local anaesthetics occurs because these drugs block cardiac Na^+ channels responsible for the initiation of normal cardiac potentials.

Local anaesthetics produce a dose-dependent delay in the transmission of impulses through the cardiac conduction system by their action on the cardiac sodium channels (Clarkson and Hondeghem, 1985; Nath et al, 1986). If the sodium channel function is depressed then cardiac conduction is slowed and, if the depression is severe enough, conduction is stopped. In our experiment mepivacaine depressed myocardial contractility, as shown in Fig. 1. Sodium channel block develops during systole and dissipates during diastole. Both mepivacaine and lidocaine can block sodium channels rapidly during systole; however, bupivacaine, a highly lipid soluble local anaesthetic, dissociates from the sodium channel much more slowly during diastole and this accounts for this drug's persistent cardiac toxicity (Atlee and Bosnjak, 1990).

In our experiment, mepivacaine reduced the force of heart contraction by 50% in less than 6.5 min. from its application on the isolated atria and also caused an immediate decrease in the force of heart contraction and a rapid recovery after its removal from the atria. It is known that a drug's potency for blocking myocardial excitability is determined both by its intrinsic potency for blocking channels during individual action potentials and by its unblocking kinetics between action potentials. This kinetic feature of drug action has important consequences as well as for the cardiotoxicity of local anaesthetics (Clarkson et al, 1984). If a kinetically slow anaesthetic such as bupivacaine gains inadvertent access to the general circulation during regional anaesthesia this may explain why this drug has been reported to be more cardiotoxic (Albright, 1979) than the kinetically faster mepivacaine and lidocaine which is a reversible and selectively antiarrythmic drug with quick binding and dissociation to and from activated channel states.

Recovery in channel block is also dependent upon the drug studied. The recovery time constants are dependent on drug structure, drug size (primarily) and lipid solubility (secondarily) (Courtney, 1980a, 1980b). In our experiment, the force of heart contraction recovers to the original value after the removal of both drugs in a similar manner.

Although no cardiac irregularities were observed when lidocaine is given intravenously (Kotelko et al, 1984) and no cardiac arrhythmias were observed in anaesthetized dogs (Liu et al, 1982; Sage et al, 1983), epinephrine added to lidocaine affects the latter's antiarrythmic properties. In our experiment, we attribute the rapid initial increase in heart rate (Fig. 2) to the presence of epinephrine in the lidocaine solution. This is confirmed by the additional experiments with the heart exposed to 60 nM epinephrine alone, which led to the same increase in frequency (10%) as in the experiments with solutions of lidocaine with epinephrine (Fig. 3). On release from the adrenal medulla, epinephrine's natural functions include the regulation of myocardial contractility and heart rate. Epinephrine stimulates beta-1 receptors to cause an increase in systolic blood pressure, heart rate and cardiac output and speeds heart rate by accelerating the rate of spontaneous phase-4 depolarization. Norepinephrine affects a decrease of the heart rate and an increase of blood pressure for some minutes, a situation highly dangerous for elderly and hypertensive patients (Meyer, 1987). Although norepinephrine produces stronger hypertensive reactions, it has a weaker vasoconstrictor effect and it is very doubtful whether norepinephrine-containing anaesthetics offer any real advantage over those containing epinephrine (Okada et al, 1989). For all these reasons norepinephrine was excluded from our study as it is also not recommended for the daily praxis in dentistry.

The addition of epinephrine in lidocaine may explain the quick initial increase of the heart rate due to an enhancement of sympathetic activity by this agent (Kao and Jalar, 1959), but this stimulation is transient in nature lasting no more than 5 min. It is also known that extremely high concentrations of local anaesthetics will depress spontaneous pacemaker activity in the sinus node resulting in sinus bradycardia and sinus arrest.

The final reduction of the heart rate and the recovery from both local anaesthetics is an indication that the main heart pacemaker system was affected in a reversible way by both anaesthetics. The reduction in heart rate from both drugs in a parallel way possibly happens because the blocking of sodium channels by local anaesthetics may shift the threshold potential to more positive values and thus slow the automatic rate. The magnitude of this effect depends strongly on the rate the drug dissociates from blocked channels. Thus, the initial stimulation from epinephrine is only transient and the two drugs follow a common behaviour, lidocaine with epinephrine having the higher rate.

Our results show that epinephrine added to lidocaine modifies the pharmacological properties of the compound so that the final result is not a negative but a positive inotropic action. In our experiment lidocaine with epinephrine increased the force of heart contraction, which was confirmed by a transient cardiac stimulation lasting 1-5 min. following the injection of epinephrine (see Fig. 1). Epinephrine is usually added to local anaesthetic solutions to produce vasoconstriction, which limits systemic absorption by approximately one third and maintains the drug concentration in the vicinity of the nerve fibres to be anaesthetized, thus prolonging the duration of conduction block by approximately 50% (Scott, 1989). The pharmacologic effect of epinephrine includes the improvement of myocardial contractility as a positive inotrope (Goldberg and Cohn, 1987), a property that prevailed in our anaesthetic solution. In our experiment, the force of heart contraction induced by lidocaine was not only reduced (like in mepivacaine), but it was increased by about 40% (Fig. 1). As was observed regarding the frequency of heart contraction, we can also attribute the increase of the force of heart contraction to the addition of epinephrine in the local anaesthetic solution, as was shown by our experiments with epinephrine alone (30% in-

crease in power of heart contraction with 60 nM epinephrine (Fig. 3)).

Since frog hearts are considered to be physiologically similar to man's, we may conclude from the comparison in the action of the two local anaesthetics that lidocaine with epinephrine would be more beneficial than mepivacaine for the healthy human myocardium. We can argue that if there is no medical contraindication to the use of epinephrine, an epinephrine-containing solution may be of value because a sudden increase in heart rate and blood pressure is usually diagnostic of an intravascular injection. Regarding the toxicity of local anaesthetics, the decreased systemic absorption of local anaesthetic due to the vasoconstriction produced by epinephrine may also reduce the possibility of systemic toxicity.

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