Lidocaine alters the input resistance and evokes neural activity in crayfish sensory neurons

M. B. Keceli and N. Purali

Hacettepe University, Medical Faculty, Department of Biophysics, Sihhiye, Ankara, Turkey

Abstract. Lidocaine, a use-dependent Na^+ channel blocker, paradoxically evokes neural activation in the slowly adapting stretch receptor organ of crayfish at 5–10 mmol/l concentration. For elucidating the underlying mechanisms of this paradoxical effect, a series of conventional electrophysiological experiments were performed in the stretch receptor neurons of crayfish. In the presence of tetrodotoxin, lidocaine did not evoke impulse activity, however, a slowly developing and dose-dependent depolarization occurred in both the rapidly and slowly adapting stretch receptors. Similar effects were observed by perfusion of equivalent concentrations of benzocaine but not of procaine or prilocaine. Lidocaine did not evoke neural activity in the rapidly adapting neuron which fires action potential(s) in response to rapid changes in membrane potential. Slowly developing mode of the depolarization indicated the reason why only depolarization but not action potential responses were observed in the rapidly adapting neuron. The depolarizing effect of lidocaine was independent from any ionic channel or exchanger system. However, lidocaine and benzocaine but not procaine and prilocaine evoked a dose-dependent alteration in the input resistance of the neuron. It was proposed that the principal mechanism of the effect could stem from a change in the physical properties of the neuronal membrane.

Key words: Lidocaine — Stretch receptor — Sensory neuron — Input resistance — Benzocaine

Introduction

Slowly and the rapidly adapting stretch receptor neurons of the crayfish provides an exceptional experimental model to investigate the mechanisms of adaptation in a primary sensory neuron (Purali 1997). Previously it has been reported that the adaptive properties could be altered substantially by using some chemicals (Purali and Rydqvist 1998). In a former study, lidocaine has been used to suppress the typical non-adapting impulse response, observed solely, in the slowly adapting neuron. Paradoxically, 5–10 mmol/l lidocaine perfusion evoked a spontaneous neural activity in the slowly adapting neuron, however, a similar effect was not observed in the rapidly adapting neuron (Purali 2002). Excitatory effects of lidocaine have previously been reported in various neuronal preparations (Tanaka and Yamasaki 1966; Warnick et al. 1971; Seo et al. 1982; Richards and Dawson 1986; Brau et al. 1998; Onizuka et al. 2005). Genesis of those effects has been interpreted as being a consequence of either a blockage of a potassium channel population or a disinhibition of a neuron activity in a neuronal circuitry.

Aim of this study is to explain the possible mechanisms underlying the unexpected excitatory effect of lidocaine in slowly adapting stretch receptor neuron. Further, it is also worthwhile to investigate why the excitatory effect of the lidocaine is absent in the rapidly adapting neuron. In the present work, the conventional electrophysiological techniques were used to investigate the effects of lidocaine, benzocaine, prilocaine and procaine in various experimental conditions comparatively both in the slowly and rapidly adapting neurons.

Correspondence to: Nuhan Purali, Hacettepe University, Medical Faculty, Department of Biophysics, 06100 Sihhiye, Ankara, Turkey
E-mail: npurali@hacettepe.edu.tr

Materials and Methods

The experiments were carried out in the slowly and rapidly adapting stretch receptors of the crayfish *Astacus*

leptodactylus. In the use of experimental animals, the guidelines by Hacettepe University have been followed and Ethics Committee approval has been obtained. The stretch receptor pairs were prepared from the second to fourth abdominal segments of the crayfish as described previously (Purali 2002, 2003). An intracellular recording microelectrode was inserted into the neuronal soma to record either membrane potential or current. In voltage clamp experiments, leak and capacitative currents were corrected for by the method of Binstock and Goldman (1971). Experiments were carried out at room temperature (20–22°C) in the recording chamber of a volume of 0.5 ml at a constant perfusion rate.

Solutions

Composition of control solution (van Harreveld 1936) was (in mmol/l): 200 NaCl, 5.4 KCl, 13.5 $CaCl_2$, 2.6 $MgCl_2$ and 10 HEPES. 20 and 200 mmol/l tetraethyl ammonium chloride (TEA) solutions were prepared by substituting equivalent moles of NaCl. For preparation of sodium-free solution, sodium was substituted with 200 mmol/l N-methyl-D-glucamine (NMDG). Isotonic calcium solution was consisted of (in mmol/l): 5.4 KCl, 160 $CaCl_2$, 10 mmol/l HEPES. All solutions were titrated to a pH 7.4 and had an osmolarity of 420 ± 10 mOsm/l. Test chemicals were added to the experimental solutions. All drugs were obtained from Sigma (Chemical Co., St. Louis, USA).

Stimulation and recording

Glass microelectrodes were manufactured from glass capillary as has been previously described (Purali 2002, 2003). The electrodes (2–5 MΩ) were routinely filled with 3 mol/l KCl solution and in some experiments low chloride electrodes were prepared using 0.62 mol/l K_2SO_4 + 8.0 mmol/l KCl filling solution. Reference electrode was an Ag/AgCl wire immersed in bathing solution. Conventional current clamp technique was employed to record the membrane potential. The signals were digitised at 5–20 kHz sampling rate depending on the experimented parameter. K^+ current were recorded using the single-electrode voltage-clamp technique (EPC8 Heka, Germany) (Sakmann and Neher 1995). Stimulus wave form was generated in a computer and applied through a DAC to the amplifier.

Statistical analysis

The results are expressed as means ± standard error of the means (S.E.M). Student's *t*-test was used to define statistical

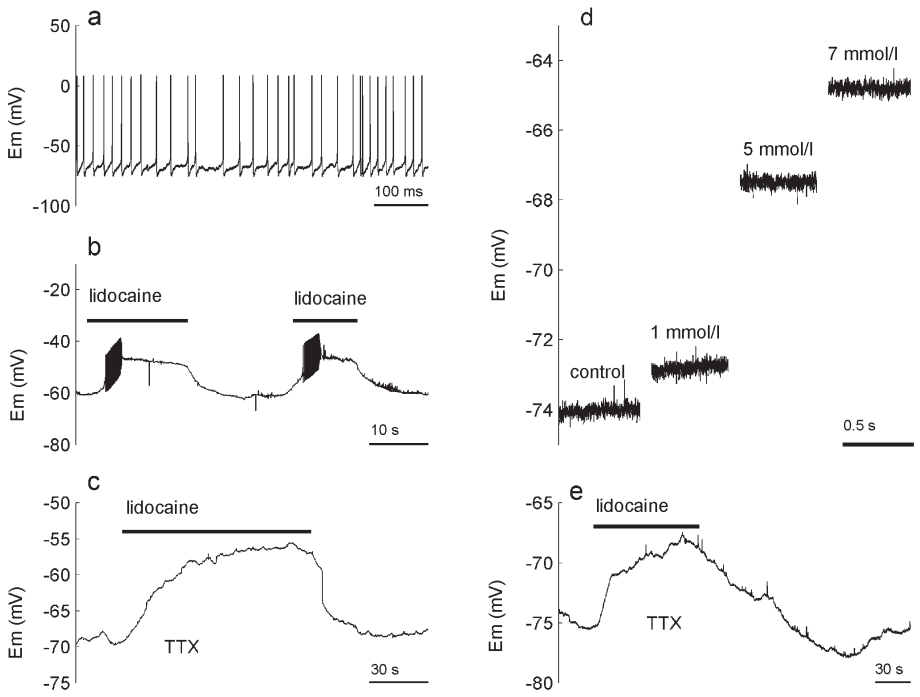

Figure 1. Effects of lidocaine exposure on membrane potential (Em). Spontaneous rhythmic (a) and bursting type (b) of neural activity induced by 5 mmol/l lidocaine exposure. Lidocaine (5 mmol/l) induced depolarization in the presence of TXT (0.3 μmol/l) in slowly (c) and rapidly (e) adapting neuron. Dose-dependent depolarization by lidocaine exposure (d). Recordings are from different slowly adapting neurons except (e) which is from a rapidly adapting neuron. Horizontal bar represents lidocaine exposure.

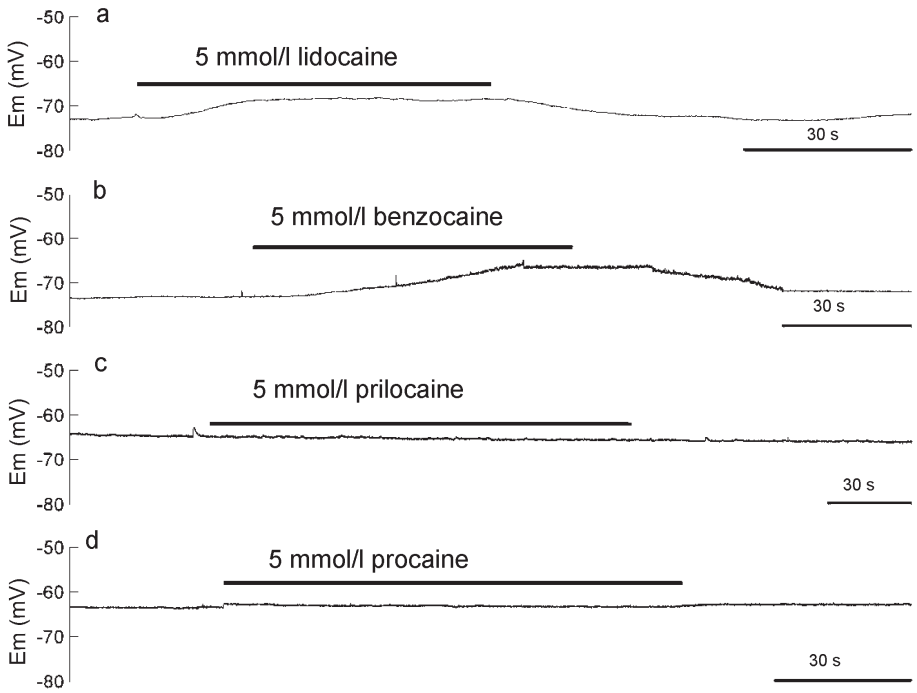

Figure 2. Effects of various local anesthetics on membrane potential (Em). TXT (0.3 μmol/l) is present in solutions and horizontal bar represents drug exposure. Recordings are from different slowly adapting neurons.

significance between the paired results. Grouped results were subjected to nonparametric Kruskal–Wallis test. Bonferroni's test was used for multiple comparison of the groups when previous test indicated a significant difference. $p < 0.05$ was considered statistically significant.

Results

Lidocaine, at 5 mmol/l or larger concentration, evoked spontaneous neural activity in the slowly adapting stretch receptor neuron. Majority of the receptor neurons developed a rhythmic spontaneous activation. However, in about 25% of the neurons a spontaneous bursting type of activation was observed (Fig. 1a,b). Firing frequency was related to the concentration of the lidocaine. The effect was removed by perfusing the preparation in control solution. In one of the slowly adapting receptor neurons with the most negative resting membrane potential, lidocaine concentration had to be increased to 7 mmol/l to induce a spontaneous activation. At higher concentrations, i.e. 10 mmol/l or larger, lidocaine blocked both the spontaneous neural activity and the evoked impulse responses to a current stimulation. However, exposing the rapidly adapting neuron to 1–10 mmol/l lidocaine did not evoke any neural activity (Fig. 1e).

In the presence of 0.3 μmol/l tetrodotoxin (TTX), a specific Na^+ channel blocker, exposing both the slowly and rapidly adapting neurons to 1–7 mmol/l lidocaine, induced a slowly developing depolarization amplitude which was washed away by perfusing the neuron in the control solution (Fig. 1c and e). The magnitude of the depolarization was dependent on the lidocaine concentration. Lidocaine at concentration 1, 5 and 7 mmol/l caused 4.3 ± 0.2 mV ($n = 11$), 7.26 ± 0.2 mV ($n = 10$), and 10.69 ± 0.3 mV ($n = 9$) depolarization, respectively (Fig. 1d). The results were significantly different comparing with the control level ($p \leq 0.001$). The differences between 1, 5 and 7 mmol/l lidocaine-exposed groups were also significant ($p \leq 0.001$).

A similar depolarizing effect was observed when the receptor neurons were perfused with 2.5–5 mmol/l benzocaine (Fig. 2b). 5 mmol/l benzocaine perfusion evoked 11.6 ± 1.7 mV depolarization ($n = 6$, $p \leq 0.001$). Properties of the depolarizing response were similar to that observed when the neuron was exposed to lidocaine. However, neither prilocaine (5–10 mmol/l, $n = 4$) nor procaine (5–10 mmol/l, $n = 4$) exposure evoked a depolarization amplitude or a form of neural activity (Fig. 2c and d).

In the presence of 5 to 10 mmol/l lidocaine, neither outward potassium currents nor the related current voltage relationship was different than that in the control solution

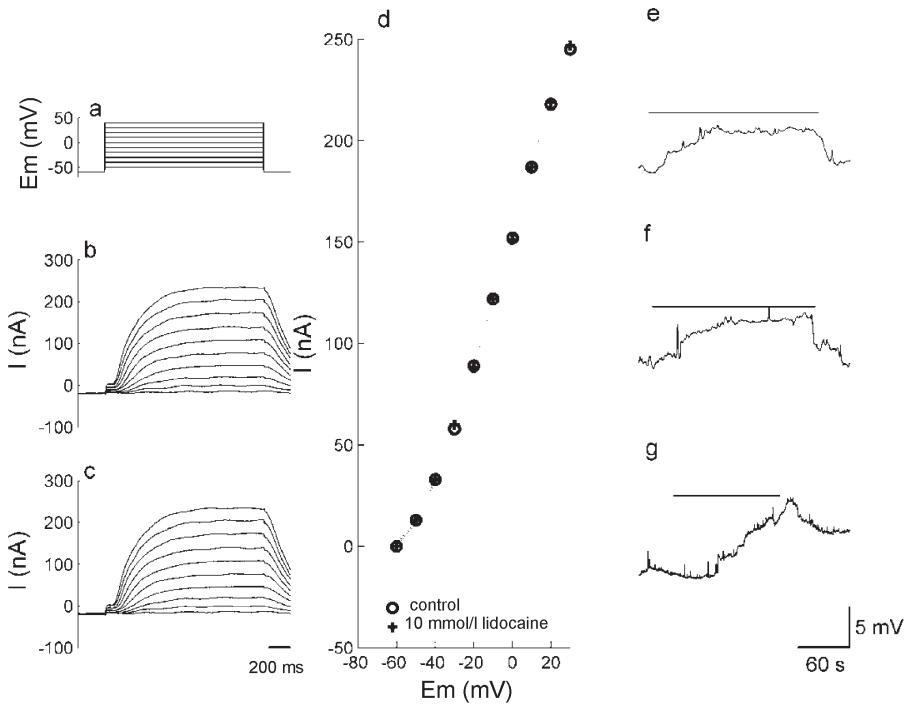

Figure 3. A family of potassium currents to voltage clamp steps (a) from resting level (–66 mV) in the control (b) and 10 mmol/l lidocaine (c) solution. Current voltage relationship for the recorded potassium currents (d). Lidocaine-induced potential responses in control (e), 20 mmol/l TEA (f) and 200 mmol/l TEA (g) solution. TXT (0.3 μmol/l) is present in solutions and horizontal bar represents lidocaine exposure. Em indicates membrane potential and recordings are from different slowly adapting neurons.

(Fig. 3a–d). Further, depolarizing effect of lidocaine exposure was observed when the potassium channels were blocked by 20 or 200 mmol/l TEA solution (n = 4, Fig. 3e–g).

The depolarizing effect of (5 mmol/l) lidocaine exposure was observed in the presence of 0.5 mmol/l verapamil (n = 3), 0.1 mmol/l picrotoxin (n = 3), or when low chloride (8 mmol/l KCl) microelectrode solution (n = 3) was used (not shown). Further, in the presence of 2 mmol/l ouabain or 2 mmol/l probenecid (n = 3), lidocaine exposure induced a depolarization amplitude similar to that in control solution (not shown). Neither NMDG (n = 4) substitution for the total extracellular sodium content nor isotonic calcium solution perfusion (n = 6) changed the lidocaine-induced depolarization (not shown). The input resistance of the neurons, as determined by injecting increasing amplitudes of a constant negative current, changed significantly in the presence of lidocaine. Membrane potential differences (ΔE) to current stimulation were recorded and compared with the ones recorded in the presence of 5 and 7 mmol/l lidocaine. As shown in the Fig. 4A, the ratio of potential difference to the current stimulus, giving the input resistance of the neuron, was 3.65 ± 0.8 MΩ (n = 9), and increased significantly to 6.93 ± 0.16 MΩ (n = 4) and to 10.89 ± 0.7 MΩ (n = 3), when 5 and 7 mmol/l lidocaine was present in the solution, respectively ($p \leq 0.001$). The increase was dependent on the lidocaine concentration and was completely reversible.

However, input resistance of the neurons was reversibly reduced from 3.56 ± 0.1 MΩ to 1.88 ± 0.05 MΩ when exposed to 5 mmol/l benzocaine (n = 5, $p \leq 0.001$) (Fig. 4B). Neither 10 mmol/l prilocaine nor procaine exposure induced a significant change in input resistance of the neurons.

Discussion

Present findings are relevant to the previous report (Purali 2002) indicating that the slowly and the rapidly adapting neurones responded differently to lidocaine exposure. Neural activity was not evoked in the rapidly adapting neuron when exposed to lidocaine. The difference may stem from the distinct adaptive properties of the slowly and the rapidly adapting neurons (Purali 1997, 2002).

Previous studies on the abdominal stretch receptor neurons of crayfish emphasizes that the rapidly adapting receptor neuron would preferably transduce the rapid changes in

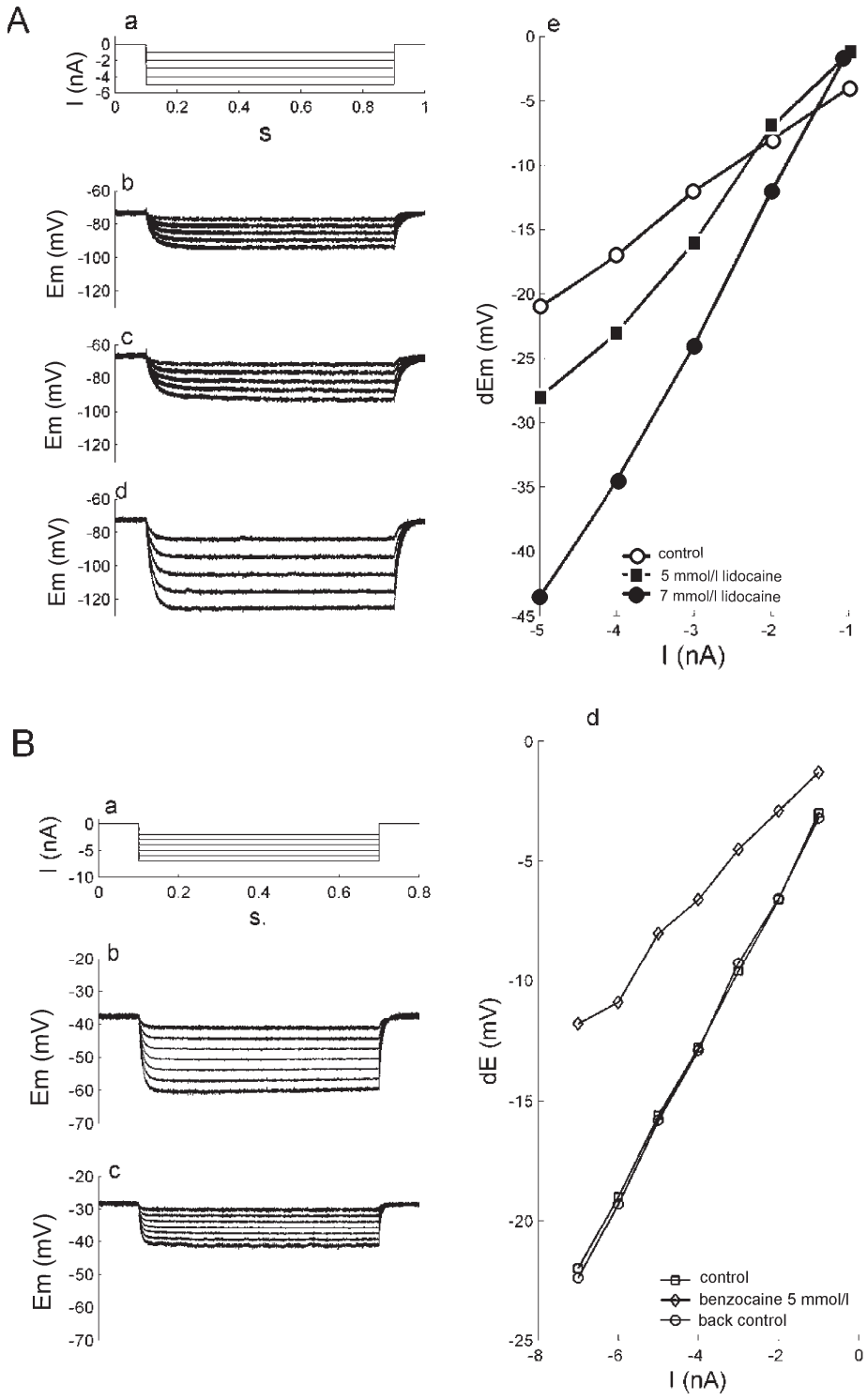

Figure 4. Comparison of the input resistance of the neurons in the presence of lidocaine and benzocaine. A. Potential responses to constant current injections (a) in control (b), 5 mmol/l lidocaine (c) and 7 mmol/l lidocaine (d) solution. The resulted current-voltage relationships (e). B. Potential responses to constant current injections (a) in control (b), 5 mmol/l benzocaine (c) solution. The resulted current-voltage relationships (d). TXT (0.3 μmol/l) was present in solutions. Em indicates membrane potential and recordings are from different slowly adapting neurons.

the membrane potential (Purali and Rydqvist 1998; Purali 2002, 2005). Thus, a slowly developing depolarization may fail to evoke any impulse response even when the threshold potential is achieved. In the slowly adapting neuron, a supra-threshold depolarization would promptly evoke a neural activity irrespective of the rate of potential change, whenever the threshold level is achieved (Purali 2002). Thus, the factor(s) inducing neural activity in the presence of lidocaine in the slowly adapting neuron might fail to generate any action potential in the rapidly adapting neuron since it has a considerably slow time course.

As can be followed in Fig. 1b,c. lidocaine exposure evokes a tonic depolarization amplitude which can be isolated by TTX treatment. The magnitude of the tonic component was similar to that obtained in TTX-free solution (cf. Fig. 1b,c). Thus, it is conceivable to assume that the major event leading to the neural activity increase should be the depolarization induced by lidocaine exposure. Lidocaine-induced depolarization may not be mediated by any mechanisms related to the sodium ions since, it is observed in sodium-free, TTX and ouabain containing solutions (Fig. 1c–e). Further, tonic depolarization is dose-dependent (Fig. 1d) as is the frequency of the evoked firing activity.

A number of authors have reported that lidocaine has a potency to block the voltage-gated potassium channels in various preparations (Richards and Dawson 1986; Brau et al. 1998). We have performed voltage and some current clamp experiments to asses the contribution of a possible outward potassium current blockage in development of the lidocaine-induced depolarization. Considering the compiled data, we presume that the lidocaine-induced depolarization may not be mediated through the potassium channels since the potassium currents are similar in control and lidocaine-containing solution (Fig. 3b,c.) and lidocaine perfusion evokes similar depolarization amplitude in control and in 20 and 200 mmol/l TEA containing solution (Fig. 3e–g).

Stretch receptor neurons receive some GABAergic inhibitory innervation (Krawitz et al. 1963; Kaila et al. 1992). It might be proposed that lidocaine may interfere with the inhibitory system. However, we presume that the relevancy of the hypothesis is less likely, since a similar depolarization amplitude is observed in presence of either verapamil (0.5 mmol/l) or picrotoxin (0.2 mmol/l) and also when low chloride electrodes were used.

In order to asses a possible interaction of lidocaine with the exchangers present in the neuronal membrane, we have performed some experiments in the presence of some exchanger-blockers (ouabain, probenecid) and in sodium-free and isotonic calcium solutions. However, in either condition, lidocaine evoked depolarization amplitude similar to that in control condition indicating the absence of such an interaction.

It was previously reported that local anesthetics interact with mechanosensitive channels (Lin and Rydqvist 1999). However, in our experimental conditions, the receptor muscle is completely slack and consequently mechanosensitive channels are at a closed state. For this reason it is less likely to propose that mechanosensitive channels would take part in the generation of depolarizing effect of lidocaine (Rydqvist and Purali 1993).

Local anaesthetics are consisted of a large group of chemicals (Hondeghem and Katzung 1980). We investigated if the depolarizing effect observed by lidocaine exposure is common to the other members of the group. At 1–10 mmol/l concentrations only lidocaine and benzocaine induced a tonic depolarization while prilocaine and procaine had no depolarizing effect (Fig. 2c,d).

Compiled data suggests that the mechanisms leading to the observed depolarization and neural activation by lidocaine and benzocaine may not be related to any sort of ionic channel or ion exchanger. Thus, conceivable to question the existence of a direct effect on physical properties of the neuronal membrane. Considering the molecular form and site of action of lidocaine, it is important to note that pKa is 7.8 and consequently 65% of lidocaine is found in polar and 35% nonpolar form in the control solution (pH 7.4). Nonpolar form may accumulate and alter the physical properties of the neuronal membrane. For testing the hypothesis, we have measured the input resistance of the neuron in the presence and absence of lidocaine since input resistance is a basic physical membrane property. As shown in Fig. 4A lidocaine exposure increases the input resistance of the neuron in a reversible and dose-dependent manner. The properties of the changes in input resistance correlates with those observed in membrane potential when neuron is exposed to lidocaine (see. Fig. 4Ae and Fig. 1d). It could be proposed that depolarizing effect of lidocaine would be related to its effect on input resistance of neuronal membrane. However, it is not possible to conclude if there is causal relationship between the increase in the input resistance and depolarizing effect of lidocaine. It should be emphasized that the input resistance is one of the principle physical properties of the neural membrane and the measuring method used in the present work is rather simple and reliable. Thus, we propose that lidocaine may alter the physicochemical properties of neuronal membrane, which may evoke the observed changes. In addition, benzocaine, which has a similar depolarizing effect, reduces the input resistance of the neuron apparently (Fig. 4B). However, procaine and prilocaine neither depolarized the neuron nor induced a change on the input resistance. Thus, depolarizing effect of the some local anesthetics is observed if they alter the input resistance of the neuron.

Compiled data indicates that the mechanisms leading to the observed effects of lidocaine exposure may not be related to any sort of ionic channel or ion exchanger. Lidocaine may mediate its effect *via* a direct effect on physical properties of

the neuronal membrane. However, further investigation is required to identify the exact molecular mechanism(s) of the interaction.

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