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Membrane effect of lidocaine is inhibited by interaction with peroxynitrite

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Abstract
Inflammation is clinically well known to decrease the efficiency of local anesthesia, an effect which has been explained mechanistically by tissue acidosis in the literature. However, recent studies offer no support to such a pharmacopathological background for anesthetic failure. Because inflammatory cells produce significant amounts of peroxynitrite, the peroxynitrite could interact with local anesthetics to decrease their effects. To examine this speculated interaction, we determined whether membrane fluidization, as one mode of local anesthetic action, was influenced by peroxynitrite. The membrane effects were analyzed by measuring the fluorescence polarization of liposomes prepared with 1, 2-dipalmitoylphosphatidylcholine. Although lidocaine, at a clinically relevant concentration, fluidized liposomal membranes, its fluidizing potency was reduced to 43.6 ± 4.4% and 58.4 ± 7.5% of that in membranes without peroxynitrite when membranes were pretreated with 50 and 250 μM peroxynitrite, respectively, for 15 min. A significant inhibition of membrane fluidization of 27.5 ± 6.8%, was also observed after reaction for 5 min. Peroxynitrite released by inflammatory cells may affect local anesthesia through a possible interaction with lidocaine, inhibiting its membrane-fluidizing effect.

Key words Lidocaine · Inflammation · Anesthetic failure · Interaction with peroxynitrite · Membrane fluidization

Inflammation interferes with the effects of local anesthetics, producing poor analgesia in patients with these agents administered into or around inflamed tissues. Especially in dentistry, this clinical feature is so problematic that teeth with pulpal, periodontal, and alveolar inflammation are difficult to anesthetize by infiltration and nerve block injections, leading to failure to obtain satisfactory pain relief [1,2]. Among pharmacopathological backgrounds of the effects of inflammation on local anesthesia proposed previously, a correlation with tissue acidosis has been most frequently cited in the literature [3,4]. In this hypothetical mechanism, the efficiency of local anesthesia is thought to be suppressed because the lowering of pH significantly decreases the levels of active nonionized molecules of drugs in inflamed tissues. However, recent studies are in conflict with this theory. The acidification was revealed to be lower only on the order of 0.5 pH units in experimentally inflamed tissues [5]. In animal experiments, tissues could rapidly buffer the excess acidity after infiltration with acidic lidocaine solutions [6] and such a pH buffering capacity was rather enhanced in inflamed tissues compared with normal tissues [7]. While membrane fluidization or disordering has been referred to as one mode of local anesthetic action [8], it was proved to occur essentially even under inflammatory acidic conditions, not supporting the conventional idea of an acidosis mechanism [9].

Peroxynitrite is a strong oxidant, formed by the reaction of nitric oxide and superoxide anion. Inflammatory cells produce a significant amount of peroxynitrite [10], which has been implicated in the pathophysiology of inflammation [11]. Peroxynitrite coexists with local anesthetics in affected tissues in infiltration and topical anesthesia where the drug solutions are applied directly into or close to the area of inflammation. Several local anesthetics inhibit lipid peroxidation [12], suggesting that they may be structurally transformed through the antioxidant action, thus decreasing their pharmacological activity. Peroxynitrite was recently reported to influence the effect of lidocaine on trigeminal nerve response [13]. Propofol, a membrane-active agent similar to lidocaine, is also known to interact with peroxynitrite and to then show structural change [14]. Therefore, local anesthetics and inflammatory-cell peroxynitrite are speculated to show an interaction which may be related to the suppression of local anesthesia. In the present

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study, we aimed to assess this speculated interaction by determining whether the effect of lidocaine on lipid membranes was influenced by peroxynitrite.

Liposomes of the lipid bilayer structure (lipid concentration, 0.14 mM) were prepared with 1, 2-dipalmitoylphosphatidylcholine (DPPC; Avanti Polar Lipids, Alabaster, AL, USA), and labeled with 1, 6-diphenyl-1, 3, 5-hexatriene (DPH; Molecular Probes, Eugene, OR, USA), as reported previously [15]. Lidocaine (final concentration, 2.0 mg·ml^{-1}) in dimethyl sulfoxide, corresponding to the concentration range of injectable preparations [3], was applied to liposomal suspensions in 16 mM sodium phosphate buffer (pH 7.4, containing 100 mM KCl). The reaction mixtures were incubated with peroxynitrite (10–250 μM) or without (control) at 37°C. Peroxynitrite in 0.1 M NaOH was added to the suspensions, with vortex-mixing for 5 s. The treatment concentrations of peroxynitrite were chosen with reference to its in vivo quantitative monitoring [16] and in vitro application [17]. The concentration of dimethyl sulfoxide was adjusted to be less than 0.5% (v/v) of the total volume so as not to influence the fluidity of liposomal membranes. After incubation for 5–30 min, DPH fluorescence polarization was measured by the method of Tsuchiya [18]. Compared with control, decreased and increased DPH polarization means the enhancement (membrane fluidization) and the reduction of membrane fluidity (membrane rigidification), respectively. Values for results are expressed as means ± SEM (n = 7). Data were statistically analyzed by one-way analysis of variance (ANOVA), followed by post-hoc Fisher's protected least significant difference (PLSD) test (StatView 5.0; SAS Institute, Cary, NC); P values of less than 0.05 were considered significant.

The blockade of sodium channels is the primary mechanism underlying local anesthesia. In addition, local anesthetics are known to affect various membrane proteins, including potassium channels, calcium channels, nicotinic receptors, muscarinic receptors, and Na, K-ATPase, which are potentially responsible for local anesthesia [19,20]. Such broad pharmacological spectra are interpretable by a common mode of action on membrane lipids [8,21]. In regard to the distribution of local anesthetics during nerve block, they have to not only pass through lipid barriers such as nerve sheaths but also penetrate into the lipid bilayers of cell membranes to reach and bind to channel proteins [3]. The drug-induced changes in membrane fluidity modify the conformation of functional proteins by altering their lipid environments. Therefore, the membrane effects of local anesthetics are both directly and indirectly associated with the function of sodium channels.

The DPH polarization changes demonstrated the membrane effect of lidocaine that fluidized DPPC membranes at a clinically relevant concentration.

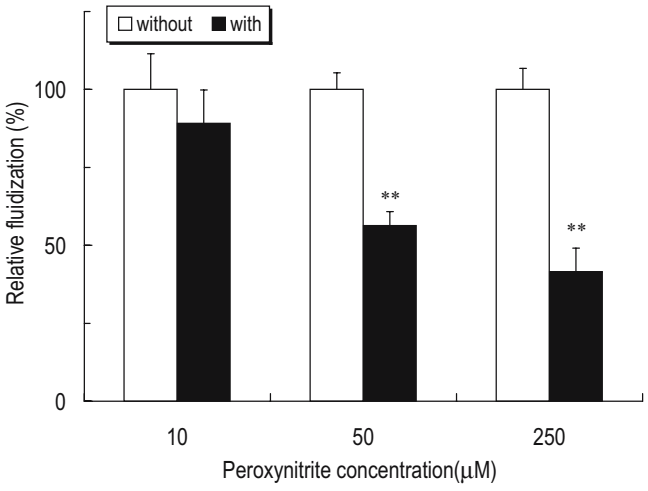

Fig. 1. Effect of peroxynitrite on lidocaine-induced membrane fluidization. Lidocaine (2.0 mg·ml^{-1}) was applied to 1, 2-dipalmitoylphosphatidyl-choline (DPPC) liposomes for 15 min with or without peroxynitrite at the indicated concentrations. DPH (1, 6-diphenyl-1, 3, 5-hexatriene) polarization change with lidocaine 2 mg·ml^{-1} in the absence of peroxynitrite was taken as 100%. Relative membrane fluidization was determined based on DPH polarization decreases. $^{**}P < 0.01$ compared with without peroxynitrite

However, when DPPC membranes were treated with lidocaine in the presence of peroxynitrite for 15 min, the lidocaine-induced fluidization was significantly attenuated in a manner that depended on peroxynitrite concentrations (Fig. 1). Treatment with 50 and 250 μM peroxynitrite reduced the membrane-fluidizing potency by 43.6 ± 4.4% and 58.4 ± 7.5%, respectively, of that without peroxynitrite.

The effect of lidocaine on DPPC membranes was inhibited by 250 μM peroxynitrite with increasing reaction time (Fig. 2). An inhibition of of 27.5 ± 6.8% was observed after 5 min, indicating that the interaction between lidocaine and peroxynitrite had proceeded rapidly. The inhibitory effect of peroxynitrite reached a plateau after 15-min reaction.

Nerve cell membranes consist of different unsaturated phospholipids [22]. Unsaturated acyl chains of phospholipids are oxidized by superoxides, consequently making membranes more rigid [23]. When one is investigating the interaction of membranes with agents that act on membranes, the polarization data should be discussed apart from the direct effect of peroxynitrite on lipid membranes. In the present study, liposomal membranes were prepared with 100 mol% DPPC in order to focus on the interaction between lidocaine and peroxynitrite.

When DPPC liposomes were treated with 250 μM peroxynitrite alone, DPH polarization increased after 30 min, but not after 5–15 min (Fig. 3). Because lidocaine was absent in the reaction mixtures, the increasing

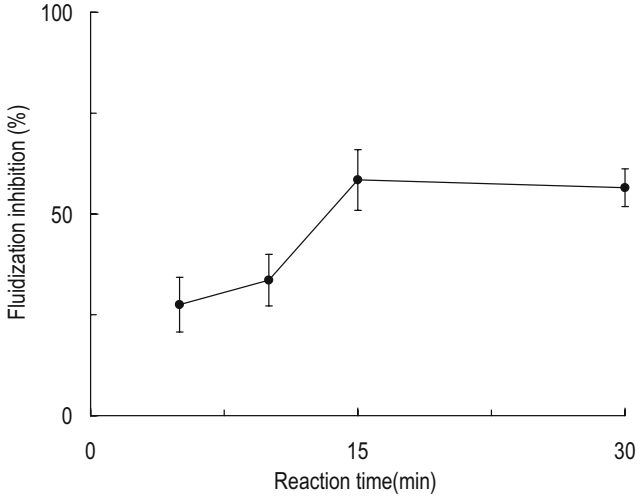

Fig. 2. Time-dependent effect of peroxynitrite on lidocaine-induced membrane fluidization. Lidocaine ($2.0\,mg{\cdot}ml^{-1}$) was applied to DPPC liposomes for the indicated times with or without peroxynitrite ($250\,\mu M$). The DPH polarization change with lidocaine $2\,mg{\cdot}ml^{-1}$ in the absence of peroxynitrite was taken as 100%. The inhibition of membrane fluidization was determined by comparing DPH polarization decreases

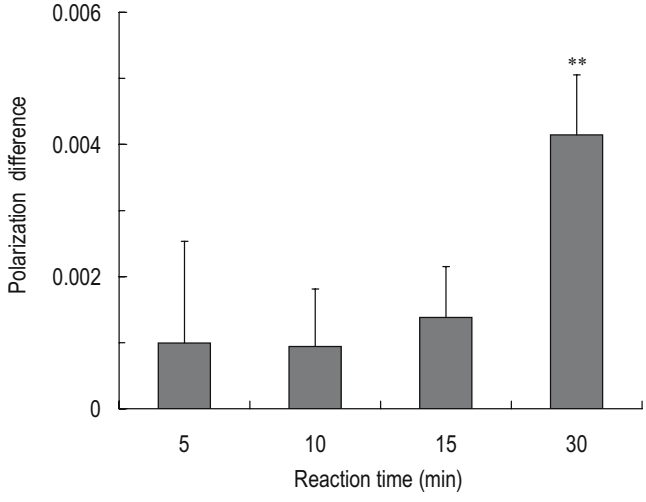

Fig. 3. Membrane DPH polarization change induced by peroxynitrite. DPPC liposomes were treated with peroxynitrite ($250\,\mu M$) for the indicated times. The DPH polarization difference was obtained by comparing results between DPPC liposomes treated with peroxynitrite and the control. $^{**}P < 0.01$ compared with control

change of polarization values was attributed to: (1) fluidity decreased by the lipid peroxidation, and/or (2) seemingly changed fluidity induced by DPH decomposition. The lipid composition of the liposomes we used was 100 mol% saturated phospholipids, so the lipid-oxidative effect of peroxynitrite was not responsible for the resulting polarization increase. The fluorophore of DPH is chemically modified during lipid peroxidation, possibly reflecting altered polarization [24]. Such a possibility was excluded by evaluating the fluidity within 15 min after the treatment of DPPC liposomes with lidocaine and peroxynitrite.

In conclusion, inflammatory-cell peroxynitrite is considered to interact with lidocaine and inhibit its membrane-fluidizing effects. This interaction may be related to the failure of local anesthetic in inflamed tissues.

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