Dual Effect of Local Anesthetics on the Function of Excitable Rod Outer Segment Disk Membrane

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The effects of local anesthetics and a divalent cation, \( \text{Ca}^{2+} \), on the function of rhodopsin were estimated from the measurements of light-induced proton uptake. The light-induced proton uptake by rhodopsin in the rod outer segment disk membrane was enhanced at lower \( \text{pH} \) (4) but depressed at higher \( \text{pH} \)s (6 to 8) by the tertiary amine local anesthetics lidocaine, bupivacaine, tetracaine, and dibucaine. The order of local anesthetic-induced depression of the proton uptake followed that of their clinical anesthetic potencies. The depression of the proton uptake versus the concentration of the uncharged form of local anesthetic nearly describes the same curve for small and large dose of added anesthetic. Furthermore, a neutral local anesthetic, benzocaine, depressed the proton uptake at all \( \text{pH} \)s between 4 and 7. These results indicate that the depression of the proton uptake is due to the effect of only the uncharged form. It is hypothesized that the uncharged form of local anesthetics interacts hydrophobically with the rhodopsin in the disk membrane. The dual effect of local anesthetics on the proton uptake, on the other hand, suggests that the activation of the function of rhodopsin may be caused by the charged form. There was no significant change in the light-induced proton uptake by rhodopsin when 1 \( \text{mM} \) of \( \text{Ca}^{2+} \) was introduced into the disk membrane at varying \( \text{pH} \)s in the absence of local anesthetics. This fact indicates that \( \text{Ca}^{2+} \) ion does not influence the diprotonating process of metarhodopsin; neither does it interfere with the local anesthetic-induced changes in the rhodopsin molecule. (Key words: Anesthetics, local: benzocaine; bupivacaine; dibucaine; lidocaine; tetracaine. Ions: calcium. Theories of anesthetics.)

Tertiary amine local anesthetics can inhibit the conduction of nerve impulses, thus preventing the rapid influx of sodium ions through sodium channels in the axon membranes. It has been demonstrated that the amount of local anesthetic molecules binding to artificial lipid and biologic membranes correlates with the anesthetic potency. Ueda et al. also reported a good correlation between the depression on the phase-transition temperature in the artificial phospholipid membrane by local anesthetics and their nerve-blocking potencies. A spin label study and proton nuclear magnetic resonance studies showed that the lipid hydrocarbon tails in the nerve membrane rotated, bent more easily, and became less ordered in the presence of local anesthetics. McLaughlin also reported that local anesthetics absorbed to lipid bilayers and changed their surface potentials. These findings suggest that membrane lipids play a role in the molecular mechanism of local anesthetic action. Electrophysiologic studies, however, have indicated that sodium channels are directly involved in the mechanism of local anesthetic action. Studies using the voltage clamping technique revealed that local anesthetics reduced the early, transient current attributable to sodium ions more than the late, steady-state current attributable to potassium ions. This fact means that local anesthetics selectively block the increase in sodium permeability by inhibiting sodium channels. The sodium channel is regarded as a membrane protein that is embedded in the nerve membrane lipids.

Although local anesthetics are certainly capable of interaction with membrane lipids, much less is known about their interaction with membrane proteins. It is, therefore, a requisite for molecular mechanism of local anesthetic action to account for the functional effects of the drug on membrane proteins. In this study, a retinal rod outer segment (ROS) disk membrane was used as a model system to determine how local anesthetics affected the function of membrane proteins.

The visual pigment rhodopsin constitutes about 90% of the ROS disk membrane protein, and the major portion of its mass is thought to penetrate into the phospholipid bilayer of the disk membrane. Rhodopsin is directly involved in early molecular events of vision. These light-induced changes result in proton uptake by the ROS disk membranes. In this study, the effects of local anesthetics on rhodopsin in the ROS disk membranes were examined by measuring the light-induced proton uptake.

Materials and Methods

The retinas were harvested from bovine eyeballs obtained from a meat processing plant. The ROS disk membranes were isolated from the bovine retinas using a linear sucrose density gradient technique according to the procedure described by Makino et al. The details of the preparations were described in our previous report. The ROS disk membranes were suspended in a 100 mM KCl solution. There was 0.28 to 0.46 mg of membrane protein.

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The light-induced proton uptake by rhodopsin in the bovine rod outer segment disk membrane suspension as a function of the pH between 4 and 8. At each pH, five measurements were done with aliquots of the same sample. The standard errors are not indicated in the graph because vertical bars will be covered by the mean values symbols. The light-induced pH increase shows the pH optimum to be 5.2.

Results

The light-induced pH change showed a rapid pH increase following a slow decrease with time, as reported previously. The extent of the light-induced pH change varied with the concentration of unbleached rhodopsin in the ROS disk membrane suspensions. The light-induced proton uptakes of ROS disk membranes were measured as a function of the pH. Illumination induced an immediate pH increase when the medium pHs were between 4 and 8. The results of such measurements are depicted in Fig. 1. The light-induced pH increase showed the pH optimum to be 5.2.

Figure 2 depicts the effects of the local anesthetics (lidocaine, bupivacaine, tetracaine, dCIbucaine, and benzocaine) on the light-induced pH increase at several pH values of the medium. The extent of the pH change is expressed as the per cent of the control. The light-induced proton uptake was depressed at higher pHs but enhanced at lower pHs by tertiary amine local anesthetics. The anesthetic-induced depression showed a dose-response. Lidocaine is the least potent anesthetic in terms of depressing and enhancing the proton uptake, and dCiBucaine is the most potent. The order of local anesthetic-induced depression of the proton uptake was the same as that of their nerve-blocking potencies. In contrast, a neutral anesthetic, benzocaine, depressed the proton uptake at all pHs between 4 and 7.

Figure 3 shows that there was no significant change in the light-induced proton uptake when 1 mM of Ca^{2+} was introduced into the ROS disk membrane suspension at pH 4, 5.5, and 7 in the absence or presence of local anesthetics, bupivacaine, and tetracaine.

Discussion

The light-induced pH increase of the ROS disk membrane suspension showed the pH optimum at 5.2. A pH
FIG. 2. The effects of the local anesthetics—lidocaine (A), bupivacaine (B), tetracaine (C), dibucaine (D), and benzocaine (E)—on the light-induced pH increase at several pH values of the medium. Each local anesthetic was added in both small (Δ) and large (□) doses: 1 and 5 mM; 0.25 and 1.25 mM; 0.24 and 1.19 mM; and 0.2 and 1.0 mM for lidocaine, bupivacaine, tetracaine, and dibucaine, respectively, and in a single dose (Δ) of 1 mM for benzocaine. The extent of the pH change is expressed as the per cent of the control (○). Data are indicated as mean ± SE except dibucaine. The n values are 4 for lidocaine, bupivacaine, and tetracaine, 2 for dibucaine, and 6 for benzocaine. The light-induced proton uptake is depressed at higher pHs but enhanced at lower pHs by tertiary amine local anesthetics. Lidocaine is the least potent anesthetic in terms of depressing and enhancing the proton uptake, and dibucaine is the most potent. Neutral anesthetic, benzocaine, depresses the proton uptake at all pHs between 4 and 7.

optimum around 5 in the light-induced pH increase was also reported by Radding and Wald. They revealed that light-induced change involved the liberation of 1 mole of a proton-binding residue with a pK of about 6.6, close to the pK of the imidazole group of histidine, between pH 4.5 and 7.5. McConnell and Bennett demonstrated a similar pH-dependence of light-induced proton uptake. Metarhodopsin II (meta II) might exist in partially protonated forms at higher pHs and lower pHs under 5, as mentioned by Bennett.

We have shown that the light-induced proton uptake was depressed at higher pHs but enhanced at lower pHs by each of the local anesthetics except benzocaine (fig. 2). The concentrations of local anesthetics used in the present study were almost equal to or 2–4 times higher than those at which local anesthetics reduced the nerve functions by 50% in vitro as referred to in Strichartz's review article. All measurements referred to in his review were made at pH 7.0–7.5. At these pHs, the light-induced proton uptake is reduced by 30–50% at the lower concentrations of added anesthetics in the present study. The dual effect of tertiary amine local anesthetics on the light-induced proton uptake suggests that the function of rhodopsin in the ROS disk membranes may be activated by the charged forms of local anesthetics but inhibited by the uncharged forms. If only the uncharged form were effective on the depression of the light-induced proton uptake, the two curves obtained at different doses should fall on the same line when the concentration is expressed as the uncharged form. Figure 4 shows that the depression of the light-
The effects of Ca\textsuperscript{2+} ions on the light-induced pH increase and the local anesthetic-induced change in proton uptake by the rhodopsin. Ca\textsuperscript{2+} at the concentration of 1 mM is introduced into the rod outer segment disk membrane suspension at pH 4, 5.5, and 7. The open and closed marks represent the Ca\textsuperscript{2+}-free and Ca\textsuperscript{2+}-contained samples, respectively. Control (O; n = 6), 1.2 mM of bupivacaine (Δ; n = 4) and 1.0 mM of tetracaine (C; n = 4). Data are indicated as mean ± SE. Differences between with and without Ca\textsuperscript{2+} ions are not statistically significant.

induced proton uptake versus the concentration of uncharged lidocaine or bupivacaine nearly describes the same curve for the small and large dose of added local

anesthetic. Furthermore, a neutral local anesthetic, benzocaine, depressed the light-induced proton uptake at all pHs between 4 and 7. These results confirm that the depression of the light-induced proton uptake is due to the effect of only the uncharged forms of local anesthetics. The molecular mechanism of local anesthetic-induced inhibition of light-induced proton uptake by the ROS disk membrane is assumed to be quite similar to that for volatile anesthetics, which were demonstrated\textsuperscript{17} to inhibit the proton uptake in a dose-dependent manner. It is implied that both volatile anesthetics and the uncharged forms of local anesthetics interact hydrophobically with the rhodopsin protein embedded in the ROS disk membrane and perturb its structure and function. It is well known that during the transition of meta I to meta II, the rhodopsin molecule takes up protons from the surrounding medium. The uncharged forms of local anesthetics might influence the major conformational change during meta I to meta II transition and, then, inhibit meta II from being protonated. Ca\textsuperscript{2+} and Hubbell\textsuperscript{18} suggested that the change in the interfacial electric potential of isolated ROS disk membranes was closely related to the change in the light-induced proton uptake. Ostrov\textsuperscript{19} also reported that changes in the pK values of ionizable groups influenced the light-induced proton uptake. We have shown that volatile anesthetics decrease the surface potential on the bovine serum albumin molecule,\textsuperscript{24} and diethyl ether increases its partial molal volume.\textsuperscript{25} From these facts, it is speculated that volatile anesthetics and uncharged forms of local anesthetics interact hydrophobically with the rhodopsin to change its conformation. This conformational change might result in a decrease in the surface charge of meta II and, then, decrease the light-induced proton uptake.

**Fig. 4.** The depression of the light-induced proton uptake versus the concentration of the uncharged lidocaine (A) or bupivacaine (B). The two curves obtained at the small (O) and large (●) doses of added local anesthetics fall nearly on the same line.
The charged forms of local anesthetics activated the light-induced proton uptake by rhodopsin in the ROS disk membranes. The charged forms of local anesthetics might interact electrostatically with rhodopsin protein and change the $pK$ values of proton-binding residues. $^2$H- and $^3$P-NMR studies$^{26}$ showed that the charged form of local anesthetics interacted with the charged head group of two zwitterionic phospholipids, phosphatidylcholine and phosphatidylethanolamine, and changed their conformation.

There have been a number of hypotheses on how local anesthetics act on axon membranes’ excitability. The most widely accepted hypothesis$^{27-29}$ is that the charged forms of local anesthetics, which penetrate into the axon in uncharged forms, interact with a sodium channel protein and inactivate. The present study shows that the charged forms of local anesthetics do not inhibit, but slightly activate, the function of rhodopsin. This result suggests that rhodopsin is not the proper substitute membrane protein for studies on sodium channels.

Although it is emphasized that only the charged forms act as local anesthetics, the uncharged forms of local anesthetics also have some nerve-blocking activity,$^{30,31}$ which represents a dual mode of action for local anesthetics. In addition to acting at specific sites, local anesthetics may be acting in the same way as volatile anesthetics do. Many studies$^{32-35}$ have suggested that the uncharged forms of local anesthetics nonspecifically affect ion permeability by perturbing sodium channels and other elements that constitute the nerve membranes. This mechanism may be quite similar to that for the local anesthetic-induced inhibition of the function of rhodopsin, as shown in the present study.

The absence of any significant effect by $\text{Ca}^{2+}$ on the light-induced proton uptake without local anesthetics indicates that $\text{Ca}^{2+}$ ions do not influence the diprotating process of meta II. Furthermore, $\text{Ca}^{2+}$ did not interfere with the local anesthetic-induced enhancement or depression of proton uptake. This fact indicates that there is no direct interaction between $\text{Ca}^{2+}$ ions and local anesthetics in the ROS disk membranes. Narahashi et al.$^{36}$ and Strichartz$^{22}$ demonstrated that $\text{Ca}^{2+}$ ions did not reverse directly the local anesthetic action in nerve membranes, and there was no specific binding of $\text{Ca}^{2+}$ to sodium channels.

References

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