Effects of the Local Anesthetic Bupivacaine on Oxidative Phosphorylation in Mitochondria

CHANGE FROM DECOUPLING TO UNCOUPLING BY FORMATION OF A LEAKAGE TYPE ION PATHWAY SPECIFIC FOR H⁺ IN COOPERATION WITH HYDROPHOBIC ANIONS

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The effects of the local anesthetic bupivacaine on the oxidative phosphorylation in rat liver mitochondria were examined. Bupivacaine caused a maximum of about 7-fold stimulation of state 4 respiration at about 3 mM, released oligomycin-inhibited state 3 respiration, and activated ATPase to a similar extent to that by the weakly acidic uncoupler SF 6847. These effects were greatly enhanced by the addition of certain hydrophobic anions such as 1-anilino-8-naphthalenesulfonate, tetraphenyl borate, and picrate. In the absence of these anions, bupivacaine did not increase the proton conductance in either energized or nonenergized mitochondrial membranes or in artificial bilayer lipid membranes and did not have any effect on the proton motive force. However, it greatly enhanced the proton conductivity of these membrane systems and collapsed the proton motive force in the presence of hydrophobic anions. The results of noise analysis of artificial lipid bilayer membranes indicated that an ion pair complex of bupivacaine with hydrophobic anions formed a leakage-type ion pathway. Thus it is concluded that bupivacaine acts as a decoupler in the absence of added hydrophobic anions but in cooperation with certain anions as an uncoupler of oxidative phosphorylation due to formation of a H⁺-specific pathway in the membranes.

Local anesthetics have diverse effects on oxidative phosphorylation in mitochondria depending on their chemical structures; they inhibit electron transport (1, 2) and F₁ ATPase (3-5), affect transport of ions such as Ca²⁺ (6-9), and cause uncoupling (10, 11). The reason for these diverse actions of local anesthetics could be that these compounds are mostly tertiary amines with pKₑ values in the range of 7-9 (12), and so either the cationic form RNH⁺ or its conjugated electrically neutral form RNH₂ or both, could have biological effects, and the overall effects should depend on the relative amounts of these molecular species and their hydrophobicities.

Garlid and Nakashima (10) reported that anions such as TPP⁻¹ and SCN⁻ are necessary for induction of uncoupling by tertiary amines including local anesthetics. However, recently Dabadie et al. (11) reported that anions are not always necessary for the uncoupling actions of local anesthetics; lidocaine induced uncoupling only in the presence of the hydrophobic anion TPB⁻, but bupivacaine induced uncoupling even in the absence of TPB⁻, although this anion potentiated the uncoupling by bupivacaine. Garlid and Nakashima (10) proposed that the inductions or enhancements of the uncoupling actions of local anesthetics by certain anions were due to formation of electrically neutral ion pair complexes between RNH⁺ and those anions that enable RNH₂ to penetrate through the mitochondrial membrane to exert its protonophoric action. According to Dabadie et al. (11), uncoupling of bupivacaine is caused by three composite mechanisms: the electrophoretic transfer of its cationic form RNH⁺ through the membrane, a protonophoric action due to shuttle-type cycles across the membrane by repeated interconversions of its RNH⁺ and RNH₂ forms, and a shuttle-type protonophoric action in association with certain anions that form ion pair complexes with RNH₂.

We are interested in whether membrane proteins are involved in the uncoupling actions of local anesthetics, because we recently found that the uncoupling actions of hydrophobic cations, such as cyanine dyes and the (o-phenanthroline)-Cu²⁺ complex, are due to modification of the state of membrane proteins such as the adenine nucleotide translocator (13-15). Thus, we studied the uncoupling of bupivacaine, which has a pKₑ of 5.17 (12), in rat liver mitochondria in the absence or presence of hydrophobic anions. We found that the action of bupivacaine is different from those of cyanine dyes and (o-phenanthroline)-Cu²⁺ and that the effect of bupivacaine without hydrophobic anions is not due to uncoupling but to decoupling, in which acceleration of state 4 respiration and activation of ATPase is not accompanied by the dissipation of the Δᵢₚᵢᵣ, but its effect was converted to uncoupling by addition of hydrophobic anions.

MATERIALS AND METHODS

Bupivacaine was a generous gift from Fujisawa Pharmaceutical Industry Co., Osaka (Japan). SF 6847 was purchased from Wako Pure Chemical Industries Co., Osaka (Japan), valinomycin from Sigma, and egg yolk phosphatidylcholine from Nippon Fine Chemical Co., Osaka (Japan). Other reagents used were of the highest grade commercially available.

Mitochondria were isolated from the liver of adult male Wistar rats as described previously (16). Respiration of mitochondria (0.7 mg of protein/ml) with succinate (10 mM) plus rotenone (1 μg/ml) as substrate was monitored polarographically with a Clark oxygen electrode (Yellow Springs Instrument Co., YSI 5331) at 25 °C in a total volume of 2.55 ml. The standard medium consisted of 200 mM sucrose, 2 mM MgCl₂, 1 mM EDTA, and 10 mM Tris-HCl buffer, pH 7.4.

ATP synthesis by mitochondria was determined from a change in
pH of the medium associated with ATP synthesis as reported by Nishimura et al. (17) at 25 °C in medium consisting of 200 mM sucrose, 20 mM KCl, 3 mM MgCl₂, and 3 mM potassium phosphate buffer, pH 7.4, in a total volume of 4.75 ml. The reaction was started by the addition of 400 μM ADP to the suspension of mitochondria (0.7 mg of protein/ml) energized with 5 mM succinate (plus rotenone at 1 μg/ml). Bupivacaine either with or without ANS⁻ was added just after addition of mitochondria to the incubation medium.

ATPase activity was determined by measuring the P-I liberated by the hydrolysis of ATP (18). Rat liver mitochondria (3 mg of protein/ml) were incubated with 10 mM ATP for 2 min in medium consisting of 150 mM sucrose, 20 mM KCl, 5 mM MgCl₂, 2 mM EDTA-2Na, and 1 mM Tris-HCl buffer, pH 7.4. Then, bupivacaine was added, and 3 min later trichloroacetic acid at a final concentration of 8% was added to stop the reaction. The concentration of P-I liberated was determined spectrophotometrically in a Shimadzu recording spectrophotometer, model UV-3000.

The proton conductivity of bupivacaine in the presence of valinomycin in nonrespiring mitochondria was determined from the decrease in the optical absorbance at 500 nm associated with swelling of the mitochondria (19, 20). Valinomycin (5 μg), bupivacaine, and certain anions were added in various orders to rat liver mitochondria (1.5 mg of protein/ml) suspended in 2.5 ml of medium consisting of 145 mM potassium acetate and 5 mM Tris-HCl, pH 7.4, in the presence of rotenone (2.5 μg) and antimycin A (10 μg).

The proton conductivity of bupivacaine in respiring mitochondria (0.7 mg of protein/ml) was determined by measuring reversal of H⁺-ejection by valinomycin in medium consisting of 200 mM sucrose, 20 mM KCl, 3 mM MgCl₂, and 3 mM Tris-HCl buffer, pH 7.4, in a total volume of 4.75 ml (21). Succinate (5 mM) plus rotenone (1 μg/ml) was used as respiratory substrate. The H⁺ movement was calibrated with oxalic acid.

Change in Δψ of respiring mitochondria by the addition of bupivacaine and ANS⁻ was determined from the Δψ and ΔpH by measuring the uptakes of [3H]TPP⁺ and [3H]acetate, respectively (22). Rat liver mitochondria (6 mg of protein/ml) were suspended in 800 μl of incubation medium consisting of 200 mM sucrose, 20 mM KCl, 3 mM MgCl₂, and 3 mM Tris-HCl, pH 7.4, with the respiratory substrate succinate (5 mM) plus rotenone (0.8 μg/ml) in the presence of either 10 μM [3H]TPP⁺ (66.3 Ci/mM) or 30 μM [3H]acetate (62.5 Ci/mM). Then, bupivacaine with or without ANS⁻ was added, and after 30 s, the mitochondria were collected by centrifugation in an Eppendorf-type centrifuge, Kubota KM-15000, and the amount of [3H]acetate incorporated was determined in an Aloka liquid scintillation counter, LSC-700. A correction for adsorption of TPP⁺ was made by a reported method (23). The volumes of the mitochondrial matrix space and sucrose space were determined as reported previously (22) by measuring the incorporations of [3H] water (10 μCi/ml) and [14C]sucrose (5 μCi/ml), respectively, in the same medium as for the measurement of Δψ.

The BLM was formed by applying a solution of egg yolk phosphatidylcholine and cholesterol (molar ratio, 1:2) in n-decane to a hole (about 1.0-mm diameter) in the wall of a Teflon test tube (24). The formation of a planar lipid bilayer was monitored by measuring interference pattern of the light and the membrane resistance at up to 1 GOhm (25). The medium consisted of 150 mM KCl and 100 mM Tris-HCl buffer, pH 7.4. Test chemicals were added on the outer side of the membrane (cis side). An electrical potential of up to 60 mV was observed in the uncoupling of cyanine dyes (15, 26, 27).

Bupivacaine also released oligomycin-inhibited state 3 respiration completely in either the absence or presence of anions (data not shown). In addition, as described later, it activated ATPase. These results showed that bupivacaine acted as an uncoupler of oxidative phosphorylation both in the absence and presence of hydrophobic anions.

Fig. 2 shows results on the dose dependence of the effect of bupivacaine on the state 4 respiratory rate. In the absence of ANS⁻, Vo, attained a maximum value, Vmax, at about 3 mM bupivacaine of about 7-fold that of state 4 respiration. The VO was slightly inhibited at concentrations of bupivacaine of more than 3 mM. ANS⁻ at 40 μM greatly potentiated the effect of bupivacaine resulting in a Vmax at about 1 mM bupivacaine, but this Vmax was about 6-fold that of state 4 respiration. This lower Vmax in the presence of ANS⁻ than in its absence was due to inhibition of the respiratory chain, because the weakly acidic uncoupler SF 6847 did not release the maximal respiration induced by bupivacaine plus ANS⁻ (data not shown).

Synthesis of ATP was inhibited in proportion with acceleration of state 4 respiration dependent on the concentration of bupivacaine both in the absence and presence of ANS⁻, as shown in Fig. 3. The inhibitory effects of bupivacaine on ATP synthesis were observed at similar concentrations to those in...
the decrease in the optical absorbance associated with swelling caused by valinomycin-mediated K⁺ influx in exchange with H⁺ efflux in medium containing potassium acetate (19, 20). As seen from Fig. 5, bupivacaine alone did not cause swelling, but it did induce swelling in the presence of ANS⁻, indicating that it induced proton conductance only in cooperation with ANS⁻. This effect was independent of the order of additions of valinomycin, bupivacaine, and ANS⁻.

The degree of swelling at a fixed concentration of ANS⁻ of 40 μM increased with an increase in the concentration of bupivacaine (data not shown). The swelling induced by 2 mM bupivacaine was greater than that induced by the combination of valinomycin and SF 6847 (cf. dotted line in Fig. 5). Similar effects were demonstrated with TPB⁻ and picrate. In the swelling induced by valinomycin and SF 6847, SF 6847 could be replaced by bupivacaine plus ANS⁻, but valinomycin could not be replaced by bupivacaine plus ANS⁻, indicating that bupivacaine plus ANS⁻ induced permeation of H⁺, but not K⁺.

Next, the proton conductive activity of bupivacaine in respiring mitochondria with succinate (plus rotenone) as substrate was examined. As shown in Fig. 6, 2 mM bupivacaine did not reverse the ejection of H⁺ caused by valinomycin, but subsequent addition of 40 μM ANS⁻ caused almost complete reversal of H⁺ ejection. ANS⁻ alone had no effect, and on the addition of bupivacaine first instead of valinomycin no ejection of H⁺ from mitochondria was observed, suggesting that proton conductive activity was responsible for the effect of

**Proton Conductive Activity**—The proton conductivity in nonrespiring mitochondria has been examined by measuring stimulation of state 4 respiration. Similar titration curves of bupivacaine in acceleration of state 4 respiration were obtained in the presence of picrate and TPB⁻ (data not shown). It is noteworthy that an increase in Vₐₐ₉ with bupivacaine in the presence of ANS⁻ was linear, as observed with weakly acidic uncouplers such as SF 6847 (28), but was sigmoidal in the absence of hydrophobic anions, as observed with cationic uncouplers (15, 26).

We recently found that uncouplings by the divalent cationic cyanine dyes tri-S-C₄(5) and tri-S-C₅(5) and the complex of (o-phenanthroline)₂-Cu⁺ were associated with mitochondrial swelling and that these uncouplings were inhibited by the SH reagent N-ethylmaleimide (15, 15, 26, 27). However, bupivacaine alone or in the presence of ANS⁻ did not cause mitochondrial swelling, and N-ethylmaleimide did not affect the bupivacaine-induced respiration, indicating that the action mechanism of bupivacaine was different from that of cyanine dyes and (o-phenanthroline)₂-Cu⁺ (data not shown).

Bupivacaine also activated ATPase, and ANS⁻ potentiated this effect of bupivacaine also, as shown in Fig. 4. The concentration of bupivacaine required for full activation of ATPase was somewhat greater than that required for full release of respiration both in the absence and presence of ANS⁻. The maximal ATPase activity was as great as that induced by SF 6847.

**Fig. 3.** Dose-dependent inhibition of ATP synthesis by bupivacaine with and without ANS⁻. The rate of ATP synthesis was measured with the indicated concentrations of bupivacaine in the absence (open circles) and presence (closed circles) of 40 μM ANS⁻.

**Fig. 4.** Dose dependence of activation of ATPase by bupivacaine with and without ANS⁻. Activity of mitochondrial ATPase was determined as the concentration of the Pi liberated by ATPase. As seen from Fig. 5, bupivacaine alone did not cause swelling, but it did induce swelling in the presence of ANS⁻, indicating that it induced proton conductance only in cooperation with ANS⁻. This effect was independent of the order of additions of valinomycin, bupivacaine, and ANS⁻.

**Fig. 5.** Effect of bupivacaine on passive swelling of mitochondria. Bupivacaine (2 mM), ANS⁻ (40 μM), and valinomycin (5 μg) were added to nonrespiring rat liver mitochondria (RLM) suspended in 145 mM potassium acetate and 5 mM Tris-HCl buffer, pH 7.4, and the swelling was measured as a decrease in optical absorption at 500 nm. The effect of the weakly acidic uncoupler SF 6847 added instead of bupivacaine and ANS⁻ at the concentration for full uncoupling (40 μM) is shown as dotted lines. Experimental conditions are described under "Materials and Methods."

**Fig. 6.** Proton conductive action of bupivacaine in respiring mitochondria. The effects of 2 μM bupivacaine and 40 μM ANS⁻ on H⁺ ejection by 25 ng of valinomycin from rat liver mitochondria (RLM) energized by succinate plus rotenone were monitored directly with a pH meter. The amount of H⁺ ejected was calibrated with oxalic acid. The rate of respiration was monitored concomitantly. For details, see "Materials and Methods."
the combination of bupivacaine and ANS\textsuperscript{−} and that the cationic form of bupivacaine was not transferred electrophoretically into the matrix space of mitochondria according to the inside-negative membrane potential (29).

Effects on Proton Motive Force—Fig. 7 shows the effect of bupivacaine without and with ANS\textsuperscript{−} on the ΔpH, Δψ, and ΔμH\textsubscript{t} of the energized mitochondria with succinate (plus rotenone) as respiratory substrate. The rate of respiration of mitochondria monitored under similar conditions is also shown in Fig. 7. In the absence of ANS\textsuperscript{−}, the ΔpH and Δψ, and thus the ΔμH\textsubscript{t}, showed almost constant values up to a bupivacaine concentration that induced full release of state 4 respiration (=3 mM). A further increase in the concentration of bupivacaine caused a decrease in the Δψ but did not have a significant effect on the ΔpH (Fig. 7A). On the other hand, in the presence of 40 mM ANS\textsuperscript{−}, a linear decrease in the ΔμH\textsubscript{t}, due mainly to a decrease in the Δψ, was observed with an increase in the concentration of bupivacaine up to 5 mM (Fig. 7B). No effect was observed of the decoupler chloroform on the ΔμH\textsubscript{t} up to the V\textsubscript{max} (Fig. 7C).

Effects on Membrane Structure—Next, we examined the effect of bupivacaine on an artificial BLM composed of egg yolk phosphatidylcholine and cholesterol (molar ratio, 1:2). As shown in Fig. 8, bupivacaine at 1 mM had little effect on the electrical conductance of a BLM, but the addition of ANS\textsuperscript{−} caused a great increase (about 150-fold) in the conductance to the same level as that induced by SF 6847. The effects of higher concentrations of bupivacaine could not be examined because of the low solubility of bupivacaine.

When an outside (cis-side) positive electrical potential of 60 mV was applied to the membrane, the electrical current fluctuation in the presence of 1 mM bupivacaine was similar to that in its absence; the fluctuation was in a range of about 50 pA (Fig. 9, A and B). However, in the presence of ANS\textsuperscript{−}, the degree of fluctuation was greater and the pattern of fluctuation was different (Fig. 9C). Fig. 10 shows the power spectra of these current fluctuations, plotted as a log of the intensity S\textsubscript{f} against the frequency f on a log scale. In the absence of bupivacaine, log S\textsubscript{f} first decreased in the range of f of 0.1 and 1 Hz and then increased up to an f of 10 Hz. However, over a wide range of f between 0.1 and 10 Hz, log S\textsubscript{f} can be regarded as independent of f (white noise or 1/f\textsuperscript{0} noise). Addition of bupivacaine caused an increase in the S\textsubscript{f} in the lower frequency region between 0.1 and 1 Hz, suggesting some perturbation, probably due to interaction of bupivacaine with the surface of lipid bilayer membrane, but the spectral pattern was essentially the same as that of the control. The presence of ANS\textsuperscript{−} in the bupivacaine solution caused a significant increase in S\textsubscript{f}, and the decrease of log S\textsubscript{f} with an increase in log f was linear with a slope of −1 in the wide frequency region of 0.1–10 Hz. This fluctuation is called 1/f noise (25, 30).

**FIG. 7.** Changes in the ΔpH, Δψ, and ΔμH\textsubscript{t} with various concentrations of bupivacaine alone (A) or with 40 mM ANS\textsuperscript{−} (B) and with chloroform (C). The changes in the respiratory rate, V\textsubscript{max}, monitored separately under similar conditions are also shown. For details, see “Materials and Methods.”

**DISCUSSION**

In this study we found that bupivacaine released state 4 respiration, oligomycin-inhibited state 3 respiration, and activated ATPase. These effects were greater in the presence of low concentrations of hydrophobic anions such as ANS\textsuperscript{−}, TPB\textsuperscript{−}, and picrate. The effects of bupivacaine with or without these anions on mitochondria seemed to be characteristic of uncoupling accompanied with dissipation of the ΔμH\textsubscript{t} necessary for ATP synthesis due to an increase in H\textsuperscript{+} conductivity of the mitochondrial membrane. However, we found that bupivacaine alone did not induce H\textsuperscript{+} conductance of membranes of either respiring or nonrespiring mitochondria but that in the presence of hydrophobic anions, it greatly enhanced the H\textsuperscript{+} conductance. Furthermore, bupivacaine alone was found to have little effect on ΔμH\textsubscript{t} at up to the concentration at which it induced full release of state 4 respiration, whereas in the presence of hydrophobic anions it dissipated ΔμH\textsubscript{t}, as in the uncoupling by weakly acidic uncouplers such as SF 6847 (21). Uncoupling without associated reduction of ΔμH\textsubscript{t}, as observed in the actions of general anesthetics such as halothane and chloroform, fatty acids such as palmitate, and derivatives of gramicidin, is termed decoupling (31–33).

Garlid and Nakashima (10) proposed a model for the protonophoric action of hydrophobic tertiary amines such as local anesthetics in combination with hydrophobic anions in which the cationic amine local anesthetic RNH\textsubscript{2} first forms an electrical neutral ion pair complex RNH\textsubscript{2}−A\textsuperscript{−} with a certain anion A\textsuperscript{−}. This reduces the polarity of RNH\textsubscript{2}−, and thus the ion-paired RNH\textsubscript{2}− can be transferred into the matrix space where it releases H\textsuperscript{+} and becomes RNH\textsubscript{2}. Upon dissociation of H\textsuperscript{+}, the ion-paired A\textsuperscript{−} also dissociates from RNH\textsubscript{2}. Then, the neutral anesthetic RNH\textsubscript{2} and A\textsuperscript{−} both return independently to the cytosolic side of the mitochondrial membrane. Transfer of A\textsuperscript{−} is electrophoretic according to the outside positive membrane potential. By the combination of the cycles of the anesthetic and anions, H\textsuperscript{+} is transported into mitochondria, resulting in dissipation of the ΔpH and Δψ. This model mimics that of the weakly acidic uncoupler cycle (21), except for the participation of the cycle of certain anions.

However, another mechanism is possible. Bupivacaine caused an increase in the electrical conductance of the BLM to the same extent as that induced by SF 6847, only in the presence of ANS\textsuperscript{−}, and it also caused fluctuation of the electrical current in combination with ANS\textsuperscript{−}. Noise analysis
FIG. 8. Effects of bupivacaine and SF 6847 on the conductance of artificial BLM composed of egg yolk phosphatidylcholine and cholesterol. Membrane conductance was monitored in the absence of bupivacaine (control, A) and the presence of 1 mM bupivacaine either without (B) or with (C) 10 μM ANS* and in the presence of 100 nM SF 6847 (D). For details, see “Materials and Methods.”

A) control

B) Bupivacaine

C) Bupivacaine + ANS

FIG. 9. Time course of membrane current fluctuation induced by bupivacaine in BLM, where the electrical potential difference was clamped at +60 mV with the cis-side positive. A, control (without bupivacaine or ANS*); B, with 1 mM bupivacaine; C, with 1 mM bupivacaine and 10 μM ANS*. For experimental conditions, see “Materials and Methods.”

of this fluctuation indicated that the log of $S_f$ (= square of the current, $A^2$) was inversely proportional to the log of the frequency $f$ between 0.1 and 10 Hz. Decrease of log $S_f$ with log $f$ with a slope of −1 over such a wide range of $f$ is called $1/f$ noise and is also observed for the effect of tri-S-C$_6$(5) in combination with P, (25). This noise is thought to be due to formation of leakage type ion pathways or open channels (25, 30). Fluctuation in ion transport by ion carriers such as valinomycin is reported to give $1/f$ noise (35), whereas that through open-close type channels, as induced by gramicidin A, gives $1/f^2$ noise, where the slope of the power spectrum is −2 (36, 37).

Thus, induction of $H^+$ conductance across the mitochondrial membrane by bupivacaine in cooperation with hydrophobic anions is suggested to be due to formation of pathways for ion transport by an ion pair complex between bupivacaine and the hydrophobic anion. This mechanism is supported by the finding that the hydrophobic anions caused the $H^+$ conductivity of bupivacaine in nonrespiring mitochondria, where electrophoretic transfer of anions from inside to outside the mitochondrial membrane can be regarded as difficult due to the absence of a high outside-positive membrane potential. As bupivacaine and ANS* in combination with valinomycin induced swelling, but the two former in combination with SF 6847 did not, the pathway formed by the local anesthetic and the hydrophobic anion should be specific for $H^+$ transfer.

Dabadie et al. (11) reported that bupivacaine acted as an uncoupler either alone or in cooperation with TPB−, associated with induction of $H^+$ conductance and reduction of $\Delta \Psi$. These results are in contrast with our results. However, it seems strange in their findings that the degree of swelling induced by valinomycin and bupivacaine plus TPB− was dependent on the order of their additions (Fig. 3 in Ref. 11) and that 3 mM bupivacaine in the presence of 5 μM TPB− collapsed the $\Delta \Psi$ to the surprisingly small value of −20 mV (Table I in Ref. 11). Furthermore, they claimed that uncoupling of bupivacaine was partly due to the electrophoretic transfer of bupivacaine cation into the mitochondria. This is also inconsistent with our present results, because addition of bupivacaine to the energized mitochondrial suspension did not cause any ejection of $H^+$ from mitochondria, as observed with valinomycin and hydrophobic cations (21, 29, 38). From the present results, induction of decoupling or uncoupling by bupivacaine should depend not only on the absence or presence of added anions but also on the tightness of mitochondrial structure. Thus, possibly these discrepant results were due to differences in tightness of the membrane structure of the mitochondria, and the mitochondria used by Dabadie et al. (11) were more sensitive to perturbation induced by bupivacaine.

From the present results we conclude that the effect of the local anesthetic bupivacaine on oxidative phosphorylation is in principle based on the type of its perturbation of mitochondrial membrane structures. When bupivacaine forms a hydrophobic ion pair with hydrophobic anions, it penetrates deep into the membrane, where it induces fluctuation of membrane bilayer structures to form a leakage type ion pathway specific for $H^+$ causing uncoupling. On the other hand, perturbation induced by bupivacaine alone seems to be restricted at the membrane surface or the region close to the outer surface of the membrane, because bupivacaine itself can probably not penetrate into membrane bilayers. This type of perturbation will cause the decoupling. At present the mechanism of decoupling is not clear, but from the present results it is suggested to take place by modification of the membrane-water interactions.
Decoupling and Uncoupling Actions of Local Anesthetic interface or of structures in the region near the outer surface of the mitochondrial membranes. According to Ueda and Kamaya (39), liberation from the membrane surface of the electrostricted water that supports a tight membrane structure is a key event in the action of anesthetics. Studies on the mechanism of decoupling are under way.

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Effects of the local anesthetic bupivacaine on oxidative phosphorylation in mitochondria. Change from decoupling to uncoupling by formation of a leakage type ion pathway specific for H+ in cooperation with hydrophobic anions.

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