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Effects of Local Anesthetics on Electron Transport and Generation of Superoxide Radicals in Mitochondria

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ABSTRACT

Local anesthetics were investigated for their effects on mitochondrial electron transport system, production of superoxide radical from submitochondrial particles and malondialdehyde production through lipid peroxidation. Local anesthetics had various effects on activities of enzymes in electron transport chain. The activities of NADH dehydrogenase, NADH oxidase and NADH-ubiquinone oxidoreductase were effectively inhibited by lidocaine, procaine and dibucaine but slightly influenced by cocaine. The activities of succinate dehydrogenase, succinate-cytochrome c oxidoreductase and succinate-ubiquinone oxidoreductase were inhibited by lidocaine and dibucaine, but the succinate oxidase activity was stimulated by local anesthetics. Both dihydroubiquinone-cytochrome c oxidoreductase and cytochrome c oxidase activities were inhibited by local anesthetics. In these reactions, the response of Complex I segment to local anesthetics was greater than other Complex segments. Local anesthetics inhibited both the superoxide production from submitochondrial particles supplemented with succinate or NADH and the enhanced production of superoxide radicals by antimycin. The malondialdehyde producion by oxygen free radicals was inhibited by local anesthetics. These results suggest that the inhibition of superoxide and malondialdehyde production caused by local anesthetics may be brought by suppression of the electron transport in mitochondria at sites in or near complex I segment.

Key Words: Local anesthetics, Electron transport, Superoxide generation, Lipid peroxidation

INTRODUCTION

Local anesthetics are found to interfere electron transport and oxidative metabolism in mitochondria (Haschke and Fink, 1975: Chazotte and Vanderkooi, 1981: Casanovas *et al.*, 1983) and this effect on metabolism was suggested to be involved with their nerve blocking action. This metabolic effect of local anesthetics on mitochondria poses an intriguing question of their effect on producion of oxygen free radicals in mitochondria. The catalytic activities of many cellular enzymes involved with electron transport processess and autoxidation of cell components yield free radical intermediates (Forman and Boveris, 1982), and mitochondria have been recognized as effective sources of O_2^- and H_2O_2 (Boveris and Chance, 1973: Boveris *et al.*, 1976) which are generated through electron transport and oxidative processes in mitochondria (Boveris and Chance, 1973: Boveris *et al.*, 1976: Forman and Boveris, 1982).

Then the inhibitory effect of local anesthetics on mitochondrial oxidative processes presents possibility that these agents may influence that free radical formation indirectly through their metabolic effect. Evidence is available in support of this possibility. It was found that hemolysis of red cells was protected by local anesthetics (Roth and Seeman, 1971). It is possible that the formation of oxygen free radicals which are known to be implicated in tissue damages in various patho-

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logical conditions which include toxicity of certain drugs (Trush *et al.*, 1982), ischemia of heart and brain (Demopoulos *et al.*, 1980: Hess *et al.*, 1981), aging (Leibovitz and Siegal, 1980) and inflammation (Fantone and Ward, 1982) may have been influenced by the metabolic effect of local anesthetics.

Thus, in the present study effects of local anesthetics on electron transport, oxidative mechanism and oxygen free radical production in mitochondria were investigated. Lipid peroxidation of mitochondrial membrane by oxygen radicals generated at the inner membrane as affected by local anesthetics was also studied.

MATERIALS AND METHODS

Lidocaine, dibucaine, procaine, NADH, sodium succinate, NADP (oxidized form), oxalacetic acid, isocitrate, INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium), dichlorindophenol, phenazine methosulfate, thenoylfluoroacetone, ubiquinone (coenzyme Q_{10}), ferricytochrome c, NBT (nitroblue tetrazolium), epinephrine bitartrate, rotenone, antimycin and TBA (2, 5-dithio-barbitutric acid) were purchased from Sigma Chemical Co.. Cocaine was obtained from Daiwon Pharmaceutical Co., Ltd.; malonic acid from Hayashi Pure Chemical industries, Ltd.. Other chemicals were of analytical reagent grade.

Preparation of rat liver mitochondria

Mitochondria were prepared from rat liver according to the method of Gazzotti *et al.* (1979). Male Sprague-Dawley rats weighing about 150 g were used. The liver was removed from the decaptitated rat and immediately introduced into ice cold medium I (0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA, pH 7.4). The liver was chopped finely with scissors and the liquid decanted. The tissue was homogenized using polytron homogenizer (Brinkman, Model PT-20). The supernatant obtained from the homogenate after centrifugation at 800 g for 10 min was centrifuged at 10,000 g for 8 min. The resulting pellet was suspended in medium II (0.25 M sucrose and 10 mM Tris-HCl, pH 7.4) and washed three times with the same buffer by centrifugation at 10,000 g for 8 min. The final pellet was suspended in medium II. Protein concentration was determined by the method of Lowry *et al.* (1951).

Preparation of submitochondrial vesicles

Mitochondria were suspended (20 mg protein per ml) in 0.25 M sucrose and 1 mM EDTA, pH 8.5. The suspension was sonicated in an ice bath at 55 watt for 12 min using a sonifier cell desruptor (Branson sonifier, Model W185D) and centrifuged for 10 min at 10,000 g to sediment still intact mitochondria. The turbid supernatant was recentrifuged at 105,000 g for 30 min. The resulting pellet was suspended in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4 and washed once in the sucrose-Tris medium. The pellet was finally suspended in the sucrose-Tris medium (Gazzotti *et al.*, 1979).

Enzyme assays

Enzyme assays in mitochondria and submitochondrial particles were performed using a Gilford UV spectrophotometer (Model 260). NADH dehydrogenase activity was determined at 420 nm in a medium consisting of 15 mM potassium ferricyanide, 4.5 mM NADH, 50 mM Tris-HCl, pH 7.4 and 18 mM triethanolamine-HCl, pH 7.8 (King and Howard, 1967). NADH oxidase and NADH-cytochrome c oxidoreductase were determined according to Hatefi and Stiggall (1978). NADH-ubiquinone oxidoreductase activity was measured in the assay medium containing 100 μM ubiquinone, 450 μM NADH, 2mM NaN_3, 50 μM potassium ferricyanide and 50 mM Tris-HCl, pH 8.0 at 340 nm (Hatefi, 1978). Succinate dehydrogenase activity was measured in the reaction mixture containing 0.1% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenylterazolium chloride, 50 mM sodium succinate and 50 mM potassium phosphate buffer, pH 7.4 at 490 nm (Pennington, 1961). Succinate oxidase and succinate-cytochrome c oxidoreductase were determined according to Vanderkooi *et al.* (1978), and Chazotte and Vanderkooi (1981), respectively. Succinate-ubiquinone oxidoreductase activity was measured in the assay medium containing 4 mM sodium succinate, 200 μM EDTA, 9.3 μM dichlorindophenol, 200 μM ubiquinone, 0.02% bovine serum albumine, 130 μM phenazine methosulfate, 200 μM thenoyltrifluoroacetone and 100 mM Tris-HCl, pH 7.4 at 600 nm (Hatefi and Stiggall, 1978). Dihydroubiquinone-cytochrome c oxidoreductase activity was determined in the assay medium containing 250 μM reduced ubiquinone, 50 μM

ferricytochrome c, 0.02% bovine serum albumine and 100 mM Tris-HCl, pH 8.0 at 550 nm (Hatefi, 1978). Cytochrome c oxidase activity was assayed in the previously described assay medium and measured at 550 nm (Errede, 1978).

Determination of superoxide radicals

The inhibitory effect of SOD on the O_2^--induced reduction of NBT to purple formazan was used to demonstrate the generation of this oxygen species (Baehner, 1975). Measurement of superoxide radicals was done in 1.0 ml reaction medium containing 100 μM KCl, 10 μM sodium azide and 50 mM HEPES-KOH, pH 7.4 with or without drugs. The reaction was started by adding 5 mM succinate or 100 μM NADH. After incubation was carried out, the reaction was stopped by addition of 1.0 ml of 1.0 N HCl. The purple color was then solubilized with 2.0 ml pyridine. Redution of NBT by superoxide radicals was measured at 490 nm and expressed as nmoles/min using the molecular extinction coefficient for blue formazan of 100 $mM^{-1}cm^{-1}$ (Green and Hill, 1984).

Superoxide radicals assay by adrenochrome method

The co-oxidation of epinephrine induced by superoxide radicals was followed in a dual wavelength spectrophotometer (Aminco-Chance dual wavelength split beam recording spectrophotometer) at 480-575 mm (Boveris, 1984). The reaction mixture contained submitochondrial particles (0.5 mg/ml), 230 mM mannitol, 70 mM sucrose, 500 μM epinephrine and 50 mM Tris-HCl, pH 7.4 with or without local anesthetics. The reaction of radical formation was initiated by addition of 5 mM succinate (or 500 μM NADH). In order to investigate effects of local anesthetics on enhanced superoxide production by mitochondrial respiratory chain blockers during electron donor linked superoxide production, local anesthetics and respiratory chain blockers were simultaneously added to reaction media. The extinction coefficient of adrenochrome at the wavelength pair employed was 2.86 $mM^{-1}cm^{-1}$ (Dionisi *et al.*, 1975).

Measurement of lipid peroxidation

Lipid peroxidation of mitochondrial membrane was estimated by measuring malondialdehyde concentration by thiobarbituric acid method (Bidlack and Tappel, 1973). The reaction medium consisting of mitochondria (0.2 mg/ml), 120 mM KCl, 50 mM Tris-HCl, pH 7.4 were incubated with or without local anesthetics. After reaction was started by addition of 5 mM succinate or 100 μM NADH, 1.0 ml of aliquots were mixed with 0.5 ml of distilled water and 0.5 ml of 30% trichloroacetic acid, and centrfiuged at 3,000 g for 15 min. 1.5 ml of supernatant was added to an equal volume of aqueous 0.67% TBA (2, 5-dithiobarbituric acid) and chromophore was developed by boiling in a water bath for 20 min. After cooling to room temperature, the absorbance was measured at 532 nm. The concentration of malondialdehyde was expressed in nmoles/mg protein using the molar extinction coefficient of 1.52×10^5 $M^{-1}cm^{-1}$ (Placer *et al.*, 1966).

RESULTS

Effects of local anesthetics on the electron transfer chain

Results presented in Table 1 show that the NADH dehydrogenase and NADH oxidase activities were inhibited by procaine, cocaine, lidocaine and dibucaine. At 5 mM of concentration, lidocaine inhibited the NADH oxidase activity markedly. On the other hand, cocaine had no significant effect on these enzyme activities. Table 1 indicates that succinate dehydrogenase and succinate-cytochrome c oxidoreductase activities were inhibitied by lidocaine and dibucaine but not by procaine and cocaine. On the other hand, the succinate oxidase activity was rather stimulated by local anesthetics except cocaine. These results suggest that local anesthetics may act on both NADH-linked oxidoreduction at Complex I and succinate-linked oxidoreduction at Complex II. Local anesthetics enhanced succinate oxidation.

Effects of local anesthetis on enzymes which are involved in oxidoreduction at Complex I and II were further evaluated with respect to their effects on complex I and II segments. The activity of NADH-ubiquinone oxidoreductase, Complex I was inhibited by procaine, cocaine, lidocaine and dibucaine in a concentration of 5 mM by 17%-34% (Table 2). The activity of succinate-ubiquinone oxidoreductase, Complex II was also influenced by all of the above agents and was inhibited by 11%-13% at the same concentration.

The dihydroubiquinone-cytochrome c oxidoreductase activity which is known to be an effective

Table 1. Effects of local anesthetics on the activity of enzymes at complex I and II in mitochondrial electron transport

Additions		NADH oxidase	NADH dehydrogenase	Succinate oxidase	Succinate-cytochrome c oxidoreductase	Succinate dehydrogenase
None		43.8 ± 1.9	275.5 ± 22.8	0.048 ± 0.006	76.1 ± 0.6	80.1 ± 1.4
Procaine	1 mM	43.3 ± 2.9	219.4 ± 10.0	0.041 ± 0.005	–	76.6 ± 9.4
	5 mM	37.6 ± 0.5	199.4 ± 10.5	0.057 ± 0.004	71.0 ± 2.3	81.2 ± 8.7
Cocaine	1 mM	42.8 ± 5.2	230.4 ± 14.3	0.052 ± 0.004	–	71.2 ± 6.3
	5 mM	39.5 ± 2.9	207.5 ± 10.5	0.046 ± 0.011	73.2 ± 1.6	73.5 ± 11.6
Lidocaine	1 mM	38.6 ± 2.4	199.1 ± 8.6	0.071 ± 0.005	–	62.9 ± 8.2
	5 mM	26.7 ± 1.9	178.9 ± 12.9	0.074 ± 0.009	65.4 ± 3.8	52.6 ± 11.2
Dibucaine	1 mM	38.1 ± 2.9	217.5 ± 14.8	0.066 ± 0.005	40.7 ± 2.3	56.6 ± 8.0
	5 mM	–	–	–	–	25.5 ± 6.9

NADH oxidase and NADH dehydrogenase activities are expressed in nmol/min/0.1 mg protein. Succinate oxidase activity is expressed as absorbance at 550 nm/min/0.1 mg protein. Succinate-cytochrome c oxidoreductase and succinate dehydrogenase activities are in nmoles/min/0.1 mg protein/min. Each value represents mean ± S.E. of 6 experiments.

Table 2. Effects of local anesthetics on complexes at mitochondrial respiratory chain

Additions		NADH–ubiquinone oxidoreductase	Succinate–ubiquinone oxidoreductase	Dihydroubiquinone–cytochrome c oxidoreductase	Cytochrome c oxidase
None		69.1 ± 2.1	13.5 ± 0.6	17.5 ± 0.2	57.6 ± 1.7
Procaine	1 mM	60.2 ± 2.7	12.5 ± 1.2	–	51.7 ± 2.0
	5 mM	54.2 ± 2.5	11.9 ± 1.0	15.2 ± 0.6	47.3 ± 1.4
Cocaine	1 mM	59.8 ± 2.1	12.8 ± 0.7	–	53.1 ± 2.5
	5 mM	57.3 ± 2.4	12.1 ± 1.1	16.9 ± 0.3	52.1 ± 1.7
Lidocaine	1 mM	58.7 ± 3.5	11.9 ± 0.8	–	50.3 ± 2.4
	5 mM	45.8 ± 2.8	9.3 ± 0.9	14.0 ± 0.8	47.6 ± 1.9
Dibucaine	1 mM	57.1 ± 3.9	13.2 ± 1.5	15.4 ± 0.5	50.9 ± 3.1
	5 mM	–	–	–	48.0 ± 1.6

Complexes activities are expressed in nmoles/min/0.1 mg protein.
Each value represents mean ± S.E. of 6 experiments.

generator of O_2^- and H_2O_2 (Forman and Boveris, 1982) was inhibited by procaine, cocaine, lidocaine and dibucaine by 3%–20% in the presence of the stated concentrations of agents (Table 2).

The activity of cytochrome oxidase was also inhibited by local anesthetics at 5 mM of concentrations by 9%–17%.

Effects of inhibitors of respiratory chain and oxidative phosphorylation on superoxide production from mitochondria

Amounts of superoxide produced by liver submitochondrial particles in the presence of succinate and NADH as electron donors were 1.33 and 4.53 nmol/min/mg of protein, respectively. As shown in Table 3, the above superoxide production was stimulated by both antimycin, a Complex III inhibitor at respiratory chain and cyanide, a Complex IV inhibitor. Superoxide production from submitochondrial particles with succinate was inhibited by malonate, Complex II inhibitor, whereas superoxide production in the presence of NADH was inhibited by rotenone, Complex I inhibitor.

Table 3. Effect of respiratory chain inhibitors on superoxide production from submitochondrial particles supplemented with electron donors

Additions	NBT reduction (nmol/min/mg protein)	
	Succinate	NADH
None	1.33 ± 0.04	4.53 ± 0.19
Rotenone	1.17 ± 0.07	3.12 ± 0.22
Malonate	0.96 ± 0.11	4.29 ± 0.17
Antimycin	2.85 ± 0.08	10.07 ± 0.36
Cyanide	1.84 ± 0.13	5.32 ± 0.08

NBT reduction by submitochondrial particles in the absence of electron donors was 0.26 nmol/min/mg protein. The reaction medium containing 0.5 mg/ml of submitochondrial particles, 100 μM NBT, 150 mM KCl, 10 μM sodium azide and 50 mM HEPES-KOH, pH 7.4 in the presence or absence of respiratory chain inhibitors. The reaction was intiated by the addition of 5 mM succinate or 100 μM NADH as electron donor. Each value represents mean ± S.E. of 5 experiments.

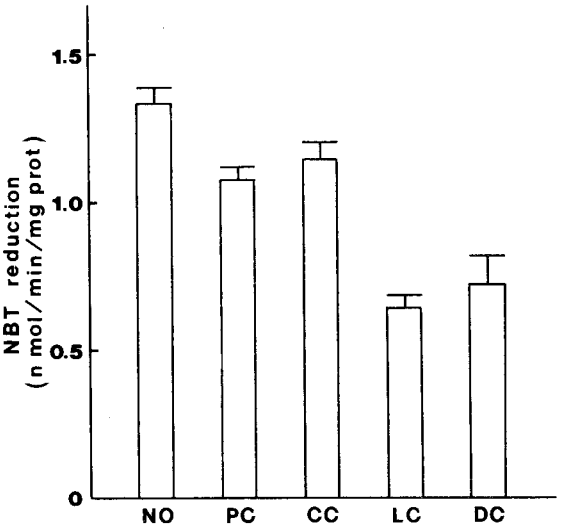

Fig. 1. Effects of local anesthetics on superoxide production with succinate. Submitochondrial particles (0.5 mg/ml) were incubated with 5 mM succinate in the presence of 5 mM local anesthetics at 37°C Each value represents mean ± S.E. of 6 experiments. NO, no addition ; PC, procaine ; CC, cocaine ; LC, lidocaine ; DC, dibucaine.

Effects of local anesthetics on NBT reduction and adrenochrome formation from epinephrine

Superoxide production from submitochondrial particles supplemented with succinate or NADH was significantly affected by procaine, lidocaine and dibucaine. As shown in Fig. 1 and 2, these agents at concentration of 5 mM inhibited the superoxide production by 20%-52%, while cocaine had no significant effect.

As shown in Fig. 3, inhibitory effect of local anesthetics on the reduction of NBT by superoxide produced from mitochondria was confirmed by their effect on adrenochrome formation. Superoxide dismutase inhibited adrenochrome formation, indicating O_2^- involvement. The rates of adrenochrome formation by submitochondrial particles in the presence of succinate or NADH were decreased by local anesthetics (data not shown).

The rate of adrenochrome formation in succinate or NADH supplemented submitochondrial particles was enhanced by antimycin and this enhancement was decreased in the presence of local anesthetics (see Fig. 3). The stimulatory effect of cyanide on adrenochrome formation was

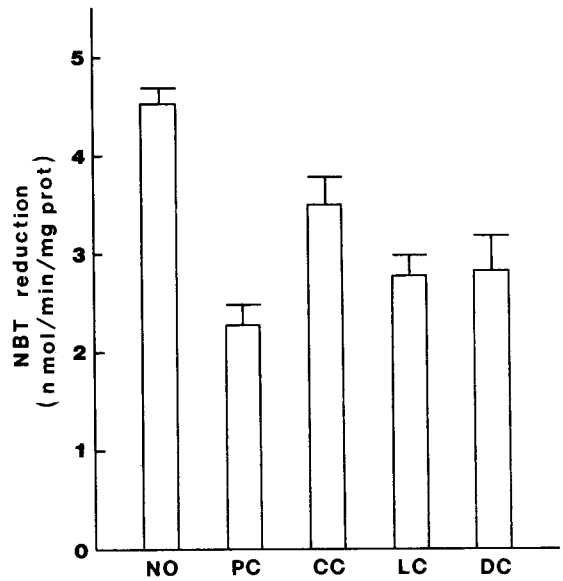

Fig. 2. Effects of local anethetics on superoxide production with NADH. Submitochondrial particles (0.5 mg/ml) were incubated with 100 μM NADH in the presence of 5 mM local anesthetics at 37°C. Each value represents mean ± S. E. of 6 experiments.NO, no addition ; PC, procaine ; CC, cocaine ; LC, lidocaine ; DC, dibucaine.

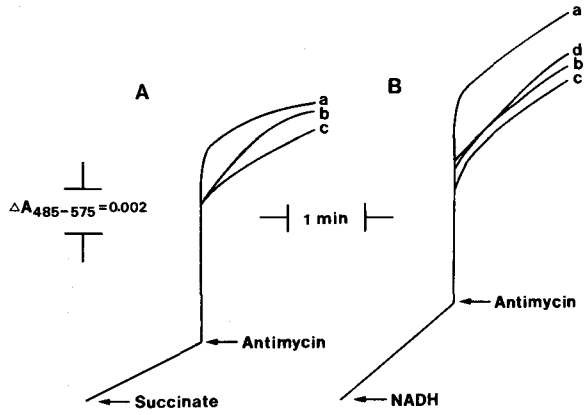

Fig. 3. Effects of local anesthetics on adrenochrome formation. Submitochondrial particles supplemented with succinate or NADH. During succinate (A) or NADH (B) linked superoxide production, 100 μM antimycin and 5 mM local anesthetics simultaneoulsy were added to reaction medium. a, antimycin ; b, antimycin plus lidocaine ; c, antimycin plus dibucaine ; d, antimycin plus procaine.

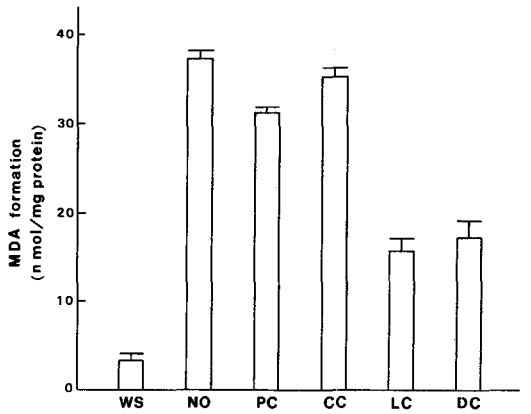

Fig. 4. Effects of local anesthetics on lipid peroxidation with succinate. Concentration of all drugs is 5 mM. The reaciton medium containing mitochondria (0.5 mg/ml), 5 mM succinate and local anesthetics. After incubation for 30 min at 37°C, malondialdehyde produced was measured. Each value represents mean ± S.E. of 5 experiments. WS, without succinate and drugs ; NO, no addition of drug ; PC, procaine ; CC, cocaine ; LC, lidocaine ; DC, dibucaine.

also inhibited by local anesthetics (data not shown).

Effects of local anesthetics on lipid peroxidation by oxygen free radicals

The production of malondialehyde from mitochondria which is an indication of lipid peroxidation by oxygen free radicals was effectively decreased by local anesthetics at 5 mM concentration. Lipid peroxidation caused by succinate or NADH-linked oxygen radicals was significantly inhibited by procaine, lidocaine and dibucaine (Fig. 4, 5). Procaine had more pronounced effect than lidocaine or dibucaine in NADH supplemented reactions.

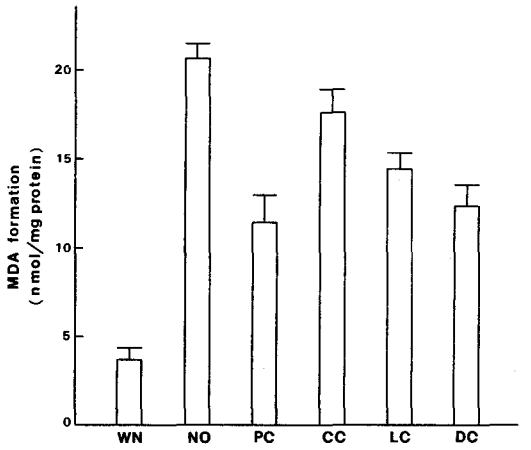

Fig. 5. Effects of local anesthetics on lipid peroxidation with NADH. The reaction medium containing 0.5 mg/ml of mitochondria, 100 μM NADH and 5 mM local anesthetics. After incubation for 10 min at 37°C, malondialdehyde produced was measured. Each value represents mean ± S. E. of 5 experiments. WN, without NADH and drugs ; NO, no addition of drug ; PC, procaine ; CC, cocaine ; LC, lidocaine ; DC, dibucaine.

DISCUSSION

Lidocaine was found to inhibit electron transport and uncouple oxidative phosphorylation reversibly in porcine brain mitochondria at concentration that closely correlate those blocking axoplasmic transport (Haschke and Fink, 1975). Thus, it is suggested that lidocaine inhibits rapid axonal transport by depressing oxidative metabolism. The effect of local anesthetics on mitochon-

drial metabolism was ivestigated. Effects of local anesthetics on the electron transfer carrier indicated that several sites or regions of the mitochondrial electron transfer chain are affected by local anesthetics (Table 1, 2). The activities of NADH dehydrogenase, NADH oxidase, and Complex I segment were all effectively inhibited by lidocaine, procaine and dibucaine but were only slightly inhibited by cocaine. The activities of succinate dehydrogenase, succinate-cytochrome c oxidoreductase and Complex II segment were all inhibited by lidocaine and dibucaine, but the succinate oxidase activity was instead stimulated by local anesthetics. The dihydroubiquinone-cytochrome c oxidoreductase, a Complex III and cytochrome c oxidase, enzymes which are contained at a Complex IV activities were also inhibited by local anesthetics. In these reactions, the response of Complex I to local anesthetics was greater than other Complex segments. Thus, these data suggest that the site which has the greatest sensitivity to the inhibitory effect of local anesthetics may be in or near Complex I. This also indicates that electron transport is effectively blocked by these agents at the NADH dehydrogenase level and at oxidoreducion process of cytochrome c.

Mitochondrial oxygen radical generation is supported by succinate or NAD-linked substrates and it is inhibited by rotenone. Accordingly, the mitochondrial generator of H_2O_2 seems to be an autoxid izable component of the respiratory chain acting between the rotenone-and the antimycin-sensitive sites (Losche *et al.*, 1971: Boveris and Chance, 1973). It has been established that O_2^- is an essential precursor of mitochondrial H_2O_2 (Dionisi *et al.*, 1975). It is well known that the respiratory chain-linked production of O_2^- is one of improtant sources of this radical in ceullular oxidative metabolism. Most of O_2^- and its product, H_2O_2 can be removed by antioxidant enzymes, namely superoxide dismutase, catalase and glutathione (Nohl and Jordan, 1980).

The results presented in Table 3 show that superoxide production from submitochondrial particles supplemented with NADH is inhibited by rotenone but not by malonate. On the other hand, succinate-linked superoxide production was inhibited by malonate but not by rotenone. Thus, Complex I and II were selectively affected by their specific blockers and effects of Complex I and II of blockers on the electron transfer chain appear to vary according to electron donors employed. Local anesthetics were found to inhibit superoxide production in submitochondrial particles as shown in Fig. 1 and 2. Since the inhibition of elctron donor-linked superoxide production was coincided with the inhibition of enzyme activities involved in electron transfter at Complex I and II segments, the inhibitory effect of local anesthetics on superoxide production may be ascribed to their inhibitory effect on enzyme activities at Complex I and II segments. On the other hand, both antimycin, a blocking agent at electron transport from cytochrome b to cytochrome c and cyanide, a blocking agent at electron transport from cytochrome c to molecular oxygen stimulated succinate or NADH-linked superoxide stimulation (Table 3). This enhanced superoxide production by antimycin was effectively inhibited by local anesthetics as shown in Fig. 3. Similary, effect of cyanide on superoxide production was also inhibited by local anesthetics (data not shown). Accordingly, these results suggest that local anesthetics may suppress superoxide production through the blocking effect on the transfer of electron from other Complexes into Complex III rather than it is direct action on Complex III. Also, local anesthetics may suppress the superoxide production from submitochondrial particles supplemented with succinate or NADH through their inhibitory actions on electron transfer carrier at several sites. Effects of local anesthetics on superoxide production appear to be proportional to the degree of their inhibition on enzyme activities at the electron transport chain (Fig. 1, 2; Table 2) at complex I and II.

Oxygen free radicals are highly reactive and can cause oxidative degradation of most types of cellular macromolecules including lipids (Kellogg and Fridovich, 1977). On account of their high content of polyunsaturated fatty acid (Mimnaugh et al., 1985: Kappus, 1986), mitochondria are peroxidized readily by oxygen free radicals, such as O_2^- and particulary $OH\cdot$. As shown in Fig. 4 and 5, oxygen free radicals produced by addition of succinate or NADH in submitochondrial particles were shown to attack mitochondrial fraction as demonstrated by lipid peroxidation. In the damage observed, both O_2^- and H_2O_2 are considered to be involved because the lipid peroxidation was prevented by SOD or catalase (data not shown). The production of malondialdehyde from mitochondrial and extramitochondrial fraction was inhibited by local anesthetics. This effect appeared to concide with their effect on superoxide production by submitochondrial particles

supplemented with electron donors (Fig. 1, 2, 4, 5) . On the other hand, it was found in experiment not presented here that local anesthetics did not have the quenching effect on oxygen free radicals produced by xanthine/xanthine oxidase system or by the U.V. irradiation. Therefore, it is suggested that the inhibition of lipid peroxidation by local anesthetics may be mediated through the suppression of generation of oxygen free radicals by these agents in mitochondria.

The results obtained in this study suggest that the inhibitory effect of local anesthetics on superoxide generation and lipid peroxidation in energy supplemented mitochondria may be attributable to their interference of electron transport steps prior to antimycin sensitive sites, in or near Complex I segment.

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= 국문초록 =

국소마취제가 Mitochondria에서의 전자이동 및 Superoxide Radicals의 생성에 미치는 영향

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국소마취제가 mitochondria에서의 전자이동 및 superoxide 라디칼의 생성 그리고 지질의 과산화에 따른 malondialdehyde 생성에 미치는 영향을 관찰하였다.

국소마취제는 전자이동계의 효소활성도에 영향을 나타내었다. NADH dehydrogenase, NADH oxidase와 NADH-ubiquinone oxidoreductase의 활성도는 lidocaine, procaine과 dibucaine에 의하여 효과적으로 억제되었고 cocaine에 의하여 약간 억제되었다. Succinate dehydrogenase, succinate cytochrome c oxidoreductase 와 succinate-ubiquinone oxidoreductase 활성도는 lidocaine 과 dibucaine에 의하여 억제되었으나 succinate oxidase는 국소마취제에 의하여 활성화되었다. 국소마취제는 dihydroubiquinone-cytochrome c oxidoreducatse와 cytochrome c oxidase의 활성도를 억제하였다. 이와 같은 반응에서 국소마취제에 대한 complex I segment의 반응이 다른 complex segment 보다 크게 나타났다.

국소마취제는 succinate 또는 NADH에 의한 superoxide 생성과 이에 대한 antimycin의 자극효과를 억제하였다. 또한 국소마취제는 산소라디칼에 의한 지질의 과산화를 억제하였다.

이상의 결과로부터 국소마취제는 mitochondria의 전자전달 과정중 Complex I segment에 또는 인접한 부위에 작용하여 전자이동을 억제함으로써 superoxide 생성과 지질의 과산화를 억제할 것으로 시사되었다.