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Laboratory Report

Lidocaine Effects on Brain Mitochondrial Metabolism in Vitro

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Both lidocaine and anoxia inhibit rapid axonal transport. In an attempt to elucidate the mechanism of this action of lidocaine, its effect on mitochondrial respiration was studied. The local anesthetic produces a dose-dependent inhibition of oxygen consumption (50 per cent inhibition at 8mM) by porcine brain mitochondria when glutamate, but not when succinate, serves as the substrate. This indicates electron transport is blocked at the NADH dehydrogenase level. Potent uncoupling of oxidative phosphorylation is observed with both substrates. All of the effects are readily reversible upon removal of the anesthetic. It is concluded that lidocaine apparently inhibits rapid axonal transport by depressing oxidative metabolism. (Key words: Anesthetics, local, lidocaine; Nerve, metabolism, lidocaine; Metabolism, mitochondrial, lidocaine.)

CELLULAR PROCESSES that utilize ATP are very sensitive to its availability. Rapid axoplasmic transport evidently is such a process, since metabolic inhibitors like dinitrophenol and anoxia that decrease the availability of ATP rapidly block movement of various substances along on axon.[1]

Axoplasmic transport in the rabbit vagus nerve is reversibly blocked by lidocaine in an *in-vitro* system.[2] Since it was already known that several types of anesthetics inhibit mitochondrial metabolism,[3-5] the effects of lidocaine on a mitochondrial system were investigated in the present study. Porcine brain was selected as a representative neuronal source of mitochondria, isolation from peripheral nerve in quantity being impractical. Lidocaine was found to inhibit electron transport and uncouple oxidative phosphorylation reversibly in porcine brain mitochondria at a concentration that closely correlates with that blocking axoplasmic transport. Inhibition by this local anesthetic was localized to the NADH dehydrogenase complex.

Materials and Methods

Porcine brain mitochondria were prepared according to the method of Basford,[6] with the exception that bacterial protease type VIII from Sigma (St. Louis) was used, resulting in a better yield. Tissue was removed and chilled (0 C) within 30 min after death of the animal.

Oxygen consumption and P/O ratio of the freshly prepared brain mitochondria were measured manometrically in a Gilson differential respirometer at 30 C. The conditions of the assays were as described by Basford,[6] with either glutamic acid or succinic acid serving as substrate. Lidocaine hydrochloride was added, when desired, from a solution previously adjusted to *p*H 7.4.

To ascertain whether the lidocaine-mediated inhibition of oxidative metabolism was reversible, the following experiment was performed. Sufficient lidocaine (9 mM) to produce approximately 50 per cent inhibition was added to freshly prepared mitochondria, which were then diluted severalfold and centrifuged for 15 min at 27,000 × *g*. The pellet was resuspended and the centrifugation repeated. Control mitochondria not exposed to lidocaine were carried through the same procedure.

Electron transport particles (ETP) were prepared from frozen mitochondria by sub-

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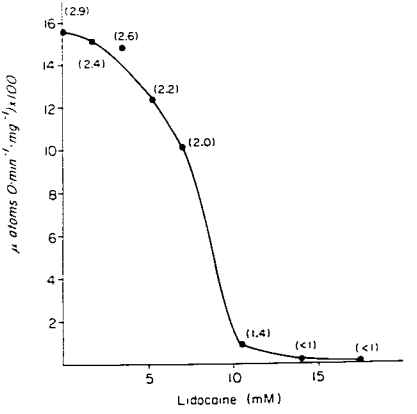

FIG. 1. Inhibition of mitochondrial oxygen uptake by lidocaine. The number in parentheses at each point on the curve indicates the P/O. Each point is the average of values from at least four repetitions.

jecting a suspension (10–15 mg/ml protein in medium A[6]) to sonication at 0 C for a total of 8 min at a power setting of 2.3 amps with a Branson Sonicator (micro tip). The sonicated suspension was then centrifuged at 27,000 $\times g$ for 20 min and the opaque supernatant solution was used for the assays. The ETP prepared in this manner will oxidize NADH with ferricyanide, cytochrome C, or molecular oxygen serving as the electron acceptor. The oxygen reduction activity is inhibited by cyanide.

The NADH dehydrogenase complex of the ETP was assayed spectrophotometrically with either cytochrome C or ferricyanide serving as electron acceptor. The reaction mixture contained 100 μl 1 per cent cytochrome C (horse heart); 50 μl 1 per cent NADH; 20 μl 0.1 M $Na\ N_3$; 10 μl appropriately diluted enzyme and buffer with or without the anesthetic to a total of 1.0 ml. The buffer was 50 mM Tris HCl, *p*H 7.4. The change in absorbance (A) at 550 nm was followed to measure the reduction of cytochrome C. Activities were expressed by the following relationship: (ΔA) (0.0262) = mM NADH oxidized. When ferricyanide was used as the electron acceptor, the cytochrome C was replaced by 100 μl of a 5-mM potassium ferricyanide solution and NaN_3 was omitted. The change in absorbance at 420 nm was followed and activity was calculated as follows: (ΔA) (0.5) = mM NADH oxidized.

Results

The effects of lidocaine on oxidative phosphorylation by purified brain mitochondria were studied with glutamate serving as the substrate. As shown in figure 1, addition of increasing concentrations of lidocaine to the mitochondrial assay system results in a dose-dependent decrease in the rate of oxygen consumption. Approximately 50 per cent inhibition of electron transport rate is observed at a lidocaine concentration of 8 mM (0.23 per cent).

In addition to the inhibition of oxygen consumption, there is a dose-dependent uncoupling of oxidative phosphorylation by lidocaine, evidenced by the decreasing P/O (ratio of inorganic phosphate uptake into ATP to oxygen utilized). This effect becomes apparent at approximately 5–7 mM lidocaine (fig. 1) and appears to be nearly complete at concentrations of 10 mM or more.

To localize further the site of inhibition of oxidative metabolism caused by lidocaine, alternative substrates that, when oxidized, feed electrons into the pathway at different locations may be employed. Succinate has been used as an alternative substrate since

electrons coming from its oxidation enter the electron transport after the first oxidative phosphorylation site, and table 1 indicates that as the concentration of lidocaine is increased to 21 mM, there is no significant decrease in oxygen consumption. However, one can see from table 1 that, again, a strong uncoupling effect occurs with lidocaine concentrations of 7 mM or more.

The reversibility study showed that O_2 uptake and P/O returned to control levels after removal of the anesthetic by washing. Reversibility was found with both succinate and glutamate as substrates.

The experiments described above with whole mitochondria indicated that the lidocaine inhibition of oxidative metabolism occurs at a site in the electron transport chain at the level of the NADH dehydrogenase. To study this enzyme complex more specifically, the mitochondria were disrupted by sonication and electron transport particles were isolated. With this sub-mitochondrial fraction, the NADH dehydrogenase activity may be easily assayed with various electron acceptors serving as substrates (*e.g.*, cytochrome C and ferricyanide). NADH dehydrogenase activity assayed with cytochrome C is inhibited by amytal or rotenone, but activity with ferricyanide is not inhibited.[7] In the present experiments, when cytochrome C or molecular oxygen was employed as the electron acceptor, dose-dependent inhibition of NADH dehydrogenase activity was seen (fig. 2). With both of these acceptors approximately 50 per cent inhibition occurred at a lidocaine concentration of 1–2 mM. One should note that 50 per cent inhibition of

TABLE 1. Effect of Lidocaine on Mitochondrial Respiration with Succinate as the Substrate*

Lidocaine Concentration			
mM	Per Cent	gatoms O min⁻¹ mg	P/O
0	0	0.097	1.9
7.0	(.2)	0.070	1.1
10.5	(.3)	0.098	0.6
14.0	(.4)	0.110	0.9
21.0	(.6)	0.070	0.1

* Each point is an average of values from two experiments.

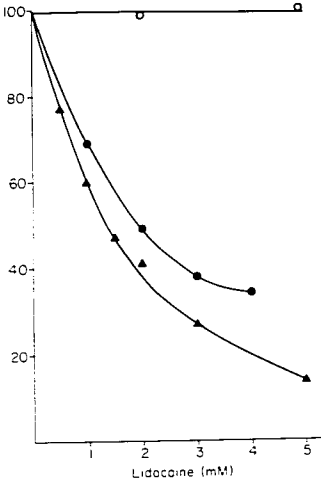

FIG. 2. Inhibition of NADH dehydrogenase activities by lidocaine. The following electron acceptors are employed in the assay: (○) potassium ferricyanide, (●) cytochrome C and (▲) molecular oxygen.

NADH dehydrogenase in intact mitochondria required a concentration at least five-fold higher. Lidocaine in concentrations as high as 15 mM had no effect on the enzymatic activity when ferricyanide was used as the electron acceptor, thus the data obtained with the sub-mitochondrial particles indicate that inhibition by lidocaine is similar in character to inhibition produced by rotenone or amytal.[7]

Discussion

It has been demonstrated by Fink and co-workers that exposure of vagus nerve *in vitro* to lidocaine causes reversible inhibition of rapid axoplasmic transport.[2] In seeking an explanation for this effect of lidocaine on the intact nerve, the present study evaluated the inhibitory effects of lidocaine on oxidative metabolism. Rapid axoplasmic transport was inhibited at concentrations of anesthetic between 7 and 10 mM, the same concentration

range where, in the present study, approximately 50 per cent inhibition of the mitochondrial respiration was observed. Therefore, inhibition of rapid axoplasmic transport might well be accounted for simply by a reduced supply of ATP in the axon, since Ochs *et al.* showed a dependence of rapid axoplasmic transport on ATP.[1]

Inhibition of mitochondrial respiration by lidocaine appeared to be a readily reversible process. This also correlated with observations of a reversible block of rapid axoplasmic transport in the intact nerve.[2]

Since all of the experiments described here were carried out on a purified *in-vitro* system, one must be cautious in drawing conclusions as to the relevance of this inhibition in an *in-vivo* situation. However, the concentration of lidocaine often injected near a nerve in a clinical block is 35 mM (1 per cent), and therefore the possibility that a transient inhibitory effect on mitochondrial respiration and rapid axoplasmic transport might occur does exist. Experiments confirming this possibility in an *in-vivo* system have been reported.[3] It should be mentioned that the concentration range of lidocaine causing inhibition of mitochondrial metabolism is relatively high compared with the concentrations required to block conduction in nerve fibers.[9]

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Erratum

An error appeared in the article by Greenstein, *et al.*, Metabolism *in Vitro* of Enflurane, Isoflurane, and Methoxyflurane (ANESTHESIOLOGY 42:420–424, 1975). The first sentence of the abstract should read: "Specific activities of methoxyflurane, enflurane, and isoflurane defluorinases were measured in microsomes prepared from the livers of Fischer 344 rats; the ratio of these activities was 23:3:1."