INHIBITION OF MITOCHONDRIAL ANION PERMEABILITY BY LOCAL ANAESTHETICS

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1. Introduction

Local anaesthetics such as nupercaine have been shown to have a protective action on the structure and energy-linked functions of rat liver mitochondria during incubation at 30°C or prolonged storage at 0°C [1,2]. This action has been attributed to the inhibition of endogenous Ca^{2+}-activated phospholipase A by the local anaesthetics [3,4]. These observations have provided a logical basis for the incorporation of nupercaine in the medium for experiments on mitochondrial Ca^{2+} uptake [5] in order to overcome the problem of mitochondrial fragility encountered in such experiments [6]. Local anaesthetics appear to have other actions on mitochondria such as competition for cation binding sites [7] and stimulation of Ca^{2+} uptake [8,9].

Although rat liver mitochondria are usually considered to have a low permeability to most anions, other than by highly specific carriers [10], rat liver mitochondria have been reported [11] to become permeable to Cl^- at high pH (pH 8–9) and conditions reported [12] under which beef heart mitochondria become permeable to Br^-, succinate, fumarate and citrate as well as Cl^-. We have reported [13] investigations of the anion permeability of rat liver mitochondria and rationalisation of the observations in terms of a pH-dependent anion-conducting pore in the mitochondrial inner membrane. This pore appears to open progressively with increasing pH over the range pH 7–9 and also depends on the presence of free Ca^{2+} inside the mitochondria.

This paper reports our finding that the anion permeability of mitochondria at alkaline pH is inhibited by several local anaesthetics in contrast to their effects or lack of effects on NO_3^-, phosphate and ribose transport.

Abbreviations: Butacaine, 3-(*p*-aminobenzoxy)-1-di-*n*-butylaminopropane; nupercaine, 2-butoxy-*N*-(2-diethylaminoethyl) cinchoninamide; procaine, *p*-aminobenzoyldiethylaminoethanol, tetracaine, *p*-butylaminobenzoyl-2-dimethylaminoethanol, FCCP, carbonylcyanide *p*-trifluoromethoxy phenylhydrazone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid

2. Materials and methods

Butacaine sulphate and tetracaine hydrochloride were obtained from Sigma (London) Chem. Co., procaine hydrochloride and ribose from BDH Chem. Ltd, nupercaine (cinchocaine hydrochloride) was a gift from CIBA Labs, Horsham. Ammonium isethionate was obtained from Stuart Kinney Co., Hepes from Hopkin and Williams Ltd, FCCP from Boehringer Corp. (London) Ltd. Other reagents were of A.R. or biochemical grade.

Mitochondria were prepared and their protein concentration assayed essentially as in [14] except that 0.5 mM EGTA was included in the homogenisation and first wash media. Light scattering measurements were made in a continuously stirred cuvette thermostatted at 25°C as in [14]; 4.0 ml medium contained 1.5 μg each of rotenone and antimycin; mitochondria, 1.5–2.0 mg protein, were added to give an initial absorbance of 0.75 to 0.95. (The amount of mitochondria added was constant for any set of experiments and batch of mitochondria.)

3. Results

The electrogenic uniport mode of movement of Cl^- across the mitochondrial membrane at pH 8 is shown by the rapid decrease in light scattering produced by adding uncoupler, FCCP, to the mitochondria suspended in an NH_4Cl medium, fig.1(A). The protons conducted by the uncoupler balance the pH difference produced by entry of NH_3 and the electrical charge difference produced by Cl^- uniport so that the overall process is equivalent to entry of NH_4Cl which thus fails to provide osmotic balance, and this in turn allows the mitochondria to swell and thus scatter less light. When a local anaesthetic such as nupercaine is added to mitochondria suspended in an NH_4Cl medium there is a small, virtually instantaneous, decrease in light scattering and a slight stimulation of the rate of decrease of light scattering, fig.1(b). However, fig.1(b–e) also show that the rate of light scattering decrease produced by addition of uncoupler is markedly inhibited by the presence of nupercaine. This inhibition occurs rapidly and is observed regardless of whether the nupercaine is added before, fig.1(b,d), or after, fig.1(c,d), the uncoupler. The virtually immediate onset of inhibition shows that it does not involve inhibition of a phospholipase since such action would prevent a time-dependent change in permeability. The procedure of adding the local anaesthetic 15 s after the mitochondria and 15 s before uncoupler, as shown in fig.1(b) was adopted in order to observe the effect of addition and also because some local anaesthetics produced progressive damage to the mitochondria when added at high concentration.

Using this procedure experiments were carried out with a range of concentrations of butacaine, nupercaine, procaine and tetracaine, the results of which are presented as Dixon plots in fig.2. All inhibited Cl^- entry, the concentrations producing 50% inhibition being: nuper-

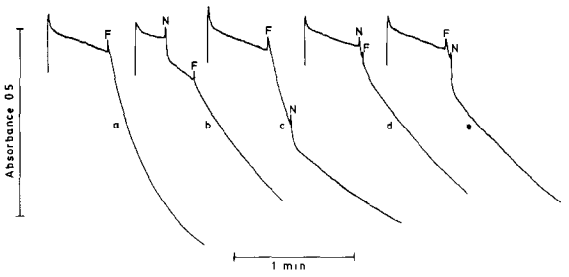


Fig.1. Inhibition of Cl^- entry by nupercaine. In all cases the medium was 0.1 M NH_4Cl adjusted to pH 7.9 with NH_3 solution. Experiments were initiated by addition of mitochondria as shown by the vertical rise at the left of each trace, N marks the addition of nupercaine to 0.6 mM final conc, F marks the addition of FCCP to 5 μM final conc. (a) No nupercaine added. (b) Nupercaine added 15 s after the mitochondria, FCCP added 15 s after the nupercaine. (c) Nupercaine added 15 s after FCCP. (d) Nupercaine added 2 s prior to FCCP. (e) Nupercaine added 2 s after FCCP. Other conditions as in section 2.

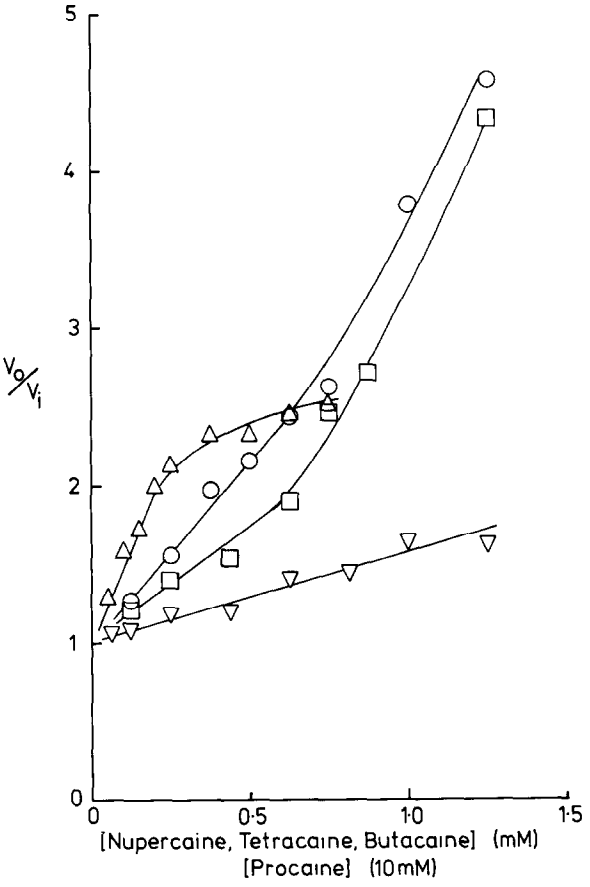


Fig.2. Dixon plot of the inhibition of Cl^- entry by local anaesthetics. Experimental conditions were as in fig.1, the order and timing of additions being as in fig.1(b). Inhibition was estimated from initial rates after the addition of FCCP, v_i rate with inhibitor present, v_o rate in absence of inhibitor. (△) Nupercaine; (○) tetracaine; (□) butacaine; (▽) procaine.

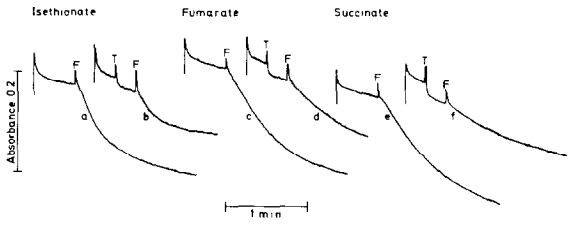


Fig.3. Inhibition by tetracaine of the entry of isethionate, fumarate and succinate anions at alkaline pH. Conditions and order of additions as fig.1(b). (a,b) Ammonium isethionate, 100 mM (pH 8.0). (c,d) Ammonium fumarate, 85 mM (pH 8.2). (e,f) Ammonium succinate, 85 mM (pH 8.5). T marks the addition of tetracaine to 0.5 mM final conc., F the addition of FCCP to 5 μM final conc.

caine, 0.2 mM; tetracaine, 0.4 mM; butacaine, 0.7 mM; procaine, 17 mM.

The entry of other anions by uniport at alkaline pH is also inhibited by local anaesthetics and as examples the inhibition of isethionate, fumarate and succinate entry by tetracaine are shown in fig.3. In contrast the entry of NO_3^- is only partially inhibited, fig.4(a,b), NO_3^- appears to have two modes of uniport entry, the alkaline pH-dependent pore and another process operative at pH 7 and below [13], and only the former is inhibited by local anaesthetics. Figure 4 also shows that the phosphate–hydroxide exchanger carrier is insensitive to tetracaine (c–e) while the entry of ribose is stimulated (f,g).

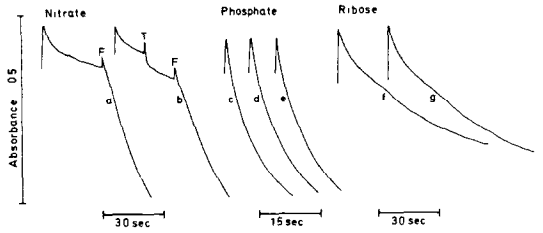


Fig.4. Effect of tetracaine on NO_3^- entry, phosphate hydroxide exchange and ribose entry. Conditions as in fig.1. (a,b) Ammonium nitrate 0.1 M, (pH 8.0), T indicates addition of tetracaine to 0.5 mM, F the addition of FCCP to 5 μM. (c–e) Ammonium phosphate, 80 mM (pH 7.5): (c) tetracaine absent; (d) 0.5 mM tetracaine present; (e) 1.0 mM tetracaine present. (f,g) Ribose, 230 mM with 10 mM Hepes–KOH buffer (pH 8.0): (f) tetracaine absent; (g) 0.5 mM tetracaine present.

4. Discussion

The distinction between the inhibition of the pH-dependent anion uniport and the lack of marked effect on NO_3^- transport and phosphate–hydroxide exchange together with the stimulation of ribose entry suggests that the interaction of local anaesthetics with the pH-dependent anion conduction system is more specific than some general effect on membrane stability produced by dissolution of the anaesthetic in the lipid of the membrane. Furthermore, simple adsorption of the local anaesthetics would make the surface charge more positive (or less negative) and this would be expected to increase rather than decrease the anion permeability as discussed [15] where the inhibition of chloride transport in red blood cells by local anaesthetics was investigated.

The apparent involvement of free intramitochondrial Ca^{2+} in the anion conduction system [13], considered together with the observation [7] of competition between local anaesthetics and Ca^{2+} for cation binding sites and the observation [8,9] of Ca^{2+} uptake stimulation by butacaine, may indicate that displacement of Ca^{2+} from an activating site is involved in the inhibition by local anaesthetics.

These observations indicate that addition of local anaesthetics to the medium for their protective action on mitochondria must be viewed with caution for, while there may be an inhibition of endogenous Ca^{2+}-activated phospholipase, in short-term experiments reduction of fragility may involve inhibition of anion movement and these effects on anion movement may well affect the apparent charge stoichiometry of Ca^{2+} transport.

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