The Interaction of Organic Cations with the Mitochondrial Membrane

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Summary. Weak and strong organic bases behave in an opposite manner in respect to several mitochondrial functions. The former induce a catalytic exchange with K^+ in valinomycon-treated, respiratory-inhibited mitochondria, and act as uncouplers in respiring mitochondria. The latter induce a stoicheometric exchange with K^+ and are actively taken up by respiring mitochondria.

The use of organic cations, local anesthetics[1-8], aliphatic and aromatic amines[6-16], and strong bases[17-22], is expanding continuously in order to clarify the molecular aspect of active transport in mitochondria and energy transducing systems. In the present study, a large number of organic cations have been tested. The experiments indicate that organic cations may be classified into two classes, which differ in the presence of a dissociable proton in the charged group of the cations.

Methods. Rat liver mitochondria were prepared by standard procedures. Oxygen consumption was measured polarographically with a Clark electrode. Absorbance changes at 546 nm were measured, simultaneously with K^+ and H^+ changes, in an Eppendorf photometer, as previously described[23]. ATPase activity was measured according to PULLMAN et al.[24]. All experiments were carried out at room temperature. Chemicals were of the highest purity commercially available. The local anesthetics used were: butacaine, dibucaine, lidocaine, phenacaine, procaine and tetracaine. The aromatic and aliphatic amines used were: dimethylbenzylamine, dibenzylamine, methyldibenzylamine and triethylamine. Other weak bases: acridine orange, atebrin and neutral red. The strong bases used were: tetraethylammonium, tetrapropylammonium, tetrabuthylammonium, trimethylphenylammonium, benzyldimethylphenylammonium, benzyltriethylammonium, safranine, pinacyanole and ethydium bromide. Abbreviations used: TPB, tetraphenylboron; DNP, dinitrophenol.

Results and discussion. Addition of organic cations to valinomycin-rotenone treated mitochondria caused a K^+ release. The rate of release increased with the cation concentration and the hydrophobicity of the cations. Addition of TPB or picrate caused a stimulation of the rate of K^+ release; TPB was two order of magnitude more effective than picrate. Figure 1 A shows that the addition of a strong base, such as tetrapropylammonium, induced a stoicheometric K^+ release. Neither pH nor absorbance changes were detected. This suggests a 1:1 exchange of K^+ with the strong base. On the other hand, weak bases, such as dibucaine (Figure 1 B) caused a slow but complete release of K^+, an absorbance increase and a hydrogen ion uptake. This suggests a catalytic effect of the weak bases. Two hypothesis may be considered. The charged form of the weak base is translocated into the matrix in exchange with K^+, it loses its proton and the neutral form flows out down the concentration gradient and takes up a H^+ again. The alternative hypothesis is that high concentra-

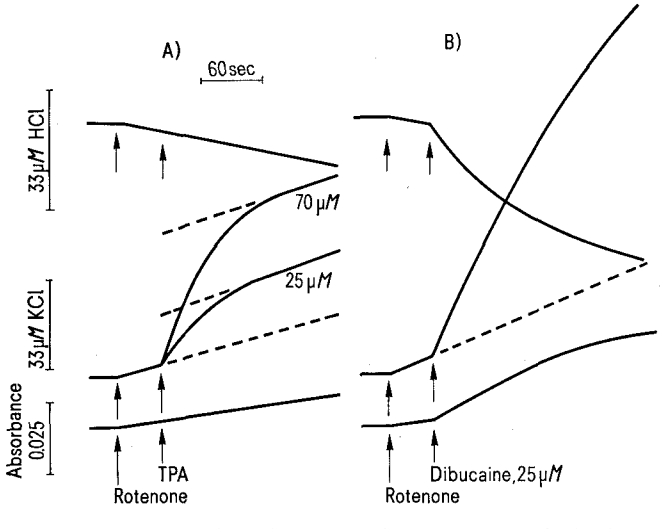

Fig. 1. Organic cation-K^+ exchange in rotenone inhibited mitochondria in the presence of valinomycin. The medium contained: 0.2 *M* sucrose, 20 m*M* LiCl, 1 m*M* *Tris*-Pi, 5 μ*M* TPB, 0.17 μg valinomycin/ml, 2 μ*M* rotenone, 400 μ*M* KCl, 2.3 mg protein/ml, pH 7. Total volume 3 ml. The upper, medium and lower traces refer to the hydrogen ion uptake, K^+ release and absorbance increase, respectively. TPA: tetrapropylammonium.

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Inhibition of P/O ratio and enhancement of the ATPase activity

	Activation ATPase (%)	Inhibition ADP/O (%)	Activation respiration (%)
Dinitrophenol (5 μM)	41	42	110
Phenacaine (0.5 mM)	42	16	44
Butacaine (0.5 mM)	15	19	64
Dibucaine (1 mM)	31	21	35
Acridine orange (10 μM)	18	14	41
Atebrine (0.2 mM)	27	12	23
Methyldibenzylamine (1 mM)	44	38	50
Triethylamine (5 mM)	19	26	36

The medium for the ATP-ase activity was: 0.12 M sucrose, 50 mM *Tris*-Cl pH 7, 6 mM ATP, 3 mM $MgCl_2$, 32 µg/ml piruvate kinase and 0.66 mg/ml of phosphoenol piruvate. Total volume 1 ml. The medium for the ADP/O and respiratory rate experiments are the same of Figure 2.

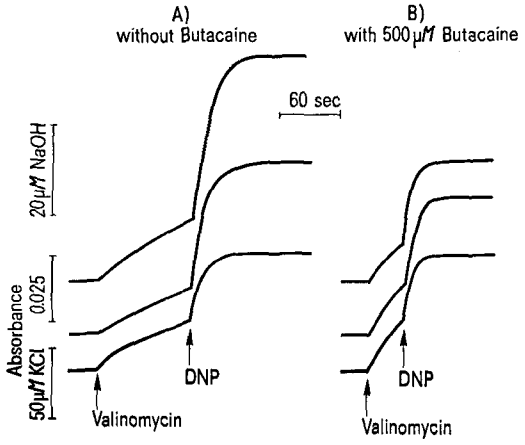

Fig. 2. Organic cation-K^+ exchange in rotenone inhibited mitochondria. The medium contained: 0.07 M sucrose, 7 mM *Tris*-Cl 2 μM rotenone, 1.5 mg protein/ml, 0.4 mM KCl, final pH 6.8. Total volume 3 ml. The valinomycin addition was 0.17 µg/ml, DNP was 50 μM. In B) 500 μM Butacaine was present. The upper, medium and lower traces refer to the hydrogen ion uptake, absorbance increase and K^+ release, respectively.

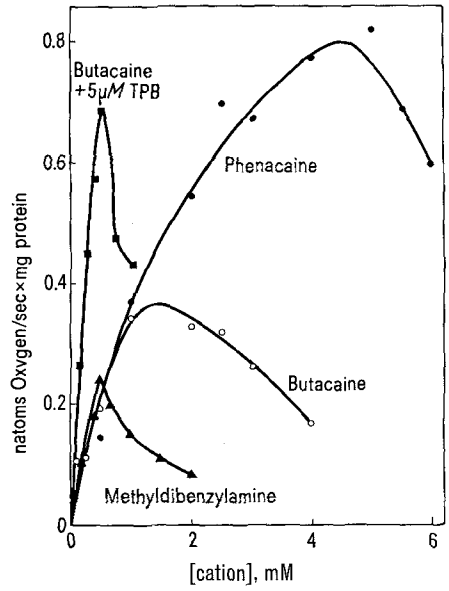

Fig. 3. Respiratory rate response on the concentration of organic cations. The medium was: 0.2 M sucrose, 5 mM *Tris*-Pi, 5 mM HEPES, 2 mM succinate-*Tris*, pH 7.2, 2.7 mg protein/ml. Final volume 2 ml. In ordinate the difference between respiratory rate after and before the cation addition is shown.

tions of organic molecules in the membrane cause damage and increase in hydrogen ion permeability. Figure 2 A and B shows a comparison between the effect of valinomycin and DNP on K^+ release, hydrogen ion uptake and absorbance changes in the absence and presence of 500 μM Butacaine, respectively. The amount of K^+ released was the same in both cases, but the extent of shrinkage and of H^+ uptake were lower with butacaine. This indicates that the K^+ release in the presence of weak bases is a K^+-weak base exchange rather than a K^+-H^+ exchange. The acidification of the matrix space permits accumulation of the weak base in the cationic form.

The inhibition of K^+ efflux by local anesthetics is a well known phenomenon[1–3]. Local anesthetics inhibit the spontaneous K^+ release rate and the K^+ release rate induced by FCCP and FCCP + valinomycin[2]. We have confirmed these results and observed that a strong inhibition occurred also with other hydrophobic weak bases. However, weak bases exert a strong interference with both K^+ and H^+ glass electrodes. If a drift is present in the electrodes, due to low current leaks, the addition of weak hydrophobic bases stabilizes the response by inhibiting the electrode drift. The electrode effect may explain why JUDAH et al.[1], using flame photometry techniques, showed no inhibition of spontaneous K^+ release. We have been unable to decide whether the inhibition of K^+ release is a true inhibition or an electrode effect. Addition of TPB abolishes the inhibition of K^+ release due to local anesthetics and causes an enhancement of the rate of K^+ release.

The main evidence for an active transport of organic strong bases is: 1. swelling of the mitochondrial matrix. 2. reversibility of swelling due to uncouplers, 3. state 4-state 3-state 4 transition of respiration similar to that due to Ca^{2+} or K^+ plus valinomycin, 4. acidification of the external medium[17]. Active transport of tetrapropylammonium has been reported[25].

The above parameters have been confirmed for all strong bases in the present study. High concentrations of lipophilic cations, such as tetrabuthylammonium, increase the state 4 respiration. Uncoupling by lipophilic strong bases has already been reported[17,19], and explained as structural or conformational alterations of the mitochondrial membrane.

Weak bases cause an activation of respiration which does not level off. Figure 3 shows that weak bases caused first an enhancement and then an inhibition of the respiratory rate. TPB increased both the oxygen uptake and inhibition. That the increase in respiration is indicative of a real uncoupling is confirmed also by measurements of the ADP/O ratios and ATPase activity. Increasing concentrations of weak bases result in inhibition of the P/O ratio and enhancement of the ATPase activity (see Table).

Uncoupling is unlikely to be due to membrane damage in view of the fact that cations similar in structure, such as methyldibenzylamine and benzyldimethylphenylammonium, have opposite effects on respiring mitochondria. The uncoupling of the weak bases seems to be related to the dissociable proton. A 'cycling' mechanism, similar to that occurring in the passive exchange of K^+, may be a simple explanation of the uncoupling. The cationic form is actively taken up, and flows out after releasing the proton into the matrix. Another explanation is that the weak base accepts or provides protons to some membrane region, where the hydrogen ion activity is responsible of the uncoupling.

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