Lipophilic Interactions of Organic Cations with Mitochondrial Inner Membranes during Respiratory Control*

(Received for publication, May 22, 1973)

KENNETH S. ROGERS AND EDWIN S. HIGGINS

From the Department of Biochemistry, Medical College of Virginia, Health Sciences Division of Virginia Commonwealth University, Richmond, Virginia 23298

SUMMARY

Twelve different lipophilic organic cations (tetraethyl-, tetrapropyl-, tetrabutyl-, tetrapentyl-, tetrahexyl-, tetraheptyl-, decyltrimethyl-, cetyltrimethyl-, and benzyltrimethylammonium bromides; and dibenzyl trimethylammonium chloride, N-1-dodecylnicotinamide chloride, and cetylpyridinium bromide) depressed respiratory control in rat liver mitochondria. Evaluation of mitochondrial responses in terms of linear free energy processes for the six symmetrical tetraalkylammonium bromides indicated that the NADH dehydrogenase receptor site for inhibitor (diminution of control of glutamate respiration) was more lipophilic than the succinate dehydrogenase receptor site (reduction of control of succinate respiration). (Both receptor activities have been located on the mitochondrial inner membrane by other workers.) Prior incubation of intact mitochondria with a tetraalklylammonium halide up to an hour at 30°C did not alter the results.

Depression of respiratory control by the symmetrical quaternary ammonium halides, e.g. tetrabutylammonium bromide, occurred by two different mechanisms depending upon inhibitor concentrations: (a) inhibition of phosphorylation at low concentrations of the alkyl bromide and (b) uncoupling of oxidative phosphorylation at high concentrations of the alkyl bromide. Depression of respiratory control by the nonsymmetrical organic cations occurred by either inhibition of electron transport, action as an inhibitor of phosphorylation, or as an uncoupler. The mechanism of inhibition was dependent upon the organic structure of these amphipathic molecules.

cetyltrimethylammonium bromide, decyltrimethylammonium bromide, cetylpyridinium bromide, and N-1-dodecylnicotinamide chloride were tested for inhibition of mitochondrial respiratory control. This work continues our investigation of the action of detergents and related compounds on mitochondrial efficiency.

Previously (9), sodium octyl, decyl, dodecyl, and tetradecyl sulfates were tested for uncoupling activity against energy-linked functions of rat liver mitochondria. All four detergents diminished respiratory control with either succinate or l-glutamate as substrate. The detergents augmented mitochondrial swelling and, at high concentrations depressed energized (adenosine triphosphate, ATP) contraction. The potency of the detergents for both depression of respiratory control and inhibition of energized recovery from swelling was a function of the number of methylene groups in the alkyl sulfate. This was equated with the lipophilicity log P (2, 7, 8) of the molecules as measured by their partitioning P across the interfacial barrier of 1-octanol and water. Uncoupling and inhibition of recovery from swelling required lesser amounts of a molecule that was lipophilic than one that was hydrophilic. The quadratic correlation of log P with uncoupling activity reflected interaction of the anionic detergents with mitochondrial inner membrane constituents (3, 6). Thus, the lipophilicity of the uncoupler dictates the magnitude of effect and the ability of the reagent to combine with a relatively lipid-like or nonpolar membrane site. Similar conclusions were recently documented by Stockdale and Selwyn (9).

Uncoupling activity of 23 substituted phenols were correlated with the lipophilicity and electron-donating or -accepting character of the substituent. As the extent of lipophilicity and electron-withdrawing capacity of the substituent increased, then the potency of the phenol as an uncoupler during succinate oxidation was increased proportionately.

EXPERIMENTAL PROCEDURE

Liver mitochondria, obtained from 150- to 200-g male Sprague-Dawley rats (Holtzman Co.) fed Purina rat chow and water ad libitum, were prepared after the rats were fasted 24 hours, following a modified method of Hogboom et al. (10). The 0.25 M sucrose solution contained 0.5 mM ethylenediaminetetraacetic acid (EDTA). The liver was homogenized in a Teflon and glass Duvall tissue grinder and centrifuged for 10 min at 700 × g. The precipitate was washed once and the combined supernatants were centrifuged for 10 min at 8500 × g. After two washings the

* This work was supported in part by an award from the Bureau of Alcohol Studies and Rehabilitation, Department of Health, Commonwealth of Virginia.

We have used a “structure-activity” approach (1-3) to analyze mitochondrial membrane functions during respiratory control studies (4, 5) in the presence of a homologous series of symmetrical tetraalkylammonium bromides. In addition, other compounds such as dibenzyldimethylammonium chloride, benzyltrimethylammonium bromide, and the cationic detergents...
mitochondrial pellet was resuspended in the sucrose-EDTA solution and used immediately. All preparative operations were at 0-4 °C.

Tetraethyl-, tetrapropyl-, tetrapentyl-, tetrahexyl-, decyltrimethyl-, and tetrabutylammonium bromides, and 2,4-dinitrophenol were purchased from Eastman Organic Chemicals. Other reagents such as adenosine diaphosphate (ADP), L-glutamic acid, sodium succinate, crystalline bovine serum albumin, cetyltrimethylammonium bromide, and cetylpyridinium bromide were obtained from Sigma Chemical Co. N-1-Dodecylhexanoyl chloride was prepared in this laboratory and was identical with an authentic sample received from Dr. Bruce Anderson, Virginia Polytechnic Institute and State University. Benzyltrimethylammonium bromide and dibenzylidimethylammonium chloride monohydrate were from Aldrich Chemical Co. Sodium deoxycholate, EDTA, and sucrose were from Fisher Scientific Co. Mannitol was obtained from Pfannstiel Laboratories.

Mitochondrial respiratory control (control of respiration by phosphate acceptor, ADP) was assayed polarographically (11) at 30 °C with a Yellow Springs Instrument model 50 biological oxygen monitor and a Clark fixed voltage polarographic probe. State 3 respiration (rate of substrate oxidation in presence of phosphate acceptor) and State 4 respiration (after exhaustion of ADP) were used for calculation of the respiratory control ratio during oxidation of L-glutamate and succinate. Mitochondrial protein was estimated in the presence of 1% deoxycholate by a biuret method (12) with crystalline bovine serum albumin as standard.

Inhibitor and Membrane Lipophilicity Calculations—Insight into the molecular events that occur during ligand-protein combination or “drug”-receptor interaction can be obtained by studying the biological response as a function of the lipophilic, electronic, or steric characteristics, or all of these, of the compound investigated (13, 14). Thus

$$pI_{50} = \log P - b (\log P)^2$$

where is the concentration of chemical responsible for producing an inhibition of a response by 50% and P is the negative logarithm of that concentration. P represents the “equilibrium” or ratio of chemical concentrations in the nonpolar to polar phases and is the partition coefficient of the chemical for the phases 1-octanol and water. From a molecular orbital theory point of view (15) partitioning into an aqueous phase is considered charge-controlled ($\sum Q^+\sum Q^-$), while partitioning into a nonpolar phase is considered as polarizability-controlled ($\sum \sigma \sum \sigma'$). The relative contribution of each factor determines the partition coefficient observed for a compound. The logarithm of P (log P) represents a lipophilic index of a chemical and the ability of that chemical to cross, or combine with, nonpolar or lipid-like membranes (2, 3, 7). The propensity for hydrophobic interaction is reflected in the positive character of log P. A hypothetical value of log P indicating membrane lipophilicity may be obtained by taking the first derivative of the quadratic equation and setting the quantity (d log P/d log P) equal to zero (6, 7).

The ability of the quadratic equation to describe the potency of the tetralkylammonium halides as inhibitors of respiratory control was evaluated statistically using the method of least squares in a multiple linear regression model. Solution of the model equation was accomplished with use of a Monroe 1860-24 programmable calculator. Comparison of the statistical values (16): $F$, significance of the equation; $r$, the multiple correlation coefficient for the fit of experimental points to the equation; $t$, contribution of each variable to the equation, and S.E., standard error of the inhibition estimate by the equation (predictability) allowed determination of whether the equation is satisfactory as a statistical and theoretical model for the inhibitor interaction with rat liver mitochondrial membranes.

Log P values for the chemical series, tetralkylammonium bromides, were calculated from the additive character of log P (8, 13) and the experimental values for the partitioning of trialkylethylammonium iodides (17) as ion pairs between the immiscible phases 1-octanol and water.

$$\log P = 0.2807 C - 5.2533 \quad (n = 4; \quad r = 0.993; \quad F = 74; \quad S.E. = 0.159)$$

$P$ values are expected (2, 8) a linear relationship was observed between the number of carbon atoms (C) in the quaternary salt and its log P value (Equation 1) as given in Table I. The correlation coefficient value of 0.993 indicated a good fit; a value for $r$ of 1.000 would be a perfect fit of the data to the line described by the equation. From Equation 1, substitution of the number of carbon atoms in the symmetrical tetraklylammonium bromides permitted calculation of their log P values and these are presented also in Table I. Replacement of an iodide with a bromide ion does not significantly alter log P for a compound (8).

**RESULTS**

Evaluations of respiratory control as affected by 12 different quaternary ammonium halides were conducted in the presence of dialyzed crystalline bovine serum albumin. Albumin has been found to stabilize mitochondrial functions (5) and provide for greater efficiency of respiration coupled to phosphorylation (6).

**TABLE I**

<table>
<thead>
<tr>
<th>No. of carbon atoms</th>
<th>Log P observed</th>
<th>Log P calculated</th>
<th>$\Delta$ log P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraethylammonium iodide</td>
<td>8</td>
<td>-2.82</td>
<td>-2.94</td>
</tr>
<tr>
<td>Tripropylammonium iodide</td>
<td>11</td>
<td>-2.19</td>
<td>-2.07</td>
</tr>
<tr>
<td>Tributylammonium iodide</td>
<td>14</td>
<td>-1.30</td>
<td>-1.20</td>
</tr>
<tr>
<td>Tripentylammonium iodide</td>
<td>17</td>
<td>-0.22</td>
<td>-0.33</td>
</tr>
<tr>
<td>Tetrapropylammonium bromide</td>
<td>12</td>
<td>-1.78</td>
<td>-1.84</td>
</tr>
<tr>
<td>Tetrabutylammonium bromide</td>
<td>16</td>
<td>-0.62</td>
<td>-0.70</td>
</tr>
<tr>
<td>Tetrpentylammonium bromide</td>
<td>20</td>
<td>0.54</td>
<td>0.52</td>
</tr>
<tr>
<td>Tetrhexylammonium bromide</td>
<td>24</td>
<td>1.70</td>
<td>1.72</td>
</tr>
<tr>
<td>Tetraheptylammonium bromide</td>
<td>28</td>
<td>2.86</td>
<td>2.93</td>
</tr>
</tbody>
</table>
The influence of tetrabutylammonium bromide upon mitochondrial respiratory control during glutamate and succinate oxidations. Oxygen consumption (nanogram atoms of oxygen per min per mg of mitochondrial protein) was monitored at 30° by a Clark fixed voltage electrode. The reaction mixture of 3 ml contained 0.33 M mannitol; 3.5 mM potassium phosphate (pH 7.4); 3.5 mM potassium chloride; 0.33 mM disodium ethylenediaminetetraacetic acid (EDTA); 4 mg of dialyzed bovine serum albumin; and approximately 2.5 mg of mitochondrial protein as intact rat liver mitochondria. Glutamate or succinate was added in a volume of 20 l to give a final concentration of 1.4 mM and ADP was added in a volume of 30 l to give a final concentration of 0.13 mM. Respiratory control was measured as the ratio of velocity of oxygen consumption during oxidation of substrate in the presence of ADP to that obtained after exhaustion of ADP. The mean from at least four different mitochondrial preparations is presented with two standard errors of the mean in brackets as a single data point for a given concentration of tetrabutylammonium bromide and approximately 2.5 mg of mitochondrial protein as intact rat liver mitochondria. Glutamate or succinate was added in a volume of 20 l to give a final concentration of 1.4 mM and ADP was added in a volume of 30 l to give a final concentration of 0.13 mM. Respiratory control was measured as the ratio of velocity of oxygen consumption during oxidation of substrate in the presence of phosphate acceptor to that obtained after exhaustion of ADP. The mean from at least four different mitochondrial preparations is presented with two standard errors of the mean in brackets as a single data point for a given concentration of tetrabutylammonium bromide. $I_{50}$, $I_{30}$, $I_{20}$, and $I_{10}$ values refer to the respective concentrations of quaternary ammonium salts that diminished respiratory control by 50, 30, 20, and 10%, respectively. Note that the $I$ values for a given percentage of inhibition were less when glutamate was the substrate than when succinate was the substrate for oxidation.

Furthermore, the protein did not alter the inhibition pattern observed with detergents (6). Assay of respiratory control involved the measurement of substrate-stimulated oxygen consumption in presence of ADP and subsequent to its exhaustion. This was a more sensitive and precise criterion of the integrity of mitochondrial membranal function than the separate determinations of oxygen uptake and phosphorus esterified (P:O ratios) (4).

The influence of tetrabutylammonium bromide on the mitochondrial properties of respiratory control are summarized in Figs. 1 and 2. Respiratory control was depressed during both succinate and glutamate oxidations. Unlike inhibition by sodium alkyl sulfates, where respiratory control ratios for succinate and glutamate were depressed equally by a given concentration of detergent (6), a greater amount of tetrabutylammonium bromide was needed to produce equal inhibition during succinate oxidation, e.g. 0.6 mM compared to 0.1 mM. It was of interest to ascertain if the depression of respiratory control by this halide was through action as an electron transport inhibitor (5), as an uncoupler of oxidation from phosphorylation (5), or as an inhibitor of phosphorylation but not of oxidation (5). In the first situation both State 3 and State 4 respiratory rates would be depressed; in the second case, State 4 is elevated and State 3 remains the same or may be slightly depressed; and lastly, State 3 is depressed during inhibition of phosphorylation and State 4 is unaffected.

Two different concentrations of tetrabutylammonium bromide, 0.5 to 4.0 mM, sufficient to depress respiratory control during succinate oxidation by approximately 45 and 65% (Fig. 1) were evaluated in terms of inhibition mechanism. These results are summarized in Fig. 2. TBAB did not inhibit electron transport since State 4 respiration was not depressed by either concentration. On the other hand, 0.5 mM TBAB inhibited phosphorylating oxidation since State 3 respiration was depressed selectively (compare Curve C with that of B) and the organelles responded to stimulation by 2,4-dinitrophenol. On the other hand, 4 mM TBAB uncoupled respiration from phosphorylation since it produced maximal respiratory stimulation which was unaffected by ADP or DNP. In a similar experiment (not shown), TBAB at low concentration (50 μM) inhibited phosphorylating oxidation with glutamate as substrate also. Therefore, depression of respiratory control in the presence of glutamate or succinate by tetrabutylammonium bromide occurred by two different mechanisms, depending upon inhibitor concentrations: (a) inhibition of phosphorylation at low concentrations of the alkyl bromide, and (b) uncoupling of oxidative phosphorylation at high concentrations of the alkyl bromide.

Bakkeva et al. (18) have reported that dibenzyldimethylammonium, tetrabutylammonium, and triphenylmethylphosphonium halides, upon addition to liver mitochondria in the presence of succinate or ATP, produced responses similar to natural penetrating cations (Ca$^{2+}$ or K$^{+}$), e.g. acidification of the incubation mixture, transient increase in ATPase, and State 4 oxidation rate, and swelling of the mitochondrial matrix in presence of phosphate. Their organic cation to mitochondrial protein ratios were much lower than ours (about 0.02 μmole of TBAB per mg of mitochondrial protein compared to 0.6 and 4.8 μmole per mg in Fig. 2) and for that reason they may not have observed the
Depression of mitochondrial respiratory control during glutamate and succinate oxidations by six lipophilic tetraalkylammonium bromides

The effects of six spherical quaternary amines on a general mitochondrial property are summarized. Negative logarithmic values of I_0.5, I_1.0, and I_2.0 (pI_0.5, pI_1.0, pI_2.0, respectively) were obtained from data plots similar to the one given in Fig. 1. Log P' values, an index of lipophilicity, were obtained from calculations given in Table I. Calculated values of pI_0.5 were obtained from Equations 5 and 9 in Table III. The ratio (pI_0.5 calculated to pI_2.0 observed) expressed the correlation between calculated (calc.) and observed (obs.) values.

### Table II

<table>
<thead>
<tr>
<th>Glutamate respiration</th>
<th>pI_0.5</th>
<th>pI_1.0</th>
<th>pI_2.0</th>
<th>log P' (calc)</th>
<th>log P' (obs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraethylammonium bromide</td>
<td>-2.820</td>
<td>0.890</td>
<td>0.730</td>
<td>0.610</td>
<td>0.520</td>
</tr>
<tr>
<td>Tetrabutylammonium bromide</td>
<td>-2.84</td>
<td>0.79</td>
<td>1.02</td>
<td>2.79</td>
<td>2.52</td>
</tr>
<tr>
<td>Tetrapentylammonium bromide</td>
<td>-0.624</td>
<td>4.744</td>
<td>4.624</td>
<td>4.52</td>
<td>0.003</td>
</tr>
<tr>
<td>Tetrahexylammonium bromide</td>
<td>0.045</td>
<td>10.409</td>
<td>6.904</td>
<td>8.047</td>
<td>7.47</td>
</tr>
<tr>
<td>Tetraheptylammonium bromide</td>
<td>1.705</td>
<td>22.555</td>
<td>5.924</td>
<td>8.255</td>
<td>2.07</td>
</tr>
<tr>
<td>Tetrapentylammonium bromide</td>
<td>2.865</td>
<td>8.570</td>
<td>5.052</td>
<td>5.375</td>
<td>18</td>
</tr>
</tbody>
</table>

Succinate respiration

| Tetraethylammonium bromide | -2.820 | 0.738 | 0.600 | 0.520 | 0.53 | 1.09 |
| Tetrabutylammonium bromide | -1.781 | 0.991 | 1.861 | 1.801 | 1.88 | 2.04 |
| Tetrapentylammonium bromide | -0.823 | 5.093 | 6.363 | 3.833 | 2.233 | 0.97 |
| Tetrahexylammonium bromide | 0.544 | 4.444 | 3.144 | 2.144 | 4.14 | 1.09 |
| Tetraheptylammonium bromide | 1.705 | 15.48 | 9.682 | 8.974 | 4.83 | 1.69 |
| Tetrapentylammonium bromide | 2.865 | 0.705 | 0.555 | 0.425 | 0.305 | 0.27 | 0.99 |

Depression of mitochondrial respiratory control during glutamate and succinate oxidations by six lipophilic tetraalkylammonium bromides—The effects of tetraethyl-, tetrabutyl-, tetrapentyl-, tetrahexyl-, and tetraheptylammonium bromides on the respiratory properties of rat liver mitochondria are presented together with partition coefficient logarithms of the halides in Tables II and III. Comparison of the State 3 and State 4 respiratory rates of the depressed mitochondria with those values obtained in absence of inhibitor (not shown) indicated that like tetrabutylammonium bromide, the symmetrical tetraalkylammonium bromides first proceeded respiratory control as phosphorylation inhibitors and then at higher inhibitor concentrations depressed respiratory control as uncouplers of oxidative phosphorylation. Thus 20% inhibition (pI_0.5) occurred when the compound acted as a phosphorylation inhibitor and 50% inhibition (pI_1.0) occurred when the compound acted as an uncoupler. Nevertheless at each level of inhibition, the biological data were satisfactorily described by the equations of lipophilicity given in Table III. Whether the reagent acted mechanistically as a phosphorylation inhibitor or as an uncoupler, the potency of its inhibition of respiratory control was correlated with the lipophilicity of the inhibitor. Thus smaller amounts of tetraalkylammonium bromide were required to produce 20% depression of respiratory control than were needed with tetrabutylammonium bromide; e.g., in glutamate oxidation (Table II), compare pI_1 of 5.85 (1.4 PM) with pI of 0.80 (0.13 µM). The ability to relate by equations the depression of respiratory control to the increased lipophilicity of inhibitory organic cations was not affected by the substrate used for providing the "reducing equivalents," i.e., glutamate or succinate. The data were satisfactorily correlated by the quadratic Equations 2 through 9 given in Table III. Calculated values of pI_0.5 from Equations 6 (glutamate respiration) and 9 (succinate respiration) (Table III) may be compared with the corresponding experimental values in Table II. Predictability was good for inhibition during glutamate respiration and it was better for inhibition during succinate respiration.

Estimation of Succinate Dehydrogenase and NADH (Nicotinamide Adenine Dinucleotide) Dehydrogenase Lipophilicity—Log P' values were calculated from the first derivative of Equations 2 through 9 in Table III. Regardless of the extent of inhibition produced by the homologous series of spherical and symmetrical tetraalkylammonium bromides, inhibition of respiratory control during succinate oxidation provided a log P' value of 4.08 ± 0.04 (mean ± standard error of mean) and depression of respiratory control was not affected by the substrate used for providing the "reducing equivalents," i.e., glutamate or succinate. The data were satisfactorily correlated by the quadratic Equations 2 through 9 given in Table III. Calculated values of pI_0.5 from Equations 6 (glutamate respiration) and 9 (succinate respiration) (Table III) may be compared with the corresponding experimental values in Table II. Predictability was good for inhibition during glutamate respiration and it was better for inhibition during succinate respiration.

"Unpublished data indicated that electron transfer to the NADH dehydrogenase site was not affected by the quaternary ammonium compounds since pI_0.5 values for tetrabutylammonium bromide were not significantly different, irrespective of the oxidizable substrate (glutamate, β-hydroxybutyrate, pyruvate plus malate, or isocitrate). Furthermore, in vitro inhibition of gluta-

TABLE III

<table>
<thead>
<tr>
<th>Glutamate respiration</th>
<th>pI_0.5</th>
<th>pI_1.0</th>
<th>pI_2.0</th>
<th>log P' (calc)</th>
<th>log P' (obs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraethylammonium bromide</td>
<td>-2.820</td>
<td>0.890</td>
<td>0.730</td>
<td>0.610</td>
<td>0.520</td>
</tr>
<tr>
<td>Tetrabutylammonium bromide</td>
<td>-2.840</td>
<td>0.79</td>
<td>1.02</td>
<td>2.79</td>
<td>2.52</td>
</tr>
<tr>
<td>Tetrapentylammonium bromide</td>
<td>-0.624</td>
<td>4.744</td>
<td>4.624</td>
<td>4.52</td>
<td>0.003</td>
</tr>
<tr>
<td>Tetrahexylammonium bromide</td>
<td>0.045</td>
<td>10.409</td>
<td>6.904</td>
<td>8.047</td>
<td>7.47</td>
</tr>
<tr>
<td>Tetraheptylammonium bromide</td>
<td>1.705</td>
<td>22.555</td>
<td>5.924</td>
<td>8.255</td>
<td>2.07</td>
</tr>
<tr>
<td>Tetrapentylammonium bromide</td>
<td>2.865</td>
<td>8.570</td>
<td>5.052</td>
<td>5.375</td>
<td>18</td>
</tr>
</tbody>
</table>

Succinate respiration

| Tetraethylammonium bromide | -2.820 | 0.738 | 0.600 | 0.520 | 0.53 | 1.09 |
| Tetrabutylammonium bromide | -1.781 | 0.991 | 1.861 | 1.801 | 1.88 | 1.04 |
| Tetrapentylammonium bromide | -0.823 | 5.093 | 6.363 | 3.833 | 2.233 | 0.97 |
| Tetrahexylammonium bromide | 0.544 | 4.444 | 3.144 | 2.144 | 4.14 | 1.09 |
| Tetraheptylammonium bromide | 1.705 | 15.48 | 9.682 | 8.974 | 4.83 | 1.69 |
| Tetrapentylammonium bromide | 2.865 | 0.705 | 0.555 | 0.425 | 0.305 | 0.27 | 0.99 |

Log P = a log P' - b (log P')^2 + c
tory control during glutamate oxidation provided a log $P^*$ value of $2.02 \pm 0.06$. These values may be used to estimate the lipophilicity of the membrane receptor which combined with the inhibitor. Hansch and Glave (7) have reported for the toxic interaction of organic ligands with Gram-positive bacteria, Gram-negative bacteria, and hemolysis of the red blood cell by various organic congeners, that upon solution of the respective quadratic equations using log $P$ in correlation of biological response, calculated log $P^*$ values appeared to decrease with an increase in lipid content of the cell wall. That is, log $P^*$ equaled 5.87 for cells containing about 2.5% lipid in the cell wall, 4.37 when cell walls contained about 25% lipid, and 3.50 when cellular membrane contained about 50% lipid (erythrocyte). Therefore we may estimate that the site of depression of respiratory control during glutamate oxidation (presumably associated with the NADH dehydrogenase site) has more nonpolar or lipid character than the site of inhibition during succinate oxidation.

If we may extrapolate from log $P^*$ the lipid content of inhibitor site by using the data of Hansch and Glave

$$\% \text{ lipid} = 115 - 19 \log P^* (n = 3; r = 0.98;$$

$$P = 20; \text{S.E.} = 6) \quad (10)$$

then the inhibitor binding site for tetramethylammonium bromides during succinate oxidation may contain 35% lipid whereas the inhibitor binding site for these compounds during glutamate oxidation may contain 75% lipid. Caution must be used in the use of these numbers since the compounds used to describe the equations were not the ones used in our experiments and it has been shown that different log $P^*$ values are obtained from the same biological response when different series of structurally dissimilar chemicals are used (19). (For example, a log $P^*$ value of 2.3 equivalent to a membrane lipid content of 70% was observed using sodium alkyl sulfates as uncouplers of oxidative phosphorylation by mitochondria during both glutamate and succinate oxidations (6).) Furthermore our values do not apply to the lipid composition of the entire mitochondrial inner membrane but only where the receptor sites are located. The lipid content of the mitochondrial outer and inner membranes has been reported to be 50% and 20%, respectively (20).

Nevertheless, the use of a selected series of quaternary ammonium compounds, the spherical and symmetrical tetramethylammonium bromides, have distinguished a difference in the nonpolar or lipid-like character of inner membrane-bound NADH dehydrogenase (21) (glutamate oxidation) and inner membrane-bound succinate dehydrogenase (22). This was in agreement with previous observations that succinic dehydrogenase is more easily extracted from mitochondrial membranes than NADH dehydrogenase with non-lipid (aqueous) solvents (23).

Depression of Mitochondrial Respiratory Control during Glutamate or Succinate Oxidation by Six Different Nonsymmetrical Quaternary Ammonium Halides — Décyltrimethylammonium bromide, cetyltrimethylammonium bromide, N-1-dodecylnicotinamide chloride, and cetylpyridinium bromide were cationic detergents that formed critical micelles (24). Critical micelle concentrations were measured at room temperature (about 25°C) with a De Novo tensiometer for surface tension (25) and values were obtained in water similar to those in the literature (24). Micelles, as detected by plotting surface tension as a function of the logarithm of a chemical's concentration (not shown), were not found for benzyltrimethylammonium bromide, dibenzylidimethylammonium chloride, or for the tetraalkylammonium bromides. Concentrations employed for surface tension measurements were limited by saturation concentrations at room temperature. Since these compounds did not form a homologous series, no attempts were made to interpret their inhibition of respiratory control as a function of lipophilicity of the inhibitor.

Comparison of the data summarized in Table IV indicated that with the exception of N-1-dodecylnicotinamide, smaller amounts of quaternary salt were required to produce 50% depression of respiratory control during glutamate oxidation than were required during succinate oxidation. These results were similar to those recorded for the spherical and symmetrical series of tetraalkylammonium bromides. Important differences were noted in the mechanism of inhibition by these amphipathic molecules. Replacement of a trimethylammonium residue with a pyridinium residue (cetyltrimethylammonium bromide versus cetylpyridinium bromide) caused a change from uncoupling of oxidative phosphorylation to action of the molecule as an inhibitor of electron transport. Furthermore, replacement of a pyridinium hydrogen with a carbamido group at position 3 changed the type of respiratory control depression in a concentration-dependent fashion, to first inhibition of phosphorylation and then, at higher concentrations of detergent, to uncoupling of oxidative phosphorylation. These phenomena occurred during both glutamate and succinate respiration. These results confirm the previous observations by Anderson et al. (26, 27) that glutamate respiration in mitochondria and submitochondrial particles was depressed by N-1-dodecylnicotinamide chloride. On the other hand, we also find that succinate respiration is also depressed in mitochondria whereas Anderson's group did not note a depression in oxygen absorbed during succinate oxidation. Variation in concentration of inhibitor cannot account for the difference. Thus the reason for the different results is not known at the present time. The benzyl compounds (Table IV) were gen-

<table>
<thead>
<tr>
<th>Compound</th>
<th>$pK_{1/2}$</th>
<th>$pK_{max}$</th>
<th>Dominant mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Décyltrimethylammonium bromide</td>
<td>5.55</td>
<td>4.41</td>
<td>Uncoupling</td>
</tr>
<tr>
<td>Cetyltrimethylammonium bromide</td>
<td>5.80</td>
<td>4.66</td>
<td>Uncoupling</td>
</tr>
<tr>
<td>1-Dodecylnicotinamide chloride</td>
<td>4.51</td>
<td>4.51</td>
<td>Concentration-dependent inhibition, then uncoupling</td>
</tr>
<tr>
<td>Cetylpyridinium bromide</td>
<td>5.32</td>
<td>4.68</td>
<td>Electron transport inhibition</td>
</tr>
<tr>
<td>Benzyltrimethylammonium bromide</td>
<td>2.96</td>
<td>2.14</td>
<td>Electron transport inhibition (glutamate), uncoupling (succinate)</td>
</tr>
<tr>
<td>Dibenzylidimethylammonium chloride</td>
<td>3.40</td>
<td>2.22</td>
<td>Uncoupling</td>
</tr>
</tbody>
</table>
Two sites in mitochondria were localized on the inner membrane of rat liver mitochondria with tetrabutylammonium bromide (TBAB). The influences of the differences in the lipophilicity of these sites with respect to glutamate oxidation (NADH dehydrogenase) and with respect to succinate oxidation (succinate dehydrogenase) were different in their response to combination with tetraalkylammonium bromides or without preincubation at either temperature. It is our belief that 30 and certainly 60 min were sufficient times (1) to allow diffusion of the inhibitor.

The difficulty in interpreting our observations of uncoupling and release from respiratory control in terms of the chemiosmotic hypothesis (28, 29) of oxidative phosphorylation is apparent to us. These cationic tetraalkylammonium compounds, unlike anionic detergents such as oleate or alkyl sulfates (6), might not be expected to function as uncoupling agents if the postulation is valid that uncouplers act as lipophilic proton carriers, thereby discharging the mitochondrial proton gradient and membrane potential. Although possessing the required lipophilicity, these substances are structurally incapable of accepting and discharging protons. Hence, a basis for structural or conformational alterations of macromolecules or of the inner membrane itself cannot be envisioned as a possible mechanism for the dissociation of electron transport from phosphorylation.

It is not our intention to propose a new model for functioning mitochondrial membranes at this time. However we would like to emphasize that structure-activity studies with these spherical, symmetrical tetraalkylammonium bromides may be a useful and sensitive tool whereby membrane lipophilities may be probed during their catalytic functioning, and thus more insight may be gained as to the molecular events which occur during important biochemical processes. From such insight, perhaps newer and better membrane models may be synthesized.

Acknowledgments—The expert technical assistance of W. H. Friend and Mrs. Regina Foster is gratefully acknowledged.

REFERENCES
Lipophilic Interactions of Organic Cations with Mitochondrial Inner Membranes during Respiratory Control
Kenneth S. Rogers and Edwin S. Higgins


Access the most updated version of this article at [http://www.jbc.org/content/248/20/7142](http://www.jbc.org/content/248/20/7142)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/248/20/7142.full.html#ref-list-1](http://www.jbc.org/content/248/20/7142.full.html#ref-list-1)