Differential inhibition of mitochondrial respiratory control by N-1-substituted, 3-,4-substituted pyridinium halides

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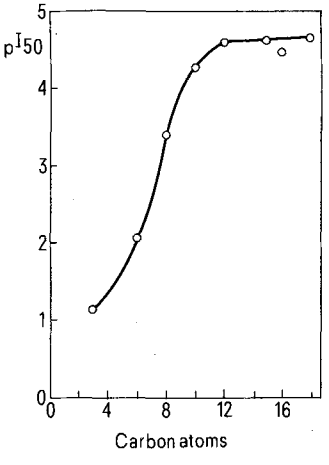
Summary. Several N-1-alkyl-, 3-, and 4-carbamidopyridinium halides were synthesized and determined to be inhibitors of mitochondrial oxidative phosphorylation. L-Glutamate respiration was most depressed by N-1-dodecylpyridinium bromide whereas succinate respiration was most depressed by N-1-dodecylisonicotinamide bromide. Combination of inhibitors with mitochondrial sites may involve lipophilic interactions as modified by steric restrictions.

Tetraalkylammonium bromides have been used to probe the lipophilic characteristics of NADH dehydrogenase and succinate dehydrogenase sites in rat liver mitochondria[1,2]. Results with these phosphorylation inhibitors indicated that inhibition of NADH dehydrogenase respiration required lower concentrations than for inhibition of succinate respiration. This selectivity was not shown by N-1-dodecylnicotinamide chloride. This compound was equally effective as a phosphorylation inhibitor with either type of substrate. In order to gain more information about this anomaly, we have synthesized several structural analogues of the pyridinium and nicotinamide compounds and have tested them as perturbants of rat liver mitochondrial oxidative phosphorylation.

Materials and methods. Liver mitochondria were prepared from male Sprague-Dawley rats and respiratory rates, respiratory control ratios (RCR) and mitochondrial protein were determined as reported previously[1,2]. Respiratory control was measured as the velocity ratio (RCR) of oxygen consumption during oxidation of substrate in the presence of phosphate acceptor (state 3) to that obtained after exhaustion of ADP (state 4)[1,2]. The mean from at least 4 different mitochondrial preparations was used for a given concentration of N-1-alkylnicotinamide bromide.

Preparation of N-1-alkylnicotinamide and N-1-alkylpyridinium bromides: The derivatives were prepared by reaction of the corresponding alkyl bromide (Pfaltz and Bauer, Inc.) with nicotinamide (Nutritional Biochemicals), isonicotinamide, or pyridine (Matheson, Coleman and Bell Company). The procedure was a modification of that reported by Anderson, Reynolds and Anderson[3]. The yield was usually 10–40%. For example, nicotinamide (12.2 g, 10 mmoles) was dissolved in 50 ml of 95% ethanol, then 1-bromodecane (22.1 g, 20.7 ml, 10 mmoles) was added to the solution. The mixture was refluxed for 6 days, cooled below 0 °C, and the crystalline precipitate was collected. The precipitate was dried under vacuum at 60 °C for 48 h (yield, 10 g). Recrystallization from 25 ml of 95% ethanol (heated to 60 °C) afforded 3.5 g of N-1-decylnicotinamide bromide. Results of carbon, hydrogen and nitrogen analyses, provided by the Department of Pharmaceutical Chemistry of this institution, are given in table 1.



Inhibition of mitochondrial respiratory control by N-1-alkylnicotinamide bromides. pI_{50} is the negative logarithm of inhibitor concentration (M) that depressed RCR by 50%.

Results and discussion. The influence of chain length of N-1-alkylnicotinamide bromides on mitochondrial glutamate respiration (NADH dehydrogenase activity) is summarized in the figure. A plot of pI_{50} (negative logarithm of inhibitor concentration that decreased the respiratory control ratio by 50%) versus the number of carbon atoms in the alkyl chain of the N-1-alkylnicotinamides shows sigmoidal character atypical of single-step partitioning between aqueous and nonaqueous or hydrophobic phases[4] and is reminiscent of a multistep partitioning process[1,5]. On the other hand, the plateau (carbons 12 through 18) may reflect steric limitations for optimal combination of inhibitor with the mitochondrial site.

Movement of the carbamido moiety from position 3 to 4 (table 2) increased the effectiveness of the N-1-dodecylpyridinium derivative for phosphorylation inhibition during glutamate respiration and also during succinate respiration. Moreover, this structural modification resulted in less chemical being required for inhibition of succinate oxidation than for equivalent inhibition of glutamate oxidation. This is opposite to that found for inhibition by tetraalkylammonium compounds[1] and indicates that the mechanism of inhibition of glutamate respiration by the former agents may be different from their mechanism of inhibition of succinate respiration.

The predominant response of mitochondria to these com-

Table 1. Elemental analyses of N-1-alkylpyridinium bromides

Compound	Percentage composition	
	Calculated (%)	Found (%)
N-1-Hexylnicotinamide	C 50.2	C 49.8
	H 6.6	H 6.6
	N 9.8	N 9.8
N-1-Octylnicotinamide	C 53.3	C 53.3
	H 7.3	H 7.3
	N 8.9	N 9.2
N-1-Decylnicotinamide	C 56.0	C 56.0
	H 7.9	H 8.0
	N 8.2	N 8.3
N-1-Dodecylnicotinamide	C 58.2	C 57.7
	H 8.4	H 8.4
	N 7.5	N 7.7
N-1-Hexadecylnicotinamide	C 61.8	C 61.9
	H 9.1	H 9.2
	N 6.6	N 7.2
N-1-Octadecylnicotinamide	C 63.3	C 63.6
	H 9.5	H 9.6
	N 6.2	N 6.2
N-1-Dodecylpyridinium, H_2O	C 59.0	C 58.8
	H 9.2	H 9.1
	N 4.0	H 4.2
N-1-Octadecylpyridinium, ½ H_2O	C 65.6	C 65.8
	H 10.2	H 10.6
	N 3.3	N 3.4

Table 2. Differential inhibition of mitochondrial respiratory control by N-1-substituted, 3-,4-substituted pyridinium halides

Compound	Glutamate respiration I_{50}*	Succinate respiration I_{50}
N-1-Hexadecylnicotinamide bromide	36 μM	31 μM
N-1-Dodecylnicotinamide chloride	31 μM	36 μM
N-1-Dodecylisonicotinamide bromide	17 μM	8 μM
N-1-Dodecylpyridinium bromide	2 μM	19 μM
N-1-Octadecylpyridinium bromide	5 μM	21 μM

*I_{50} refers to the concentration of inhibitor that diminished respiratory control by 50%.

Table 3. Selective inhibition of succinate oxidation in mitochondria by N-1-dodecylisonicotinamide

Inhibitor (μM)	Respiratory velocity* State 3	State 4	RCR
0	115.0 ± 4.0	22.1 ± .4	5.21 ± .16
5	59.5 ± 2.6	17.2 ± .4	3.46 ± .14
10	29.4 ± 2.6	13.9 ± .5	2.10 ± .12
15	16.5 ± 1.5	11.9 ± .6	1.38 ± .07
20	11.8 ± 1.0	10.0 ± .7	1.17 ± .07
25	8.8 ± 0.7	9.3 ± .6	0.95 ± .04

*Nanogram atoms oxygen per min per mg mitochondrial protein ± SEM. RCR is the ratio of respiratory velocity in presence of phosphate acceptor (ADP) to that after exhaustion of ADP.

pounds was a selective inhibition of the respiration stimulated by phosphate acceptor. Table 3 shows the dose related behavior of N-1-dodecylisonicotinamide bromide on succinate respiration. Phosphorylating oxidation (state 3) was markedly depressed whereas respiration in the resting state (state 4) was relatively unaffected. Similar characteristics were observed with each of the substituted pyridinium halides and with either succinate or glutamate as substrate. Since these compounds did not stimulate resting state respiration, they do not have uncoupling activity and consequently they are classified as inhibitors of phosphorylating oxidation.

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The Purkinje fiber-myocardial cell region in the goat heart as studied by combined scanning electron microscopy and chemical digestion[1]

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Summary. The 3-dimensional architecture of the junctional region between Purkinje fibers and ordinary myocardial cells has been closely studied by combined scanning electron microscopy and chemical digestion in the goat heart. It was revealed that the Purkinje fibers forming the terminal arborization of the atrioventricular bundle are followed by transitional cells which are in contact with ordinary myocardial cells.

It is well known that Purkinje fibers are electrophysiologically linked to ordinary myocardial cells of the ventricle in the heart. The transitions between the 2 types of cardiac cells have not, however, yet been perfectly elucidated on a morphological basis. In the light-microscopic studies on the Purkinje fiber -myocardial cell junction of the heart made so far, a number of authors have observed a direct continuity from Purkinje fibers to ordinary myocardial cells[3-6], whereas other authors could not confirm its existence[7]. Further, another group of authors recognized direct continuity only in man out of the 3 animal species examined[8]. Kugler et al.,[9] using the light microscope, detected an indirect transition between Purkinje fibers and ordinary myocardial cells mediated by transitional cells, in addition to direct continuity. Later, Palomo et al.[10] examined using an electron microscope the cardiac cells which could be identified as transitional cells on the basis of their electrophysiological features. To the best of our knowledge, however, there has not been any electron-microscopic evidence which shows unequivocally direct or indirect transition between the Purkinje fibers and ordinary myocardial cells.

In the present study, a combination of scanning electron microscopy and a chemical digestion procedure[11] have enabled us to study the 3-dimensional architecture of the junctional region (P–M region). As a result of the study, examples of indirect transition were found, and the steric structure of the transitional cells could be described.

Materials and methods. Hearts of adult goats were excised under nembutal anesthesia and were immersed in Karnovsky's fixative for 3 h or longer. The tissue specimens containing endocardium were dissected out from the free wall of the ventricle. The endocardial endothelium and connective tissue elements were subjected to digestion with NaClO followed by HCl[11]. The specimens were then throughly washed in physiological saline, postosmicated, dehydrated in graded ethanol series, dried by the critical point method and the examined in a JSM-25 scanning electron microscope.

Results and discussion. As a result of chemical digestion, the endocardial endothelium and the connectve tissue elements of the subendocardium were effectively removed and Purkinje fibers, myocardial cells and transitional cells were clearly visualized 3-dimensionally by examination with a scanning electron microscope. The Purkinje fibers were broader and shorter than ordinary myocardial cells. The former was found to form a subendocardial network, whereas the latter was arranged in parallel rows (fig. 1). Transitional cells frequently occurred in the P–M region, either singly or in rows of two or more cells (figs. 1 and 2). A transitional cell or rows of these cells were attached at one end to the terminal arborization of Purkinje fibers and to be in contact with ordinary myocardial cells at the other. Transitional cells were somewhat cylindrical in shape and of a thickness intermediate between Purkinje fibers