ELECTROCHEMICAL AND BIOLOGICAL OXIDATION OF 1,4-DIHYDROPYRIDINE DERIVATIVES

Antonín KURFÜRST[a], Jiří LUDVÍK[b], Pavel RAUCH[c] and Miroslav MAREK[c]

[a] *Department of Organic Chemistry, Prague Institute of Chemical Technology, 166 28 Prague 6*
[b] *J. Heyrovský Institute of Physical Chemistry and Electrochemistry, Czechoslovak Academy of Sciences, 118 40 Prague 1 and*
[c] *Department of Biochemistry and Microbiology, Prague Institute of Chemical Technology, 166 28 Prague 6*

Received March 28th, 1980

Electrochemical oxidation of 1,4-dihydropyridines *I—XII* and electrochemical reduction of the nitro group of 4-(2-nitrophenyl)-1,4-dihydropyridines *III*, *VI*, *IX* and *XII* was studied using cyclic voltametry. When comparing the electrochemical and the biological oxidation of 3,5-dimethoxycarbonyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine (*IX*, Nifedipine) and related 1,4-dihydropyridines it was found that in spite of certain common trends the oxidation of these compounds (*I—XII*) in the organism cannot be predicted unambiguously. Of the substances tested compounds *IX* and *XI* (3,5-diethoxycarbonyl-4-phenyl-2,6-dimethyl-1,4-dihydropyridine) were the least oxidizable by the respiratory chain, and at the same time they did not inhibit this enzyme system. In addition to this, compound *IX* was the least oxidizable by the microsomal system.

1,4-Dihydropyridines can be oxidized[1] to corresponding pyridine derivatives with various chemical reagents, as well as electrochemically and biologically. Some 1,4-dihydropyridines have an antihypertensive effect[2]. The most active substance of this type is 3,5-dimethoxycarbonyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine (*IX*, Nifedipine)[2,3]. In compound *IX* the antihypertensive effect is due to the inhibition of the transport of the extracellular calcium ion through the cell membrane and subsequent inhibition of myofibrial ATPase. On a physiological level the mechanism described elicits vasodilatation and blood pressure decrease[4]. The first metabolic transformation of dihydropyridines after their administration to the organism is oxidation[5]. While oxidation is a slow reaction, further degradation of the oxidation products is rapid. However, the pharmacological effect is connected only with unoxidized dihydropyridine derivatives[6]. For this reason we studied and compared electrochemical and biological oxidations of variously substituted 1,4-dihydropyridines.

EXPERIMENTAL

Material and Methods

1,4-Dihydropyridines *I*—*XII* were synthetized in the conventional manner[2] and their melting points and spectral characteristics are in agreement with the literature data. Acetonitrile was dried over calcium chloride, distilled and stored over a molecular sieve. NADH and ADP were supplied by Reanal, Hungary. The mitochondria and the microsomal system were isolated from the liver of white laboratory male rats (weight about 180 g, Large Scale Breeding of Laboratory Animals, Lysolaje near Prague). The mitochondria were isolated by differential centrifuging according to Clark[7] and their integrity was tested by measuring the ADP/O ratio. The microsomal system was isolated using the procedure described by Walkenstein[5], with the difference that the liver tissue was previously homogenized in a solution of sucrose (0·25 mol l^{-1}) and Tris-HCl (5 mmol l^{-1}, pH 7·4). The electrochemical measurements were carried out on a multipurpose polarograph GWP 673, Academy of Sciences of GDR. A three-electrode connection was made use of. The working and indicator electrodes were made of platinum, the reference electrode was of S.C.E. Cyclic voltametry was carried out in anhydrous acetonitrile at a 1 mmol . . l^{-1} concentration of the substances measured, using tetrabutylammonium tetrafluoroborate (0·1 ml l^{-1}) as the basic electrolyte. The total volume of the measured sample was 5 ml. Cyclic voltamograms were recorded at various rates from 0·5 V min^{-1} to 25 V s^{-1} in the potential range from +2 V to —2 V, using a linear X—Y writer (Endim 62002) (Academy of Sciences of GDR). At rates above 0·25 V s^{-1} the writer was connected with the memory of the Scan Recorder Model 4101 (PAR, U.S.A.). All the measurements were carried out at room temperature and under nitrogen. Biological oxidations were measured using an oxygen cell of the Clark type[8]. The incubation medium contained in a total volume of 2 ml 160 μmol of KCl, 10 μmol of phosphate buffer of pH 7 4; 40 μmol of $MgCl_2$, 40 μmol of Tris-HCl buffer of pH 7·4. The amount of the substances *I*—*XII* in the incubation medium was 1·25 μmol. The oxidation of compounds *I*—*XII* was measured both in the presence of intact mitochondria (ADP/O for succinate was 1·96 and for maleate 2·85), and in the preparations of mitochondria with a damaged ultrastructure, produced by triple freezing (—40°C) and defreezing (+4°C). The total amount of mitochondria or the microsomal enzymes in the incubation solution measured corresponded to 0·5 g of the liver tissue.

RESULTS

Cyclic Voltametry

The electrochemical oxidation of some 1,4-dihydropyridines studied was investigated both in water-containing[9] (compounds *I*, *IV*, *VII*, *X*) and anhydrous[10,11] acetonitrile (compounds *I*, *II*, *IV*, *V*, *VII*, *IX*—*XII*). The mechanism of electrochemical oxidation was discussed[12] for the case of related 1,4-dihydronicotinamides. In agreement with the results in refs[9-11] we confirmed by cyclic voltametry that compounds *I*—*XII* are oxidized by an irreversible process within the whole range of the measurement rates used. In compounds *III*, *VI*, *IX* and *XII* containing the nitro group the first reduction wave appears in the region of −1·1 to −1·4 V. From the measured values of the potential peaks E_p (Table I) the following conclusions may be drawn.

1) The ease of oxidation of compounds *I*–*XII* decreases in dependence on the substituents X in the positions 3 and 5 in the following order: $COCH_3 > COOC_2H_5 > COOCH_3 > CN$. The same dependence has been published for E_p in anhydrous acetonitrile[10], while in aqueous acetonitrile the following order[9] has been observed: $COOC_2H_5 > COCH_3 > CN$. However, the difference of E_p (or $E_{1/2}$) in substances with $X = COOC_2H_5$ and $X = COCH_3$ are very small (maximum 0·05 V), so that it may be stated with certainty only that the substituents X=CN considerably deteriorate the ability of electrochemical oxidation of 1,4-dihydropyridines in comparison with other substituents X used, the effect of which is approximately equal and dependent on the solvent used. The same order of the effect of substituents X as in electrochemical oxidation in anhydrous acetonitrile has also been published for the oxidation of 1,4-dihydropyridines with chloranil[12,13] or enzymatic oxidation[14]. The electronic effects of substituents X on electrochemical oxidation of 1,4-dihydropyridine were interpreted by means of HMO quantum chemical calculations[9]. From analogous π-SCF-MO calculations[15] it may be predicted that the ease of oxidation of compounds *I*, *IV*, *VII* and *X* decreases in dependence on the substituents X in the following order: $COOC_2H_5 > COCH_3 > COOCH_3 > CN$.

TABLE I

E_p Values Measured by Cyclic Voltametry for 1,4-Dihydropyridines *I*—*XII*

Three-electrode arrangement, S.C.E., concentration of 1,4-dihydropyridine in anhydrous acetonitrile 1 mmol l^{-1}.

Compound	Oxidation E_p, V	c	Reduction E_p, V
I	+1·28	(+1·16)[10]	—
II	+1·38	(+1·38)[10]	—
III	+1·44		−1·12
IV	+0·93	(+0·90)[10]	—
V	+1·12	(+1·13)[10]	—
VI	+1·16		−1·21
VII	+0·98	(+0·93)[10]	—
VIII	+1·18		—
IX	+1·27	(+1·16)[11]	−1·35
X	+0·96	(+0·93)[10]	—
XI	+1·16	(+1·07)[10]	—
XII	+1·25	(+1·11)[11]	−1·37

2) The ease of electrochemical oxidation of compounds *I*–*XII* decreases with increasing electronegativity of the substituent R in the position 4 as follows: $H > C_6H_5 > o\text{-}C_6H_4NO_2$. This trend is in agreement with the formerly determined results of simple polarographic measurements[10,11].

3) The ease of electrochemical reduction of the nitro group in compounds *III*, *VI*, *IX* and *XII* decreases in dependence on substituents X in positions 3 and 5 in the following order: $CN > COCH_3 > COOCH_3 > COOC_2H_5$.

Biological Oxidation

In the case of biological oxidation of the compounds *I*–*XII* studied after their administration to the organism three methods of their oxidation come into consideration: With peroxidase, the respiratory chain and the microsomal system[16]. During the oxidation of 1,4-dihydropyridines *I*, *IV*, *V*, *X*, *XII* with peroxidase[14], which is localized in leucocytes, erythrocytes and bone marrow, 1,4-dihydropyridines *I*, *IV* and *X* are oxidized most rapidly; substitution in the position 4 distinctly decreases the rate of enzymatic oxidation. This rate decreases in dependence on the substituents X in the positions 3 and 5 in the following order[14]: $COOC_2H_5 > COCH_3 >$ $> CN$. Some 1,4-dihydropyridines were found[17] to have antioxidant properties

TABLE II

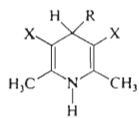
Biological Oxidizability of 1,4-Dihydropyridines *I*—*XII*

Consumption of O_2 (nmol min^{-1}) is given per 1 g of liver tissue; the data represent average values from three experiments.

Compound	*a*	*b*	*c*
I	6·6	54·9	7·9
II	10·5	64·2	8·6
III	10·0	61·6	6·6
IV	18·0	85·8	17·4
V	6·8	60·4	8·6
VI	7·4	61·8	7·9
VII	15·2	46·4	11·1
VIII	7·9	36·9	5·9
IX	6·2	64·9	6·3
X	11·8	38·6	18·0
XI	5·9	67·9	8·8
XII	8·5	50·0	6·4

[a] Oxidation with mitochondria with a damaged ultrastructure; [b] oxidizability of NADH with mitochondria with damaged ultrastructure in the presence of 1,4-dihydropyridine (for 0·625 mmol NADH in ethanol the consumption is 54 nmol O_2 min^{-1}); [c] oxidation with microsomal system.

in the peroxidation of lipids with free radicals in mitochondria in the presence of ferrous ions. In this work we turned our attention on the oxidation of compounds *I*–*XII* by mitochondria and by microsomal system. In the case of intact mitochondria we now found a 1·2–1·5 times lower rate of oxidation of compounds *I*–*XII* than in the case of the mitochondria with a damaged ultrastructure. The mitochondria with a damaged ultrastructure displayed (Table II) the fastest oxidation of compound *IV* in agreement with cyclic voltametry in anhydrous medium. Compounds *IX* and *XI* were oxidized most slowly. The sequence of oxidizability of 1,4-dihydropyridine derivatives unsubstituted in the position 4 (*I*, *IV*, *VII*, *X*) was veray similar to that of the electrochemical oxidation in anhydrous medium, *i.e.* the dependence on the substituents X in positions 3 and 5 had the following order: $COCH_3$ > > $COOCH_3$ > $COOC_2H_5$ > CN. However, for 4-substituted-1,4-dihydropyridines the effect of the substituents X was different, especially in the case of dicyano derivatives *II* and *III*.



	X	R		X	R
I	CN	H	*VII*	$COOCH_3$	H
II	CN	C_6H_5	*VIII*	$COOCH_3$	C_6H_5
III	CN	$C_6H_4NO_2$-2	*IX*	$COOCH_3$	$C_6H_4NO_2$-2
IV	$COCH_3$	H	*X*	$COOC_2H_5$	H
V	$COCH_3$	C_6H_5	*XI*	$COOC_2H_5$	C_6H_5
VI	$COCH_3$	$C_6H_4NO_2$-2	*XII*	$COOC_2H_5$	$C_6H_4NO_2$-2

In view of a possible inhibitory effect of compounds *I*–*XII* on the respiratory chain enzymes the rate of oxidation of NADH was also measured in their presence with damaged mitochondria (Table II). From the tabulated values it follows that only substances *VII*, *VIII*, *X* and *XII* distinctly inhibit the oxidation of NADH. The other substances studied not only did not affect the oxidation of NADH with the respiratory chain negatively, but in some cases (compounds *IV*, *IX*, *XI*) a higher consumption of oxygen was also observed than would correspond to the total rate of oxidation of the substance tested with NADH. This is surprising especially in the case of compounds *IX* and *XI* which are in themselves oxidized very slowly by the respiratory chain, in contrast to compound *IV*.

During the oxidation with the microsomal system substance *VIII* was the least oxidizable besides the *o*-nitrophenyl derivatives *III*, *IX* and *XII*. The effect of the substituents X in positions 3 and 5 was not as unambiguous as in the case of electro-oxidation, the order of substituents depended on the substitution in position 4.

DISCUSSION

From the electrochemical measurements that were carried out it is evident that the effect of the substituents X in positions 3 and 5 is different in the case of the electro-oxidation of the 1,4-dihydropyridine nucleus than in the case of the electroreduction of the nitro group. It may be judged that in the electrochemical reduction of the nitro group the reactivity of the substances studied is affected mainly by the steric effect of the substituents X in positions 3 and 5 of the 1,4-dihydropyridine nucleus. In contrast to this, in the electrochemical oxidation of this nucleus the reactivity of the compounds *I–XII* studied is affected mainly by the electronic effects of the substituents X in positions 3 and 5 and by the steric requirements of the substituents R in position 4.

In the biological oxidation the mentioned dependence is not as unambiguous. The substitution in position 4 of compounds *IV–XII* deteriorates the oxidizability of 1,4-dihydropyridines distinctly (in agreement with other methods of oxidation[9–11,13,14]), but in the case of dicyano derivatives *I–III* the oxidation rate is increased by the substituents in position 4. The retarding effect of the *o*-nitrophenyl group, probably due to the sterical requirement of this group in comparison with the 4-phenyl derivatives, manifested itself in the oxidation with the microsomal system. In contrast to this, in the oxidation with mitochondria this effect was no longer as distinct and unambiguous as in the case of electro-oxidation.

From a general point of view on the biological oxidation of the 1,4-dihydropyridines *I–XII* compounds *IX* and *XI* seemed most interesting by the fact that in spite of their slowest oxidation with the respiratory chain the highest rate of oxidation of NADH was measured in their presence (except for substance *IV*, which itself was most oxidizable in the respiratory chain). From a comparison of compounds *IX* and *XI* in the oxidation with the microsomal system it followed that substance *XI* is oxidized with the microsomal system distinctly more rapidly, which means that this substance will be probably also oxidized more rapidly in the organism and thus lose its biological activity also more rapidly. The results obtained of the biochemical oxidation of the compounds *I–XII* studied show that it is not possible to predict the oxidation of these substances in the organism from electrochemical data.

The authors thank Professor J. Kuthan and Professor J. Volke for stimulating discussions concerning the problems studied.

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Translated by Ž. Procházka.