INFLUENCE OF 1,4-DIHYDROPYRIDINE DERIVATIVES ON THE GENERATION OF HYDROXYL RADICALS

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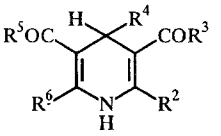
It was shown that a series of 1,4-dihydropyridine (1,4-DHP) derivatives in the NAD-H—Cu^{2+}—H_2O_2 system inhibit the formation of the hydroxyl radical (HO·), while derivatives of 1,4-DHP with electron-donor substituents in the molecule are themselves capable of generating HO· in the presence of Cu^{2+} and H_2O_2.

Derivatives of 1,4-dihydropyridine (1,4-DHP) attract attention as a new class of cardiovascular preparations (nifedipin or phenigidin; foridon or riodipin; nicardipin, etc.). Moreover, some 1,4-DHP derivatives have an antioxidant activity (diludin or dieton), which as is assumed, is the basis for their physiological activity (antimutagenic, radioprotective, etc.). The hydroxyl radical (HO·), in its turn, is one of the initiators of the peroxy oxidation process, and also participates in autooxidative degradation reactions of readily oxidizable organic compounds, including medicinal preparations. Hence, the interest can be understood in the possible participation of 1,4-DHP in reactions in which HO· is generated. As known, the 1,4-DHP derivatives react with active forms of oxygen, generated in Fenton's reactions [1]. However, in that work the reactivity with HO· was evaluated only tentatively.

In the present work, we used the generation of HO· by the NAD-H—Cu^{2+}—H_2O_2 system [2]. The measure of the generated HO· was the amount of the 4-nitropyrocatechol, formed from 4-nitrophenol [3, 4].



I - X

We first studied the influence of a series of 1,4-DHP derivatives on the generation of HO· by the NAD-H—Cu^{2+}—H_2O_2 system. The results obtained show (Table 1) that the derivatives of 1,4-DHP are capable of generating HO· in this system. In compounds I (R^4 = Me) and II (R^4 = H) the ability to inhibit and generate HO· is practically the same, although it is well known that 1,4-DHP derivatives unsubstituted at the 4-position are much more reactive in reactions with free radicals than the substituted dihydropyridines [1, 5]. Compound III (R^4 = COONa) has a relatively strong electron-donor, the carboxylate, in the 4-position [6], and correspondingly its reactivity is higher than in compound I. All this indicates that a complex process takes place in the system used, including both the reaction of dihydropyridine with HO· and the generation of HO· in parallel reactions. This action is most pronounced in compound II (R^4 = H), which is unstable on storage because of the autooxidation readily taking place.

TABLE 1. Influence of 1,4-Dihydripyridine Derivatives (1,4-DHP) on the Generation of HO· in the NAD-H—Cu^{2+}—H_2O_2 System (concentration of NAD-H $5 \cdot 10^{-4}$ mole/liter)

*Compound	R^2=R^6	R^3=R^5	R^4	c** × 10^5 mole/liter at c 1.4-DHP, mole/liter		
				10^{-3}	5×10^{-4}	10^{-4}
I	CH_3	CH_3	CH_3	7,70	6,96	6,48
II	CH_3	OCH_2COONa	H	7,08	7,61	7,62
III	CH_3	OC_2H_5	COONa	13,2	8,93	5,97
—		NAD-H (control)		—	6,35	—

*Compounds I-III — derivatives of 1,4-DHP.

**c — concentration of 4-nitropyrocatechol.

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TABLE 2. Capability of 1,4-Dihydropyridines (1,4-DHP) to Generate HO.

Compound	$R^2=R^6$	$R^3=R^5$	R^4	c** × 10^5 mole/liter (% with respect to control) at c of 1.4-DHP, mole/liter		
				10^{-3}	5×10^{-4}	10^{-4}
I	CH_3	CH_3	CH_3	2,21 (14)	1,13 (18)	0,28 (12)
II	CH_3	OCH_2COONa	H	4,51 (28)	3,85 (61)	2,04 (89)
III	CH_3	OC_2H_5	COONa	12,2 (76)	5,53 (87)	0,62 (27)
IV	CH_3	CH_3	COONa	14,9 (93)	13,1 (206)	3,63 (159)
V	CH_3	CH_3	H	5,09 (32)	2,26 (36)	0,59 (26)
VI	CH_3	OCH_2COONa	C_6H_5	4,77 (30)	3,19 (50)	1,62 (71)
VII	CH_3	OCH_2COONa	C_6H_4Cl-4	2,38 (15)	2,07 (33)	1,23 (54)
VIII	CH_3	OC_2H_5	CONHCHCOONa $(CH_2)_2COONa$	5,65 (35)	3,68 (58)	1,42 (62)
IX	CH_3	OC_2H_5	-β-$Py^+CH_3Cl^-$	1,27 (8)	1,35 (21)	1,18 (52)
X	CH_2COONa	OC_2H_5	H	11,8 (73)	6,47 (102)	1,79 (78)
—		NAD-H (control)		16,1 (100)	6,35 (100)	2,29 (100)

*Compounds I-X — derivatives of 1,4-DHP.

**c — concentration of 4-nitropyrocatechol.

Prompted by the above considerations, we tested these and other derivatives of 1,4-DHP for the ability to generate HO· in a system with Cu^{2+} and H_2O_2. The results obtained (Table 2) show that many of the 1,4-DHP derivatives tested are capable of generating HO· in this system, whereby this capability is dependent on the electron/hydrogen-donor properties of the compounds. The ability to generate HO· is most pronounced in compounds III, IV, and X, containing strong electron-donor substituents in the molecule. Compounds VII and IX have electron-acceptor substituents in the 4-position of the dihydropyridine ring, and hence their reactivity is much lower. Considering the dependence of the capability to generate the HO· on the concentration of the compounds tested, it can be readily seen that a rectilinear dependence is obtained only when 1,4-DHP carries electron-acceptor substituents and a methyl group at the 4-position (compounds I, VI, VII, IX). Compound IV — the sodium salt of 2,6-dimethyl-3,5-diacetyl-1,4-dihydroisonicotinic acid in a concentration of $5\cdot10^{-4}$ mole/liter surpasses NAD-H by more than twofold in its ability to generate HO· Further investigation of this compound should show that it can be used for the generation of HO·, because of its considerable advantage over NAD-H consisting in the ease of its synthesis [7] and stability on storage.

Thus the participation of the 1,4-DHP derivatives, especially those which are characterized by high electron-donor ability in processes where HO· is formed, is complex in character. On the one hand, the 1,4-DHP derivatives react with HO·, and on the other hand they generate HO· in a system with H_2O_2 and Cu^{2+}. Knowledge of these properties is particularly important when discussing the data on the physiological activity of 1.4-DHP derivatives, since the biological activity of these compounds is often explained by their antiradical and antioxidant activity, without providing confirmation for this conclusion.

EXPERIMENTAL

The reaction mixture in 1 cm quartz cuvette containing $5\cdot10^{-4}$ mole/liter of a $CuCl_2$ solution, 10^{-3} mole/liter of 4-nitrophenol, $5\cdot10^{-3}$ mole/liter of H_2O_2, $5\cdot10^{-4}$ mole/liter of NAD-H (in experiments on the influence of 1,4-DHP on the generation of HO·) and 10^{-3}, $5\cdot10^{-4}$, or 10^{-4} mole/liter of 1,4-DHP, respectively. Fresh solutions of the compounds were used for the experiments: the concentration of H_2O_2 was determined iodometrically, NAD-H — spectrophotometrically before the experiment. The cuvettes were thermostated at 25°C and after 30 min the optical density of 4-nitrocatechol was measured at 424 nm (ε 9900) on a Hitachi 557 spectrophotometer (Japan).

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