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[1] National Taras Shevchenko University of Kyiv, Department of Cytophysiology
[2] Medical Institute of Ukrainian Folk Medicine Association, Department of Medical Biology, Kyiv

SVITLANA YABLONSKA[1], VOLODYMYR LOZOVYY[2],
OLENA FILINSKA[1], GALYNA OSTROVSKA[1], TARAS RYBALCHENKO[1],
VICTORIA ZELENYUK[1], VOLODYMYR RYBALCHENKO[1]

Activity of membrane-bound enzymes of rat hepatocyte plasma membrane under the influence of pyridine and pyridoxine (vitamin B_6)

Wpływ pirydyny i pirydoksyny (witaminy B_6) na aktywność enzymów błon plazmatycznych hepatocytów szczura

Pyridine core is a structural component of many biologically active substances (BAS). Native derivates are vitamin PP (nicotinamide) and vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine), which in a dose-dependent manner inhibits the growth of some cancer cells [4]. Exogenous pyridine derivates are widely used in pharmacology (1,4-dihydropyridines – blocks selectively calcium channels [10], imidazopyridines – potent and selective cyclin-dependent kinase inhibitors [2], oxipyridines – reveal antioxidant activity [8], in agriculture (as insecticides and plant growth regulators (N-oxide 2,6-dimethyl-pyridine and its molecular complex [6]. But their interaction with biological membranes still has not been studied well. Membrane-bound enzymes are very sensitive indicators of cells plasma membrane (PM) condition. Sensitivity of PM to BAS, including pyridine and its derivates, depends on the structural and functional relationship between membrane-bound enzymes and lipid matrix. According to prior work, pyridine derivate N-oxide 2,6-dimethyl-pyridine incorporates into phosphatidylcholine membrane lipid matrix provoke lipids destabilization that grows with increasing of substance concentration [1]. N-oxide 2,6-dimethyl-pyridine does not produce significant changes of hepatocytes plasma membrane ecto-ATPase and Mg^{2+}, Ca^{2+}-ATPase activity *in vitro* (10^{-9} – 10^{-4} M) but disturbs Mg^{2+}, Ca^{2+}-ATPase activity after subchronic treatment of rats [5].

The aim of this work was investigation of pyridin and pyridoxine influence at the concentrations 10^{-9}–10^{-4} M on rat hepatocyte plasma membrane-bound enzymes activity (5′-nucleotidase, ecto-ATPase, Mg^{2+}, Ca^{2+}-ATPase, Na^+, K^+-ATPase).

MATERIAL AND METHODS

The influence of pyridine and pyridoxine within the concentration range 10^{-9}–10^{-4} M on the activity of membrane-bound enzymes was studied on Wistar rats hepatocyte PM fractions which had been isolated by ultracentrifugation in sucrose gradient according to the procedure of Song [11]. 5'-nucleotidase activity was measured in reaction solution containing 20 μg of PM protein, 50 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 4 mM AMP. Ecto-ATPase activity was measured with an addition of 2 mM $CaCl_2$ to the reaction mixture containing 20 μg of PM protein, 50 mM Tris-HCl, pH 7.5, 140

mM NaCl, 5 mM KCl, 100 μM NH_4VO_3, 1 mM ATP. Mg^{2+},Ca^{2+}-ATPase activity was assayed in the reaction mixture containing 20 μg of PM protein, 50 mM Tris-HCl, pH 7.5, 100 mM KCl, 3 mM $MgCl_2$, 0.06 mM EGTA, 3 mM ATP. The enzyme was activated by an addition of 40 μM $CaCl_2$. Na^+,K^+-activity was defined as the difference in enzyme activity with and without 1 mM ouabain in the reaction solution containing 50 μg of PM protein, 50 mM Tris-HCl, pH 7.4, 130 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, $5 \cdot 10^{-6}$ M SDS, 3 mM NaN_3, 3 mM ATP. The activity of the enzymes was determined using a colorimetric procedure for measuring inorganic phosphate released from ATP and AMP [7].

RESULTS AND DISCUSSION

Pyridine and pyridoxine do not cause significant changes of 5′-nucleotidase and ecto-ATPase activity in all the concentration range (Table 1). But pyridine in 10^{-5} M concentration provokes increasing of ecto-ATPase activity up to 20% in comparison with the control data.

Table 1. 5'-nucleotidase and ecto-ATPase activity of hepatocyte PM under the influence of pyridine and pyridoxine in the concentration range 10^{-9}–10^{-4} M, n=7

Concentration, M	5′-nucleotidase activity, nmol P_i/min·mg protein		ecto-ATPase activity, nmol P_i/min·mg protein	
	pyridine	pyridoxine	pyridine	pyridoxine
0 (control)	219.4±21.76	140.9±13.89	168.8±16.46	169.4±14.64
10^{-9}	225.3±30.52	126.3±5.27	157.2±12.91	155.7±15.71
10^{-8}	229.4±36.94	134.8±11.84	158.4±11.73	156.9±17.67
10^{-7}	227.7±25.65	134.2±11.45	156.3±8.43	155.8±17.70
10^{-6}	233.3±29.14	132.3±7.72	176.8±14.51	158.9±19.12
10^{-5}	237.4±37.31	128.5±6.61	190.2±22.61	150.5±19.81
10^{-4}	221.6±26.19	133.5±16.08	160.2±18.05	172.4±17.72

The examined substances stimulate some alterations of PM ion-transporting ATPases activity. Mg^{2+},Ca^{2+}-ATPase and Na^+,K^+-ATPase activities of intact PM are 41.8±4.5 nmol P_i/min·mg protein and 55.7±5.6 nmol P_i/min·mg protein, respectively. Pyridoxine produces dose-dependent changes of hepatocyte PM Mg^{2+},Ca^{2+}-ATPase activity (Fig. 1). The uncommon shape of a dose-effect curve is similar to parabola with two minimums. Pyridoxine at the concentrations 10^{-9} and 10^{-4} M causes the most significant decrease of enzyme activity (to 35%) while micromolar concentration does not cause changes of Mg^{2+},Ca^{2+}-ATPase activity. Pyridine produces some insignificant changes of Mg^{2+},Ca^{2+}-ATPase activity.

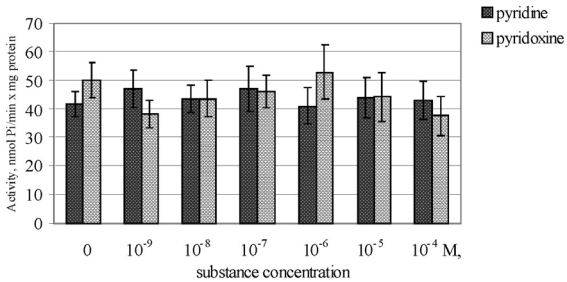

Fig. 1. Mg^{2+}, Ca^{2+}-ATPase activity of hepatocyte PM under the influence of pyridine and pyridoxine in the concentration range 10^{-9}–10^{-4} M

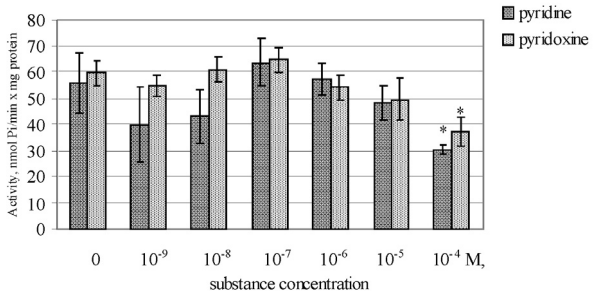

Fig. 2. Na^+, K^+-ATPase activity of hepatocyte PM under the influence of pyridine and pyridoxine in the concentration range 10^{-9}–10^{-4} M. * – $p < 0.05$

Concentration-dependent changes of Na^+, K^+-ATPase activity was noted under the influence of pyridine too (Fig. 2). Pyridine at the concentration 10^{-7} M does not change PM Na^+, K^+-ATPase activity whereas concentrations 10^{-9} and 10^{-8} M significantly disturb enzymes' activity and decrease twice in 10^{-4} M. Pyridoxine produces a gradual decrease of this activity in the studied concentration range. In the highest dose (10^{-4} M) it produces a decrease to 25%. We suggest that abnormalities of enzyme activity under the influence of pyridine and pyridoxine may be a consequence of both the direct influence on enzyme molecule and the indirect influence via changing parameters of membrane lipid matrix.

Sensitivity of membrane-bound enzymes to any chemical compounds depends on a tight structural and functional connection between enzymes and membranes lipid matrix, which is a target for different substances. Amphiphilic pyridine molecules are adsorbed at the surface of dimyristoylphosphatidylcholine liposomes, then sandwich between the choline and glycerol moieties and cause minor perturbation of phospholipids head-groups at liposome intersurface [3]. However, pyridine molecules promote penetration of water into the restricted regions in the interface and cause negligible altering of lipid packing. A small amount of pyridine gets into the hydrophobic region of the membrane but do not significantly alter the packing and dynamics of phospholipids. Pyridine, as expected, is less active to membrane in comparison with other pyridine derivate N-oxide 2,6-dimethyl-pyridine studied previously [1]. It incorporates into lipid matrix producing membrane destabilization that can cause disorders of membrane-bound ion-transporting pumps. Mg^{2+}, Ca^{2+}- and Na^+, K^+-ATPase molecules are transmembrane polypeptide chains that cross membrane 8–10 times and contain extra-membrane loops. Enzymes' activity significantly depends on the charge of the surrounding hydrophilic lipid-heads. Various responses of those enzymes to pyridine and pyridoxine influence might be caused by the difference in enzymes' structure. In contrast to Mg^{2+}, Ca^{2+}-ATPase, Na^+, K^+-ATPase molecule has additional small regulatory β-subunits with extracellular N-terminal domain [9]. Its function is regulation of the enzymes activity. Interaction of pyridine and pyridoxine with this domain might be a reason for a more significant alteration of Na^+, K^+-ATPase activity in comparison with Mg^{2+}, Ca^{2+}-ATPase and a gradual decrease of sodium pump activity with the growth of the concentration of the substance.

Consequently, pyridine and pyridoxine do not change hepatocytes PM 5′-nucleotidase, ecto-ATPase activity significantly. But ion-transporting ATPases of PM display dose-dependent changes under the influence of the studied substances. Those changes might be caused by two mechanisms of the influence of the studied substances on membrane-binding enzymes.

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SUMMARY

Enzymes' activity of hepatocyte plasma membrane was studied under the influence of pyridine and pyridoxine in the concentratin range 10^{-9}–10^{-4} M. The studied substances reveal membrane effects that depend on their ability for interaction with membrane lipid matrix. Those substances do not disturb 5′-nucleotidase, ecto-ATPase activity significantly, but produce alteration of Mg^{2+}, Ca^{2+}- and Na^+, K^+-ATPase activity of hepatocytes plasma membrane. A different response of Mg^{2+}, Ca^{2+}- and Na^+, K^+-ATPase activity to the influence of pyridine and pyridoxine might depend on their structure.

STRESZCZENIE

Badano wpływ pirydyny i pirydoksyny w stężeniach 10^{-9} – 10^{-4} M na aktywność enzymów błon plazmatycznych szczurzych hepatocytów. Badane substancje wykazywały efekt błonowy w zależności od zdolności do interakcji z lipidową matrycą błonową. Substancje te nie zaburzały znacznie aktywności 5'-nukleotydazy i ekto-ATP-azy, lecz wpływały na aktywność Mg^{2+}, Ca^{2+}- i Na^+, K^+-ATPazy. Zróżnicowana odpowiedź aktywności Mg^{2+},Ca^{2+}- i Na^+, K^+-ATPazy na działanie pirydyny i pirydoksaminy może być uzależniona od różnic w strukturze chemicznej.