Effect of Plant Alkaloids on Mitochondrial Membrane Permeability

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ABSTRACT

The study of the physicochemical properties of mitochondrial membranes shows that its inner membrane selectively allows certain compounds to pass through, for example, drugs. It is necessary to take into account the influence of pharmacological agents on the functional parameters of mitochondria not only from a toxicological, but also from a therapeutic point of view. Potential targets for several pharmacological agents have been identified at the mitochondrial level.

Keywords: mitochondria, alkaloids, protopin, conductivity, in vitro, environment , calcium ions.

Introduction. The development of modern physico-chemical biology has broadened the understanding of biochemical, physiological and biophysical mechanisms underlying cellular activity, energy synthesis and its exchange in living systems.

Cell and organelle membrane damage has been identified in various pathologies. In particular, several factors affect mitochondrial membrane damage.

Accumulation of free radicals, hydroxyl ions (OH^-) and H_2O_2 in high concentrations in the cell leads to increased LPO process, cell membrane and DNA damage. As a result of Mx breathing, unpaired electrons (ê) are released and they interact with O_2 to form superoxide ions (free radicals or reactive species of oxygen). These factors do not affect the membrane of Mx, because Mx has the necessary antioxidant protection systems. This defense system consists of high concentrations of glutathione, superoxide dismutase and catalase. The main way of release of damaging factors (radicals, superoxide anion) from the matrix of Mx can be through H_2O_2. Excessive formation of free radicals in the body and, in this regard, the increase of the LPO process, that is, under the influence of OC, biomembranes and cell functions are disrupted.

Research materials and methods. The researches were carried out in purebred white rats with a body mass of 120-140 g. Feeding of laboratory animals was carried out under normal vivarium conditions. Researches were carried out in three stages, interdependently, in vitro conditions.

Kinetics of degradation of Mx (Mx protein was 1mg/ml) was determined by changes in optical density at a wavelength of 540 nm in 3 ml cells. IM composition for energized Mx: 125 mM KCl, 2.5 mM KH_2PO_4, 1mM EGTA, 5 mM glutamate, 1 mM malate, 10

mM tris-HCl, pH 7.4; IM composition for unenergized Mx: 0.240 mM sucrose, 1 mM EGTA, rotenone 1 μM in 10 mM tris-HCl. In the environment where Ca^{2+} -EGTA buffers were used, the Ca^{2+} coconcentration was calculated using the BAD4 computer program.

The obtained results and their analysis. During the study of the effect of plant alkaloids on mitochondrial membrane permeability, in vitro studies, healthy animal livers, activated Mx membrane permeability, i.e. SsA-sensitive pore state, protopine, cryptopine, α-allocryptopine and zeravshanizine the effect of 50 μM concentration of alkaloids was studied (Table 1). In the absence of SsA-sensitive pore inducers in the incubation medium, the studied alkaloids did not affect the Mx spawning rate. The obtained results indicate that the studied compounds do not have membrane-active properties, that is, under these conditions, the studied compounds do not affect the SsA-sensitive pore in the Mx membrane.

In experiments, Mx membrane permeabilization was induced by adding small concentrations of Ca^{2+} ions to the incubation medium, and the effect of alkaloids on Mx membrane permeability under these conditions was studied (Table 1). Under these conditions, the pore goes into an open conformational state. As a result of the study, among the alkaloids used, the effect of protopine on the speed and amplitude of energized Mx barking was more evident than that of other alkaloids. In the studies, the effect of cryptopine, α-allocryptopine and zeravshanizine alkaloids on Mx membrane permeability was shown to be weaker than that of protopine (Table 1).

Table 1

Effects of plant alkaloids on membrane permeability of energized mitochondria in in vitro studies

Experience groups	100·Mx winding speed without inductors, (DE540/min)	In the presence of inductors 100·Mx throttling speed, (DE540/min)	
		Ca^{2+}	GPK
Control	10,7±1,5	32,4±2,3	58,7±3,0
Protopine 50 μM	12,8±1,6	42,1±2,6	66,2±3,2
Cryptopine 50 μM	11,4±1,5	33,9±2,0	59,1±3,8
α-Allocryptopine 50 μM	11,7±1,3	34,2±2,3	60,5±3,3
Zeravshanizin 50 μM	12,2±1,8	35,1±2,5	58,8±3,6

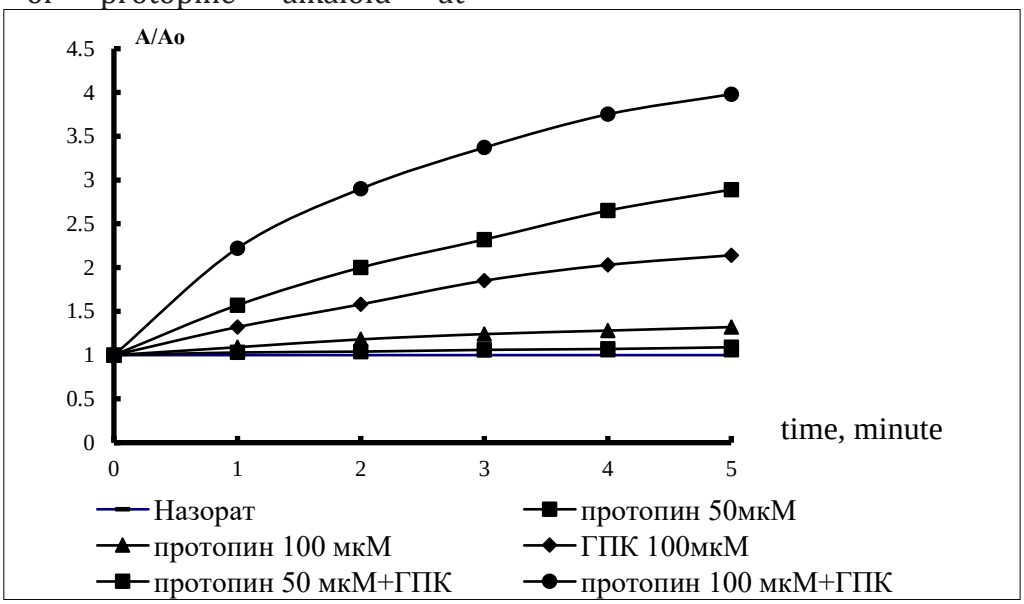
Note: IM- 125 mM KCl, 2.5 mM MKH_2PO_4, 5 mM glutamate, 1 mM malate; 5 μM Ca^{2+}, 4 mM GPK, 1 mM EGTA, 10 mM tris-HCl, pH-7.4. $P<0.05$ in all cases (n=8).

It is known that under these conditions, the change in the permeability of the Mx membrane is mainly due to the fact that the SsA-sensitive pore is in a state of low permeability. Therefore, based on the obtained results, protopine alkaloid activates the opening of the pore in this case. In order to confirm the obtained results, we continued our further studies under the conditions of using hydroperkiscumol (GPK), which is considered as an MRT inducer. Even under these conditions, the effect of protopine was more evident than that of other alkaloids (Table 1).

At the same time, in additional experiments, it was found that the used concentration of protopine alkaloid does not

affect the accumulation of LPO products. This process means that protopine alkaloid does not have a pro-oxidant effect, that is, it does not cause LPO.

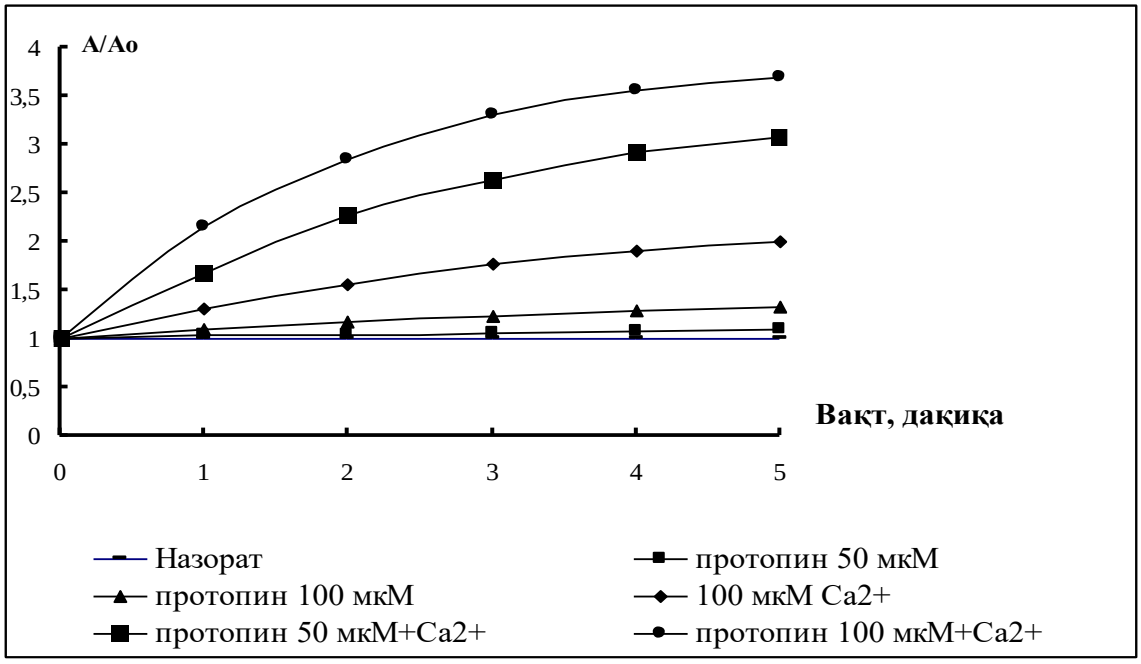
In our next in vitro studies, we studied the effect of protopine alkaloid at concentrations of 50-100 μM on the state of energized and unenergized SsA-pores. In our experiments, we studied the effect of protopine alkaloid in the presence of GPK on the state of the energized Mx membrane (Fig. 1).



Note: A/A0 is the ratio of experimental group Mx snoring rate to control group Mx snoring rate. IM- 125 mM Kcl, 2.5 mM KH2RO4, 1 mM EGTA, 5 mM lutamate, 1 mM malate, 10 mM trisHCl, pH-7.4. R<0.05 in all cases (n=8).

Figure 1. Effects of protopine on GPK-induced membrane permeability of energized mitochondria

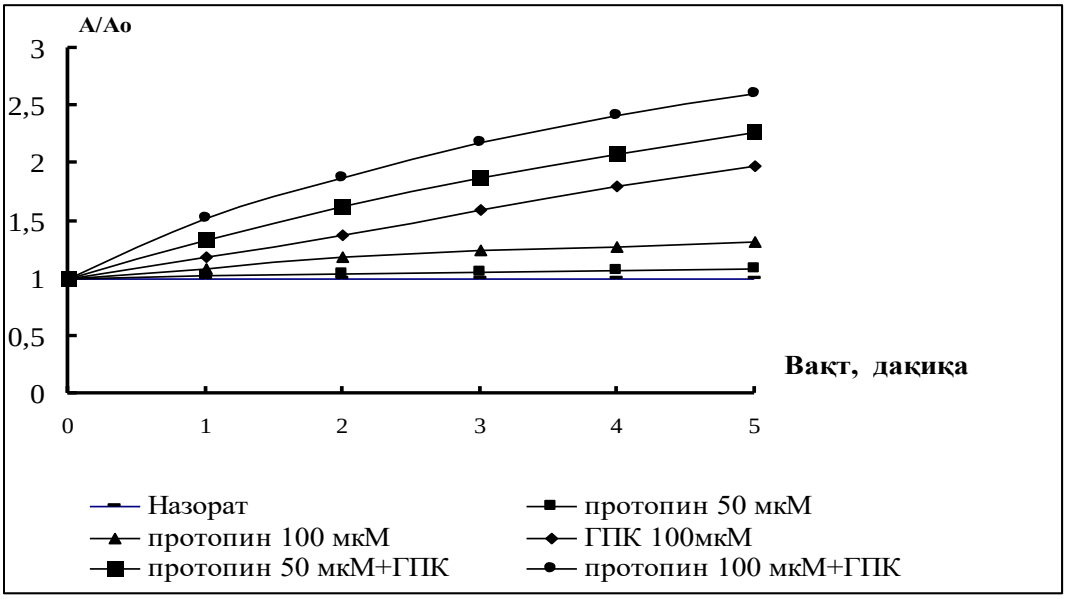
In in vitro studies, in the incubation medium, no swelling was observed in Mx of animals of the control group. Addition of 50 μM or 100 μM of the compound to the incubation medium did not significantly affect the rate of Mx shedding. Addition of GPK to the medium at a concentration of 100 μM increased the rate of Mx shedding by 2.2 times compared to the control group. Under these conditions, GPK causes the SsA-sensitive pore to open, resulting in an increased rate of activated Mx shedding (Fig. 1). In experiments, 50 μM of the compound enhanced the damaging effect of the GPK inducer on SsA-sensitive pores. Increasing the amount of compound increases the Mx gating rate and causes the Mx megachannel to open. At the next stage of our research, we will study the state of permeabilization of the energized Mx membrane with the presence of Ca^{2+} ions induced by protopine alkaloid (Fig. 2).



Note: A/A0 is the ratio of experimental group Mx snoring rate to control group Mx snoring rate. IM - 125 mM Kcl, 2.5 mM KH_2PO_4, 1 mM EGTA, 5 mM glutamate, 1 mM malate, 10 mM trisHCl, pH-7.4. R<0.05 in all cases (n=8).

Figure 2. Effects of protopine on Ca^{2+}-induced membrane permeability of energized mitochondria

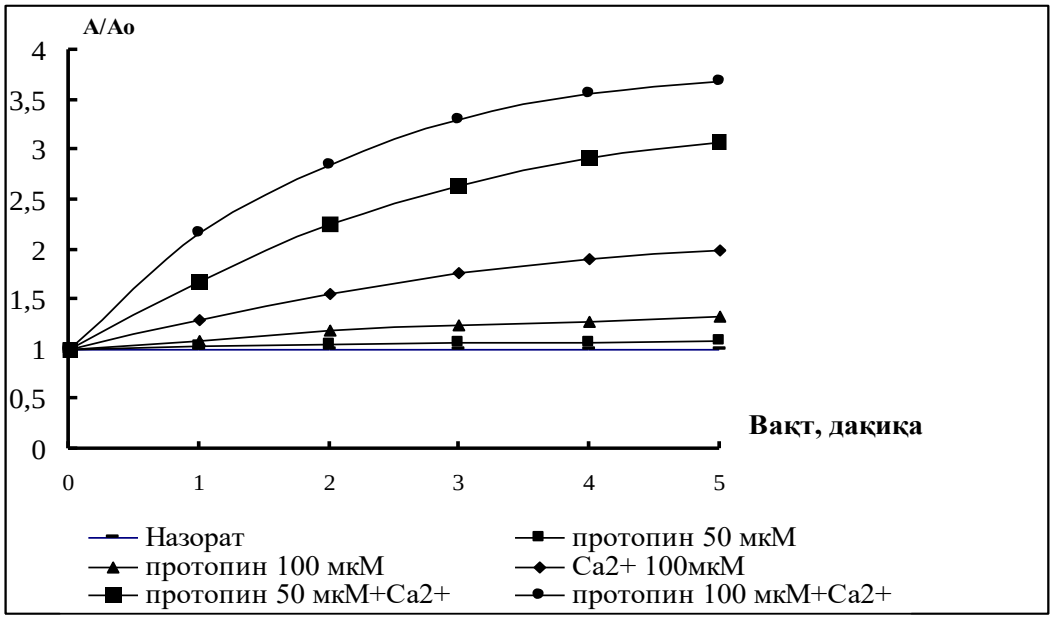
Protopine concentrations of 50-100 μM did not significantly affect the rate of Mx swelling, that is, the state of the Ca^{2+}-dependent SsA-sensitive pore. Addition of Ca^{2+} ions to the medium activated pore opening. Under these conditions, protopine combined with Ca^{2+} ions further increased SsA-sensitive pore opening (Fig. 2).



Experiments were continued in energized Mx. Protopine alkaloid increased the damaging effect of Ca2+ and GPK inducers on Mx in the state of slow conductance of SsA-pore in energized Mx, as well as in non-energized Mx (Figs. 3-; 4).

Note: A/A_0 is the ratio of experimental group Mx snoring rate to control group Mx snoring rate. R<0.05 in all cases (n=8).

Figure 3. Effect of protopine on GPK-induced permeabilization of unenergized mitochondrial membrane



Note: A/A_0 is the ratio of experimental group Mx snoring rate to control group Mx snoring rate. $R<0.05$ in all cases (n=8).

Figure 4. Effect of protopine on Ca^{2+}-induced permeability of unenergized mitochondrial membrane

Summary. In conclusion, it can be said that this alkaloid increases the effect of SsA-sensitive pore inducers Ca^{2+} ions and GPK on membrane permeability in energized and non-energized Mx. Protopine, cryptopine, α-allocryptopine and zeravshanizine alkaloids significantly increase the Ca^{2+}-accumulating capacity of Mx at a dose of 0.5 mg/kg. This effect of alkaloids is due to the decrease in conductivity and the increase in the ability to accumulate Ca^{2+} ions as a result of the transition of the SsA-sensitive pore to a closed conformational state.

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