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Full Length Research

Ultra-low doses of melafen affect the energy of mitochondria

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Addition of the organophosphorous plant growth regulator - melafen to the mitochondria incubation medium resulted in modification of energy thereof. The modification was dose dependent one. The melafen concentrations 2×10^{-12}, 2×10^{-18} and 2×10^{-21} M raised the maximum rates for oxidation of NAD^+-dependent substrates, elevated the efficiency of oxidative phosphorylation and activated electron transport in the terminal step of mitochondrial respiratory chain. Melafen stimulated electron transport during oxidation of succinate by rat liver mitochondria, but had no effect on the rate of this substrate oxidation by sugar beet root mitochondria, which was an evidence for adaptive properties of the preparation. Water stress resulted in decreasing the maximum rates of oxidation of NAD^+-dependent substrate and decreasing the electron transport rates at the end of the respiratory chain by 30%. A pretreatment of pea seeds with a 10^{-7}% solution of melafen led to elimination of differences in energetic of mitochondria of sprouts growing under standard conditions and under conditions of low moisture. By stimulating the activity of NAD^+-dependent dehydrogenases and activation electron transport in the cytochrome oxidase part of respiratory chain, melafen stimulated energy metabolism in the cells and these effects determine the adaptive properties thereof.

Key words: Cytochrome oxidase, mitochondria, oxidative phosphorylation, respiratory chain.

INTRODUCTION

Many natural and synthetic biologically active substances (BAS) exhibit their activity in a range of low (10^{-10} - 10^{-4}M) and ultra-low concentrations (10^{-20} - 10^{-11}M). The level of biological organization, at which the effect of ultra-low doses (ULD) is observed is very diverse: from macromolecules, cells, organs and tissues to animal and plant organisms and even populations (Zinkevich et al., 2002; Belov et al., 2002; Terekhova et al., 2002; Ashmarin et al., 2005). It does not follow from the aforesaid that the effect was observed at ultra-low doses of any one of biologically active substances on any one of biological objects. The observable effect at the substance concentrations 10^{-13} - 10^{-17} M and lower can not be attributed to any definite structure or a level of biological organization (Burlakova et al., 2003).

The effects of ultra-low doses of biologically active substances have common characteristics that do not depend on the substance nature. These characteristics manifest themselves most visibly in studies on dose dependences. In some cases, the dependence is bimodal: the effect increases at ultra-low doses of a preparation, then, the effect decreases, as the dose is increased and is succeeded by a "dead zone" and increases again. Sometimes, the dose dependence has a stage of "a change of sign". For example, if an inhibiting activity was observed in the region of ultra-low doses, it changed for a stimulating one as the concentration was increased, and

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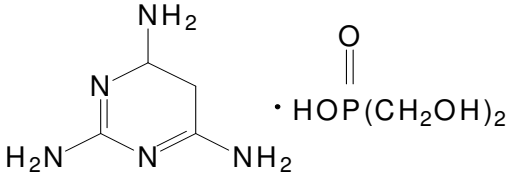
then again an inhibiting effect was observed. There are known cases when the effect did not depend on a dose within a wide concentration range. In particular, in one of the work, wherein the effect of a herbicide of the class of hydro peroxides on a plant cell culture was studied, it was discovered that the preparation has an equal effect at doses that are six orders different (10^{-13} - 10^{-7} M) and the effect is absent in the range of intermediate concentrations (Burlakova, 2003).

The nature of the dependences may be accounted for by the fact that BAS ultra-low doses have common targets. These targets may be cell and sub cellular membranes that play a key role in the cell metabolism. A change in physicochemical properties of the latter caused by various factors including that of BAS results in changing the activity of membrane-associated enzymes (Palmina, 2009).

Although there are a great number of works on effects of BAS ultra-low doses, the mechanism thereof is not well studied yet. The aim of this work is to study the biological effects of plant growth regulators at ultra-low doses.

Natural phytohormones and their synthetic derivatives play a key role in the regulation of plant metabolism in all steps of their ontogenesis (Shevelukha, 1992; Kulaeva, 2002). At present, many biologically active substances able to stimulate plant growth were identified or synthesized (Korol et al., 2001; Thomas et al., 1993; Morohashi, 1984). Application of these compounds enables possibility to prevent drowing of grain crops, accelerate germination and maturation of plants, increase their yields and improve the quality of agricultural products by increasing their resistance to pathogenic microorganisms and parasites (Khan N.A., Samiulallah, 2001; Korol' et al., 2001; Kirillova et al., 2003; Romanov, 2009).

However, the use of natural plant growth regulators (phytohormones) in agricultural practice is often coupled with difficulties, e.g., high cost of final products, fast loss of their useful properties under the action of environmental factors, etc. Therefore, a search for and the synthesis of substances able to stimulate plant growth even when used at low and very low concentrations are currently underway. Such compounds concern the melamine salt of bis (ox methyl) phosphoric acid (melafen) with the structural formula:



for an example. At very low concentrations, melafen increases the general productivity of some agricultural crops (Fattakhov et al., 2002; Kostin et al., 2006). At 3 × 10^{-9} M, it increases the photosynthesis rate and accelerates respiration of plant cells by 15% (Fattakhov et al., 2004).

The pre-sowing treatment of seeds of cereals and leguminous and solanaceous cultures with melafen results in a 5 - 25% gain in the energy of germination, an increase in the productivity and vegetative mass of plants and improvement of quality and nutritive value of the product (Fattakhov et al., 2004).

Since the activation of synthetic processes requires considerable energy expenditures, it is safe to suppose that melafen regulates vital processes of plant cells by influencing the energy metabolism therein and primarily, the energy of mitochondria. The respiratory chains of mitochondria of plants and animals are organized similarly; the main differences are in the CN-resistant electron transfer and the structure of the NADH-dehydrogenase region of the respiratory chain (Palmer, 1976). Therefore, the test subjects used were rat liver mitochondria and mitochondria of plants: a storage parenchyma of sugar beet (*Beta vulgaris* L.) and mitochondria of pea sprouts (*Pisum sativum*).

Since most of plant growth regulators possess the antistress activity, melafen may also possess such activity. Indeed, the action of stress factors promotes the generation of reactive oxygen species (ROS) in the electron transport chains of mitochondria (Baraboi, 1991; Kurganova, 2001; Cadenas et al, 1977). In the situation when the generation of reactive oxygen species increases and the antioxidant system can not cope with the increasing ROS pool, the lipid per oxidation (LPO) processes are activated in membranes (Kulinsky, 1999; Grabelnych, 2005). The antistress preparations must obviously reduce the level of LPO products in biological membranes; the effect is achieved by various pathways including restructuring the mitochondria energy. We used a model of pea seeds germination under conditions of insufficient watering - water stress. Melafen, like many other physiologically active substances, may exhibit a dose dependence of the effect on metabolic processes and the activity of the preparation may be expected for low and ultra-low doses. Based on the above assumptions, the aim of this work was to study the concentration dependences of the effect of the preparation on the energy of mitochondria and the influence of a water stress and pressuring treatment of pea seeds with melafen on the energy of mitochondria isolated from pea sprouts.

MATERIALS AND METHODS

The experiments were carried out on mitochondria from sugar beet root, sprouts of peas and rat liver.

Germination of pea seeds

Pea seeds were germinated in a control group, rinsed with water and soap and then with a 0.01% KMnO4 solution and were soaked in water for 60 min in the experimental group, the seeds were

in water for 60 min. In the experimental group, the seeds were soaked in a 10^{-7}% melafen solution for 30 min and then in water for 30 min. In a day, half of the control seeds and half of the seeds treated with melafen were carried over onto a dry filter paper in open corvettes. In two days of the "drought", the seeds were carried over into closed corvettes with a periodically damped filter paper, wherein the seeds were left for another 5 days. On the fifth day, we calculated the number of sprouting seeds, measured the length of hypocotyls and isolated mitochondria.

Isolation of mitochondria

Mitochondria were isolated from rat liver (Zhigacheva et al., 1995) by differential centrifugation. The isolation medium comprised of 0.25 M sucrose, 5 mM MOPS, pH 7.4. The primary centrifugation lasted for 10 min at 600 g; the secondary, for 10 min at 10000 g. The precipitate was suspended in 0.5 ml of the isolation medium.

Mitochondria from sugar beet root storage parenchyma (Shugaev et al., 1982) and sprouts of peas (Popov, 2003) were isolated by differential centrifugation. Epicotyls having a length of 3 to 6 cm (20 to 25 g) or 30 g of sugar beet root storage were poured with the isolation medium and disintegrated in a blender. The isolation medium comprising 0.4 M sucrose, 5 mM EDTA, 20 mM KH_2PO_4 (pH 8.0), 10 mM KCl, 2 mM 1, 4 -Dithio-dl-theritol, and 0.1% BSA (free of fatty acids) was placed in a cup of a glass homogenizer and homogenized manually for 1 min. The tissue/medium ratio was 1:2. The first centrifugation was performed at при 25000 g for 5 min. The precipitate was resuspended in 8 ml of the rinsing medium and centrifuged at 3000 g for 3 мин. The rinsing medium comprised of 0.4 M sucrose, 20 mM KH_2PO_4 (pH 7.4), 5 mM EDTA, 10 mM KCl and 0.2% BSA (free of fatty acids). Mitochondria were precipitated by centrifugation at 11000 g for 10 min. The precipitate was resuspended in 2 to3 ml of a medium comprising 0.4 M sucrose, 20 mM KH_2PO_4 (pH 7.4), 0.1% BSA (free of fatty acids) and then mitochondria were precipitated again by centrifugation at 11000 g for 10 min. Protein was determined by the biuret method.

Rate of mitochondria respiration

The rate of mitochondria respiration was measured with the aid of Clarke oxygen electrodes and LP-7 polarograph (Czechia). Sugar beet root and pea sprout mitochondria were incubated in a medium containing 0.4 M sucrose, 20 mM HEPES-Tris buffer (pH 7.2), 5 mM KH_2PO_4, 4 mM $MgCl_2$ and 0.1% BSA. The incubation medium for study of the electron transport in respiratory chain of rat liver mitochondria contained 0.25 M sucrose, 10 mM Tris-HCl, 2 mM $MgSO_4$, 2 mM KH2PO4 and 10 mM KCl, pH 7.5 (28°C).

Reagents

sucrose("Sigma-Aldrich" USA), FCCP (carbonylcyanide-p-trifluorometoxyphenylhydrazone) ("Fluka", Germany)., rotenone ("Sigma-Aldrich" USA), antimycin A ("Sigma-Aldrich" USA), ascorbate("Sigma-Aldrich" USA), malate ("Sigma-Aldrich" USA), glutamate("Sigma-Aldrich" USA), succinate ("Sigma-Aldrich" USA), BSA (Bovine serum albumine) (Fraction V, free fatty acids) ("Sigma" USA), N,N,N',N'- tetramethylphenyldiamide (TMPD) ("Sigma" USA); HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid) (BioChemika Ultra, for molecular biology) ("Fluka", Germany), MOPS (3-(N-Morpholino)propanesulfonic acid) ("Fluka", Germany), Tris (hydroxymethyl)aminjmethan)(" MP Biomedicalis, LLC", Germany); KCl (Potassium chloride purees) ("Fluka", Germany), 1,4 –dithio-dl-theritol ("Fluka", Germany).

RESULTS

The study of the effect of melafen on the energy of mitochondria was commenced with a study of the effect on the electron transport maximum rate in a respiratory chain of mitochondria of rat liver and sugar beet root storage parenchyma. The electron transport rates were studied in the presence of a FCCP (carbonylcyanide-p-trifluorometoxyphenylhydrazone) - protonophore reducing a pH gradient and providing thereby for maximum rates of oxidation of substrates. We studied also the rates of oxidation of substrates in the presence of ADP (State 3), introduction of which to the mitochondria incubation medium results in activation of ATP synthesis and, as a consequence, in a decrease in $\Delta\mu_{H^+}$ and in enhancement of the electron transport rate.

The addition of melafen to the incubation medium for the mitochondria of rat liver or sugar-beet storage roots changed the mitochondria energy. The changes were dose-dependent ones. At the concentrations 2×10^{-5} and 2×10^{-14} M, the preparation reduced rates of oxidation of NAD^+-dependent substrates by liver mitochondria by 50 and 12%, respectively and by sugar beet root mitochondria by 30% (Figure 1). The most efficient were the concentrations 2×10^{-12} M and $2 \times 10^{-18} – 2 \times 10^{-21}$. Melafen used in these concentrations increased the rates of oxidation of NAD^+-dependent substrates in the respiratory chain of liver mitochondria by 35 - 43% in the presence of ADP and by 52% in the presence of FCCP (carbonylcyanide-p-trifluorometoxyphenylhydrazone).

The respiratory control rate (RCR) increased in 1.2 - 1.4 times (Table 1). In the respiratory chain of sugar beet root mitochondria, the rates of oxidation of NAD^+-dependent substrates increased by 13 - 23% in the presence of ADP and the respiratory control rate increased in 1.3 times (2×10^{-18} and 2×10^{-21}) (Table 2).

It should be noted that in all cases the increase or decrease (2×10^{-5}; 2×10^{-14} M) in the RCR in the oxidation of NAD^+-dependent substrates was due to an increase or decrease in the rates of oxidation of substrates by liver mitochondria in the presence of ADP (in State 3).

The differences between plant and animal mitochondria were observed when the oxidation substrate used was succinate. The effect of the preparation on the oxidation of succinate by rat liver mitochondria was similar to that on the rates of oxidation of NAD^+-dependent substrates (Table 3). However, all concentrations studied had no effect on the maximum rates and the respiratory control rate in oxidation of succinate by sugar beet root mitochondria, which is an evidence for the adaptive character of the melafen effect (Table 4).

Mitochondria of storage organs are characterized by relatively low rates of oxidation of NAD^+-dependent substrates (Shugaev, 1985) Melafen enhances the activity of NADH-dehydrogenases and, evidently, activates the

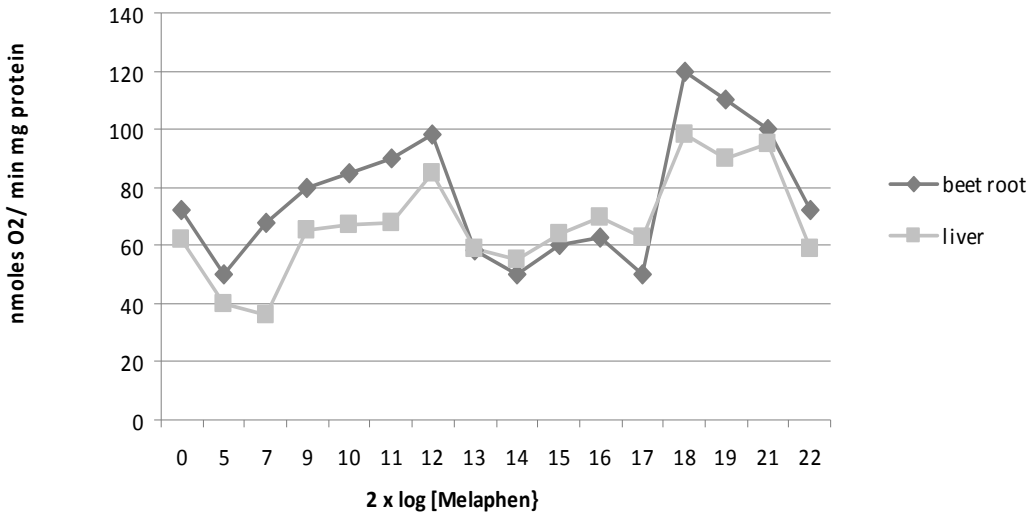


Figure 1. Effects of melafen on respiration rate of mitochondria in the presence of FCCP. Ordinate: rates of glutamate + malate oxidation in the presence of FCCP in n atoms O2/ mg protein min.. Abscissa: 2 × l g concentration of melafen. Ordinate in n atoms/ mg protein min.

Table 1. Effect of various melafen concentrations on the efficiency of oxidative phosphorylation in the respiratory chain of rat liver mitochondria in oxidation of NAD^+-dependent substrates. The respiration rate was measured in n atomsl O_2/min x mg protein**.**

Melafen, M	State 2	State 3	State 4	RCR	FCCP
-	16.0 ± 1.3 (8)	50.4 ± 2.4 (8)	20.5 ±1.5 (8)	2.45 ± 0.1 (8)	58.9 ± 1.6(8)
2×10^{-5}	14.0 ± 1.2 (5)	40.0 ± 2.5 (5)	21.0 ±1.0 (5)	1.90 ± 0.1(5)	51.0 ± 4.2 (5)
2×10^{-12}	16.5 ± 0.2 (6)	58.2 ± 1.6 (6)	19.7 ± 0.9 (6)	2.95 ± 0.10	62.9 ± 0.6 (6)
2×10^{-18}	22.1 ± 3.2 (5)	72.5 ± 4.3 (5)	21.0 ± 2.6 (5)	3.45 ± 0.2 (5)	90.0 ± 5.2 (5)
2×10^{-19}	17.5 ± 3.0 (5)	62.4 ± 2.1 (5)	24.0 ± 2.4 (5)	2.6 ± 0.2 (5)	55.0 ± 2.7 (5)
2×10^{-21}	22.5 ± 1.4 (6)	68.4 ± 4.6 (6)	22.8 ± 3.4 (6)	3.00 ± 0.2 (6)	90.0.0 ± 3.2 (6)

Incubation medium: Contain 0.25 M sucrose, 10 мM tris-HCl, 2 mM KH_2PO_4, 5 mM $MgSO_4$, 10 mM KCl, pH 7.5. Other additives: 200 µM ADP, 10^{-6}M FCCP, 4 mM glutamate, 1 mM malate.

Table 2. Effect of various melafen concentrations on the efficiency of oxidative phosphorylation in the respiratory chain of beet root mitochondria in oxidation of NAD^+-dependent substrates. The respiration rate was measured in n atomsl O_2/min x mg protein

Melafen, M	State 2	State 3	State 4	RCR	FCCP	KCN
-	15.8 ± 1.0 (6)	66.4 ± 3.9 (6)	28.8 ± 2.5 (6)	2.3 ± 0.1 (6)	72.0 ± 4.6 (6)	6.3 ± 0.4 (6)
2×10^{-5}	12.7 ± 2.4 (5)	50.1 ± 3.0 (5)	27.6 ± 2.0 (5)	1.81 ± 0.2 (5)	50.0 ± 3.8 (5)	5.9 ± 0.6(5)
2×10^{-12}	17.5 ± 2.0 (5)	75.5 ± 3.0 (5)	26.1 ± 1.0 (5)	2.90 + 0.1(5)	95.0 + 3.2 (5)	8.2 + 0.2 (5)
2×10^{-14}	12.0 ± 2.4 (6)	60.0 ± 3.0 (6)	33.5 ± 2.0 (6)	1.79 ± 0.2 (6)	48.6 ± 4.0 (6)	5.7 ± 0.4 (6)
2×10^{-18}	20.0 ± 3.2 (5)	80.0 ± 2.4 (5)	27.1 ± 1.6 (5)	2.95 ± 0.3 (5)	120.0 ± 4.0 (5)	6.0 ± 0.2 (5)
2×10^{-21}	20.5 ± 2.4 (5)	81.9 ± 4.6 (5)	27.8 ± 2.2 (5)	2.95 ± 0.2 (5)	100.0.0 ± 40 (5)	5.2 ± 0.5

Incubation medium: 0.4 M sucrose, 20 mM HEPES-tris-buffer (pH 7.2), 5 mM KH_2PO_4, 4 mM $MgCl_2$, 0.1% BSA.,: 5 mM malate, 10 mM glutamate. Other additives: 125 µM ADP, 0, 5 µM FCCP (carbonylcyanide-p-trifluorometoxyphenylhydrazone).

energy processes in cell and provides thereby for a high energy of seeds germination.

The activation of energy processes in cell is provided also by the effect of the preparation on the electron transfer

Table 3. Effect of melafen on the kinetics of consumption of oxygen by rat liver mitochondria in oxidation of succinate. The respiration rate was measured in n atomsl O_2/min x mg protein.

Melafen, M	State 2	State 3	State 4	RCR	FCCP
-	42.3 ± 3.8 (6)	113.2 ± 2.5 (6)	45.7 ± 3.1(6)	2.48 ± 0.10 (6)	129.0± 7.2 (6)
2×10^{-12}	19.0 ± 1.4 (5)	151.8 ± 1.5 (5)	46.0 ± 0.2(5)	3.30 ± 0.09 (5)	64.0 ± 1.0 (5)
2×10^{-18}	38.7 ± 4.1(5)	158.7 ± 1.4 (5)	46.0 ± 1.7(5)	3.45 ± 0.10 (5)	130.0± 2.9 (5)
2×10^{-21}	41.8 ± 3.0 (5)	136.2 ± 3.4 (5)	45.4 ±2.5 (5)	3.00 ± 0.10 (5)	123.0± 4.0(5)

Incubation medium: Contain 0.25 M sucrose, 10 мM tris-HCl, 2 mM KH_2PO_4, 5 mM $MgSO_4$, 10 mM KCl, pH 7.5. Other additives: 200 µM ADP, 10^{-6}M FCCP, 5 mM succinate.

Table 4. Effect of melafen on the kinetics of consumption of oxygen by sugar beet root mitochondria in oxidation of succinate. The respiration rate was measured in n atomsl O_2/min x mg protein.

Melafen, M	State 2	State 3	State 4	RCR	FCCP
-	42.3 ± 3.8(6)	113.2 ± 2.5 (6)	45.7 ± 3.1 (6)	2.48 ± 0.3 (6)	129.0 ± 7.2(6)
2×10^{-5}	42.0 + 2.4(5)	120.0 + 2.4 (5)	53.0 + 1.6 (5)	2.26 + 0.3 (5)	139.0 + 4.0 (5)
2×10^{-12}	45.0 + 3.2 (5)	120.0 + 2.4 (5)	53.0 + 1.6 (5)	2,26 + 0.3 (5)	123.0 + 4.0 (5)
2×10^{-18}	38.7 ± 4.1 (5)	115.0 ± 1.4 (5)	46.0 ± 1.7 (5)	2.50 ± 0.3 (5)	130.0 ± 2.9 (5)
2×10^{-21}	41.8 ± 3.0 (5)	111.7 ± 3.4(5)	45.4 ± 2.5 (5)	2.46 ± 0.1 (5)	123.0 ± 4.0 (5)

Incubation medium: 0.4 M, sucrose 20 mM HEPES-tris-buffer (pH 7.2), 5 mM KH_2PO_4, 4 mM $MgCl_2$, 0.1% BSA, 10 mM succinate. Other additives: 125 µM ADP, 0, 5 µM FCCP (carbonylcyanide-p-trifluorometoxyphenylhydrazone).

transfer rate at the terminal cytochrome oxidase region of the respiratory chain of both plant and animal mitochondria. The dependence of the electron transport rate on the concentration of the preparation was also dose-dependent one. At the concentration 2×10^{-5}; 2×10^{-14} and 2×10^{-17}M, melafen decreased the rates of oxidation of ascorbate by sugar beet root storage mitochondria in the presence of TMPD (N,N,N',N'-tetramethylphenyldiamine) and by rat liver mitochondria (2×10^{-5}; 2×10^{-17}M) by 13 and 40 - 20%, respectively. Other effect was observed on addition of 2×10^{-12}M and 2×10^{-18} - 2×10^{-21}M melafen to incubation medium. The rates of oxidation of ascorbate in the presence of TMPD by liver mitochondria increased from 82.4 ± 1.2 to 105.1 ± 1.4 nmol O_2/mg.protein.min. At the same concentrations, melafen promoted the electron transport at the cytochrome oxidase site of the respiratory chain of sugar beet root mitochondria. The rates of oxidation increased by 12 - 15%. The increase was not caused by the activation of the alternative CN-resistant oxidase (AO) of mitochondria, since the electron transport was completely suppressed by cyanide. Evidently, "a change of sign" of the effect of the preparation on the electron transport rate at the end site of the respiratory chain determines the dose dependence of oxidation rates of NAD^+-dependent substrates. The observable increase in the activity of cytochrome oxidase and oxidation rates of NAD^+-dependent substrates in the presence of melafen promotes, evidently, the energy metanolism in cell, which results in increasing the heat generation by plant cells and activation of synthetic processes described in the literature (Fattakhov et al., 2004.). Evidently, the regulation of metabolic processes in plant cell by melafen is effected due to modification of physicochemical properties of biological membranes and, consequently, of the activity of enzymes bound thereto.

We verified the supposition in respect of adaptive properties of melafen in terms of a model of mitochondria of pea sprouts growth under conditions of water stress. Low moisture led to decreasing the maximum rates of oxidation of NAD^+-dependent substrates by pea sprout mitochondria. The rate of oxidation of glutamate + malate in the presence of FCCP decreased from 105.0 ± 2.1 to 75.0 ± 3.4 n atoms of O_2/ mg protein.min. Hence, the respiratory control rate decreased from 2.27 ± 0.1 to 1.7 ± 0.2. The pretreatment of seeds with melafen prevented from drought-induced change in the efficiency of oxidative phosphorylation. In addition, the pretreatment resulted in reduction of the rates of oxidation of NAD^+-dependent substrates in the presence of ADP or FCCP to the control values. The decrease in the maximum rates of oxidation of NAD^+-dependent substrates under low-moisture conditions may be caused by a decrease in the electron transfer rate at the end of the respiratory chain. From Table 5, the rates of oxidation of ascorbate in the presence of N, N, N', N'- tetramethylphenylenediamine (TMPD) by mitochondria isolated from drought-conditioned pea sprouts were less than the control values

Table 5. Effects of melafen on the rate of electron transport in the respiratory chain of sugar beet root mitochondria in the presence of ascorbate and TMPD. The respiration rate was measured in n atomsl O_2/min x mg protein.

Group	State 2	TMPD		
		200 µM	200 µM	400 µM
Standard conditions of germination	8.0 ± 0.3 (5)	250.0 ± 10 (5)	336.0 ± 11.0 (5)	500.0 ± 32 (5)
Water stress	5.0 ± 0.2 (8)	175.0 ± 25 (8)	240.0 ± 21 (8)	356.0 ± 32 (8)
Melafen treatment + water stress	7.0 ± 0.4 (6)	242.0 ± 15 (6)	340.0 ± 17 (6)	498.0 ± 36 (6)

Incubation medium: 0.4 M sucrose, 20 mM HEPES-Tris buffer, 5 mM KH2PO4, 2 mM $MgCl_2$, 5 мМ EDTA, 10 mM ascorbate, 60 µM rotenone, 5 µM antimycin A, 0,5 µM FCCP, pH 7.4.

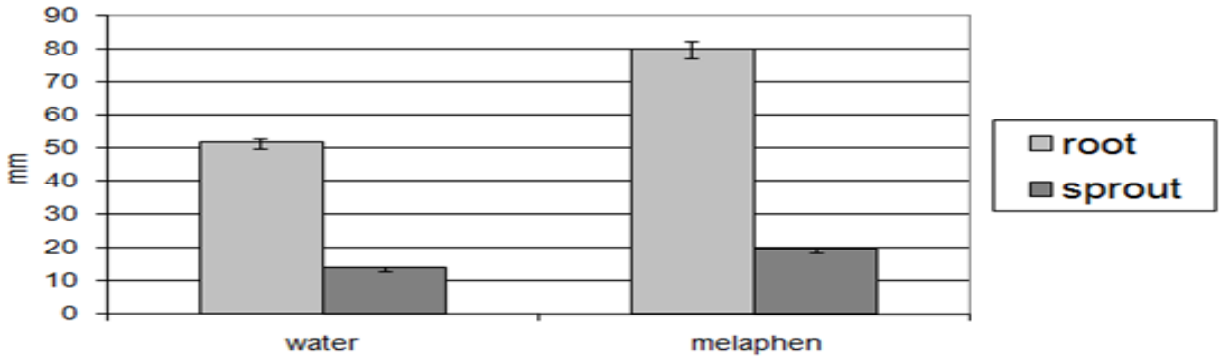


Figure 2. Growth of roots of etiolated pea germs under low - moisture conditions. Control - standard sprouting conditions; drought - sprouting of seeds under low- moisture conditions. Ordinate - length of hypocotyls, mM. The number of pea seeds in each group was 100 (n = 100); $p < 0.05$.

almost by 30%. In this case, the pretreatment with melafen is prevented from change at this site of the respiratory chain. It is safe to suppose that shifts in the physiological parameters were caused by changes in the metabolic activity of mitochondria: The pretreatment of pea seeds with melafen stimulated the growth of sprouts (by 18 - 24%) both for the control and for plants growing in drought; however, germination of treated and untreated seeds differed significantly. Under drought conditions, the germination of seeds in control decreased by 46%; that of melafen-treated seeds remained almost unchanged. Moreover, melafen at 1.5 times stimulated the growth of sprout roots under drought conditions, which is of great importance for adaptation (Figure 2). Melafen increases the maximum oxidation rates of NAD^+-dependent substrates and the efficiency of oxidative phosphorylation and thus promotes the activation of energy processes in cell and provides for a high energy of seeds germination.

DISCUSSION

The adaptive effect of the compound may be attributed to activation of the electron transfer at the end of the mitochondrial respiratory chain, which results in inhibiting the formation of reactive oxygen species (ROS) at the site of complex III (Cadenas, 1977; Sviryaeva and Ruge, 2006). Even ultra-low concentrations of melafen affect the functional state of membranes.

It should be noted that the discrete character of the concentration dependences of the effect of ultra-low doses of melafen obtained in our experiments (Tables 1 - 3 and Figure 1) is qualitatively consistent with published data on the effect of ultra-low doses of biologically active substances on living systems of various degrees of complexity (Burlakova et al., 2003; Myagkova et al., 2003; Zhernovkov, 2006). There exist a great number of hypotheses as to the mechanisms of the effect of ultra-low concentrations of BAS in the literature. According to one of the hypotheses, BAS, in a range of physiological concentrations of up to 10^{-9} M, are incorporated into membranes and interact with lipids. At concentrations of 10^{-9} - 10^{-17} M, BAS interact with membrane ligands and receptors located at particular membrane sites (rafts). Thus, the receptor signal may be amplified by a factor of 10^6 - 10^{10} due to highly efficient systems of transmission and amplification of signals (Palmina, 2009). As to the region of “virtual concentrations” (10^{-17} - 10^{-25} M), the authors

authors interpret the observable effects in terms of the concept about the effect of ultra-low doses of physical factors and chemical substances on the structure of water (Burlakova et al., 2003).

In our opinion, the results obtained for melafen may be interpreted in terms of the physicochemical behavior of highly diluted solutions of the preparation. Konovalov et al. (2008) showed that melafen in a concentration of 10^{-20} - 10^{-4} mol/l forms nanoassociates of the size of about 100 - 200 nm with participation of water. The concentration dependences obtained by the authors for a size and electrokinetic potential (ζ-potential) of nanoassociates formed in aqueous solutions of melafen at low and ultra-low concentrations (Ryzhkina et al., 2009), are comparable with the biological effects of the preparation as described in this work and published previously (Zhigacheva et al., 2008), which is the evidence for the role of melafen nanoassociates in biological effects. The most plausible evidence for the fact that ultra-low concentrations of melafen that affect the energy of mitochondria are discrete in character is the formation of associates of a different polarity and probably, of different level of complexity depending on the melafen concentration in a solution (Konovalov et al., 2008).

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