THE INFLUENCE OF CATACYN AND BENZONAL ON CA^{2+} ACCUMULATING CAPACITY OF LIVER MITOCHONDRIA IN RATS INTOXICATED WITH THE VENOM OF *NAJA OXIANA* EICHWALD

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Abstract: The present work describes the influence of benzonal and catacyn (substances which are known by antihypoxic effect) on calcium transport in mitochondria of liver cells of rats intoxicated by Central Asian Cobra venom. White rats (average weight 200–230 g) were used in the experimental studies. These animals were divided into four groups. The rats from the first, second and third groups were injected with the venom of *Naja oxiana* at 160 μg•kg^{-1} of weight, intramuscularly. Two minutes later the rats from the second and third groups were injected with 50 mg•kg^{-1} of catacyn or benzonal. The fourth group was injected with the normal saline solution. The rats were decapitated 15 min after venom injection.
It was established that after the injection of benzonal and catacyn into the rats, the Ca^{2+} accumulating capacity of liver mitochondria decreased. So, after the injection of 50 mg of benzonal per 1 kg of body weight the Ca^{2+} capacity of rat liver mitochondria dropped by 32.4% of the control level, while catacyn → caused → the drop by 26,8%. The drop of Ca^{2+} accumulating capacity of mitochondria by above-mentioned antihypoxants is connected with either the → inhibition of Ca^{2+} ion absorptive function of mitochondria, or with the increase of glycoprotein content by benzonal and catacyn, which specifically binds. Ca^{2+}, or by their activation of ryanodine receptor. The obtained results suggested the inhibition of Ca^{2+} transport to mitochondria by benzonal and catacyn. It was established that under the venom of Central Asian Cobra effect the consumption of calcium ions in rat liver mitochondria increased by 68,6% from the norm. With benzonal and catacyn it constituted only 17,4% and 20,4%, respectively. It means that the benzonal and catacyn reduce the Ca^{2+} accumulating capacity of mitochondria, i.e. almost completely reduce the negative effect of *N. oxiana* venom effect.

Key words: *Naja oxiana*, venom, liver mitochondria, calcium, benzonal, catacyn.
Słowa kluczowe: *Naja oxiana, jad, mitochondria wątroby, wapń, benzonal, katacyna.*

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1. Introduction

Benzonal (Ziyaeva, 1994; Ziyaeva et al., 1996; Ziyaeva, 1997; Yuldashev, Asanova, 2002; Shirinova, Nurdinov, 2006) and catacyn (Kurmukov et al., 1990; Nazrullaev, 1994; Asanova, 2002) are known to have a pronounced antihypoxic effect and directly affect the gas-oxygen exchange and energetic metabolism in mitochondria at hypoxia. It is known that antihypoxants (gutimin and oxybutyrate) were effective when used as a prophylactic means and after intoxication with the snake venom (Vinogradov, 1972; Valtseva et al., 1975;

Bogrova, 1976; Vinogradov, Pastushenkov, 1977; Orlov, Valtseva, 1977; Chichkanov et al., 1982; Mashkovsky, 1987; Evtodienko et al., 2000). The question of a possibility of the direct action of antihypoxants (catacyn and benzonal) on the calcium-accumulating capacity of mitochondria in different organs of animals in the context of the snake venom effect remains open.

It is well known that Ca^{2+} ions regulate many intracellular processes, including generation of energy. The regulation is realized either as a direct allosteric influence of Ca^{2+} on target enzymes or indirectly by activating/retarding different protein kinases and protein phosphatases catalyzing phosphorylation/dephosphorylation of target enzymes. It is generally accepted that Ca^{2+} ions can regulate the synthesis of ATP in mitochondria through the activation of several dehydrogenases in the Krebs cycle. It is known that Ca^{2+} can modulate the activity of translocase of adenine nucleotides. The maximum rate of ATP synthesis and hydrolysis in the mitochondria of rat liver is observed after the addition of 5.10^{-7}M of Ca^{2+} to respiring mitochondria. A decrease in the concentration of Ca^{2+} to 10-8M or increase to 5.10^{-6}M leads to the retard of the oxidative phosphorylation and hydrolysis of ATP (Evtodienko et al., 2000).

The aim of this work is to study the influence of benzonal and catacyn on the transport of Ca^{2+} in mitochondria of liver cells in control animals and those intoxicated with the venom of *N. oxiana*.

2. Methods

White rats (average weight 200–230 g) were used in the experimental studies. These animals were feed with mixed diet and kept in wooden boxes (50 by 30 cm) in a well ventilated light room. From 8 to 10 rats were kept in each box. Water and food were supplied without any restrictions.

Ten rats from each of four groups were kept in separate boxes. The rats from the first, second and third groups were injected with the venom of *N. oxiana* at 160 $\mu g \cdot kg^{-1}$ of weight, intramuscularly. Two minutes later the rats from the second and third groups were injected with 50 $mg \cdot kg^{-1}$ of catacyn or benzonal. The fourth group was injected with the normal saline solution. The rats were decapitated 15 min after venom injection. The venom of *N. oxiana* was kindly provided by the Institute of Zoology of Uzbek Academy of Sciences. We used venom samples of the 2002 collection, which were dried in the desiccator filled with calcium chloride.

The mitochondria were isolated from the liver cells of rats using the method described by Almatov et al. (1993). The transfer of Ca^{2+} through the mitochondrial membrane was registered using the pH-metric method based on the change of $2H^+/Ca^{2+}$ exchange in mitochondria. The absorption of Ca^{2+} in exchange for protons is replaced by a spontaneous release of the accumulated Ca^{2+} at a consecutive addition of several portions of calcium chloride to the mitochondrial suspension. This is connected with the damage of mitochondrial membranes caused mainly by the activation of phospholipase A2 and phospholipase B with large concentrations of Ca^{2+}, uncoupling of the oxidative phosphorylation, change in the membrane permeability and the opening of cyclosporine A-sensitive pore (Brockermeier, Pfeiffer, 1995; Madesh, Balasubramanian, 1997; Ganitkevich, 2003). The more calcium ions mitochondria can accumulate until its spontaneous release, the more stable are their membranous structures to the damaging effect of these ions. The replacement of Ca^{2+} by ions can change depending on conditions of stoichiometry. Since in the presence of phosphate as a penetrating anion the stoichiometry of $2H^+/CVa^{2+}$ change is constant and approximately equal to 1, in the experiments the incubation medium contained 120 μmol Tris-KCl, 10 μmol Tris-HCl, 5 μmol of succinate, pH 7.4, rotenone (1 $\mu g \cdot ml^{-1}$) and 1μmol of phosphate. The system was calibrated with the HCl solution at a known concentration. The protein was identified as described by Lowry et al. (1951).

3. Results and discussion

It is established that after the injection of benzonal and catacyn into the rats, the Ca^{2+} accumulating capacity of liver mitochondria decreases (Table 1). So, after the injection of 50 mg of benzonal per 1 kg of body weight the Ca^{2+} capacity of rat liver mitochondria drops by 32.4% of the control level, while catacyn causes the drop by 26,8%. The drop of Ca^{2+} accumulating capacity of mitochondria by above-mentioned antihypoxants is connected with either the inhibition of Ca^{2+} ion absorptive function of mitochondria, or with the increase of glycoprotein content by benzonal and catacyn, which specifically binds Ca^{2+}, or by their activation of ryanodine receptor (Deryabina et al., 2004). The addition of ryanodine to isolated mitochondria brought about the inhibition of the Ca^{2+} transport and inhibited the high-amplitude swelling of mitochondria.

The obtained results of the study suggest the inhibition of Ca^{2+} transfer to mitochondria by benzonal and catacyn.

In the next series of the study we studied the effect

Tab. 1. The effect of benzonal and catacyn on Ca^{2+} accumulating capacity of liver mitochondria in rats (M±m; n = 8-10)
Tab. 1. Wpływ benzonalu i katacyny na zdolność akumulacyjną Ca^{2+} mitochondriów wątroby szczurów (M±m; n = 8-10)

Preparations *Rodzaj próby*	**Ca^{2+} accumulating capacity, nmol/mg of protein** *Zdolność akumulacyjna Ca^{2+}, nmol/mg białka*
Control	82,8±3,7
Benzonal	56,0±3,2***
Control	84,3±4,4
Catacyn	61,7±3,9***

of benzonal and catacyn on Ca^{2+} accumulating capacity of animal liver mitochondria in the background of the effect of *N. oxiana* venom (Table 2). It was established that under the venom effect the consumption of calcium ions in rat liver mitochondria increased by 68,6% from the norm. With benzonal and catacyn it constituted only 17,4% and 20,4%, respectively. It means that the benzonal and catacyn reduce the Ca^{2+} accumulating capacity of mitochondria, i.e. almost completely reduce the negative effect of *N. oxiana* venom effect. In our opinion, this venom causes a progressive increase $[Ca^{2+}]_c$, which gives the signal to the Ca^{2+} cycle in the mitochondrial membrane. This increase lasts until the work of the system of input and output of Ca^{2+} leads to the critical growth of $[Ca^{2+}]_m$ to 1–3 μm. Induction of Ca^{2+} dependent non-specific permeability of the inner membrane (the so-called membranous pore) takes place under these conditions. It is accompanied with a high-amplitude swelling of mitochondria, damage of the external membrane and a release of soluble pro-apoptotic agents into the cytosol. These include cytochrome c localized in inter-membrane area, apoptosis-inducing factor, a number of caspases, which directly participate in the launch of the cascade of apoptic reactions, as well as the factor Smas/DIABLO, which promotes apoptosis and inactivates inhibitors of apoptic proteins (Deryabina et al., 2004). Thus, the injection of benzonal and catacyn into the organism intoxicated with the N. oxiana venom inhibits the transport of Ca^{2+} and a high-amplitude mitochondrial swelling.

4. References

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Tab. 2. The effect of *N. oxiana* venom on Ca^{2+} accumulating capacity of rat liver mitochondria against the effect of benzonal and catacyn (M±m; n = 8-10)
Tab. 2. Wpływ jadu N. oxiana na zdolność akumulacyjną mitochondriów wątroby szczura w obecności benzonalu i kataryny

Values *Wartości*	**Ca^{2+} accumulating capacity**, nmol/mg of protein *Zdolność akumulacyjna Ca^{2+}*, nmol/mg białka			
	Intact rats *Szczury bez intoksykacji*	Control	Benzonal	Catacyn
Liver	91,4±5,9	154,1±12,6****	107,3±8,2	110±6,7
	100	168,6	117,4	120,4

Note: *** P < 0,01; **** P < 0,001

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WPŁYW KATACYNY I BENZONALU NA ZDOLNOŚĆ AKUMULACYJNĄ MITOCHONDRIÓW WĄTROBY SZCZURÓW PO PODANIU JADU KOBRY ŚRODKOWOAZJATYCKIEJ (*NAJA OXIANA* EICHWALD)

Streszczenie

Praca przedstawia wpływ benzonalu i katacyny (substancji wykazujących efekt antyhipoksyjny) na transport wapnia w mitochondriach komórek wątroby u szczurów, którym podawano jad kobry środkowoazjatyckiej i u zwierząt kontrolnych. Szczury o przeciętnej masie 200–300 g zostały podzielone na cztery grupy. Zwierzętom z grup 1, 2 i 4 wstrzykiwano domięśniowo jad kobry w ilości 160 $\mu g\ kg^{-1}$ wagi ciała. Po dwóch minutach szczurom z grupy 2 i 3 podawano benzonal lub katacynę w ilości 50 $mg\ kg^{-1}$ wagi ciała. Czwartej grupie podawano roztwór soli fizjologicznej. Zwierzęta uśmiercano 15 minut po podaniu jadu. Badania wykazały, że po podaniu benzonalu i katacyny zmniejszała się zdolność akumulacyjna mitochondriów wątroby w stosunku do jonów Ca^{2+}, odpowiednio o 32,4 i 26,8%. Spadek ten powodowany przez wyżej wymienione substancje był związany albo z hamowaniem funkcji absorpcyjnej mitochondriów w stosunku do jonów wapnia, albo przez zwiększenie zawartości glikoprotein po wpływem benzonalu i kataryny, które specyficznie absorbowały Ca^{2+}, lub w wyniku aktywacji przez receptor ryanodinowy. Uzyskane wyniki sugerowały hamowanie transferu Ca^{2+} do mitochondriów przez benzonal i katacynę. Wykazano, że jad kobry środkowoazjatyckiej zwiększa przyswajanie jonów wapnia w mitochondriach wątroby szczurów o 68,6%. Przy udziale benzonalu i katacyny wzrost ten wynosi odpowiednio, 17,4 i 20,4%. Oznacza to, ze benzonal i katacyna redukują zdolność akumulacyjną mitochondriów w stosunku do jonów wapnia, co wskazuje na niemal całkowitą redukcję ujemnego wpływu jadu *N. oxiana* w tym aspekcie.