EXPERIMENTAL WORKS

UDC 577.27

doi: https://doi.org/10.15407/ubj95.02.024

CHOLINE DERIVATIVES AS NATURAL LIGANDS OF MITOCHONDRIAL NICOTINIC ACETYLCHOLINE RECEPTORS

O. LYKHMUS, M. IZMAILOV, M. SKOK✉

Department of Molecular Immunology, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;
✉*e-mail: skok@biochem.kiev.ua*

Received: 16 March 2023; **Revised:** 28 April 2023; **Accepted:** 05 June 2023

Nicotinic acetylcholine receptors (nAChRs) regulate mitochondria-driven apoptosis; however, their intracellular ligands are unknown. In the present paper, we show that choline and its derivatives (phosphocholine (PC), L-α-glycerophosphocholine (G-PC) and 1-palmitoyl-sn-glycero-3-phosphocholine (P-GPC)) dose-dependently influence cytochrome c release from isolated mouse liver mitochondria. Choline inhibited Ca^{2+}-stimulated cytochrome c release, while PC attenuated wortmannin-induced cytochrome c release. Small doses of G-PC and P-GPC (up to 0.1 μM) were protective against either Ca^{2+} or wortmannin, while larger doses (up to 1 μM) stimulated cytochrome c release by themselves. Choline and PC disrupted interaction of VDAC1, Bax and Bcl-2 with mitochondrial α7 nAChRs and favored their interaction with α9 nAChR subunits. It is concluded that choline metabolites can regulate apoptosis by affecting mitochondrial nAChRs.

K e y w o r d s: nicotinic acetylcholine receptor, mitochondria, cytochrome c, choline, choline derivatives, apoptosis.

Mitochondrial apoptosis pathway is initiated by cytochrome *c* (Cyto *c*) release from mitochondria intermembrane space followed by apoptosome formation and caspases activation. Nicotinic acetylcholine receptors (nAChRs), classically attributed to neurons and muscle cells, are now found in many non-excitable cells and are involved in regulation of multiple biological processes like cell proliferation and survival, immune response and cytokine production [1-3]. In addition to cell plasma membrane, functional nAChRs were found in the outer membrane of mitochondria where they regulate Cyto *c* release under the effect of pro-apoptotic signals like Ca^{2+} or reactive oxygen species by interacting with pro-apoptotic protein Bax and voltage-dependent anion channel (VDAC1) and influencing the activity of intramitochondrial kinases [4, 5]. However, in contrast to the plasma membrane nAChRs activated by acetylcholine released into extracellular space, the nature of ligands affecting mitochondrial nAChRs inside the cell is less clear. The most obvious candidate is choline, an agonist for α7 nAChRs, which is abundantly present inside the cell and is metabolized by mitochondria. In addition, numerous choline metabolites derive from the synthesis and catabolism of phosphatidylcholine, the major phospholipid component of eukaryotic membranes. The published evidence identified phosphocholine and dipalmitoylphosphatidylcholine as novel nicotinic agonists that elicit metabotropic activity at monocytic nAChRs containing α7, α9 and α10 subunits [6].

In the present paper, we put an aim to investigate if choline and its derivatives (phosphocholine (PC), L-α-glycerophosphocholine (G-PC) and 1-palmitoyl-sn-glycero-3-phosphocholine (P-GPC)), influence Cyto *c* release from mitochondria, affect mitochondrial nAChRs interaction with VDAC1, Bax and Bcl-2 and, therefore, can be regarded as natural nAChR ligands regulating mitochondrial apoptosis pathway.

Materials and Methods

Materials. All reagents including PNU282987 (P2303), choline, phosphocholine (P0378), L-α-glycerophosphocholine (G5291) and 1-palmitoyl-sn-glycero-3-phosphocholine (L5254), as well as VDAC1-specific antibody (V2139) were purchased from Sigma-Aldrich (Saint Louis, USA). α-Conotoxin PeIA cloned from Conus pergrandis [7], was synthesized in the Department of Molecular Bases of Neurosignalization in Schemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS and was a kind gift of Prof. V. Tsetlin. Antibodies against Bax (PA5-11378), Bcl-2 (PA5-27094) and Neutravidin-peroxidase conjugate (A2664) were from Invitrogen and were purchased from ALT Ukraine Ltd (representative of Thermo Fisher Scientific in Ukraine). Antibodies against α7 and α9 nAChR subunits were obtained and characterized in our laboratory [8, 9]. The antibodies were biotinylated according to a standard procedure.

Animals and procedures. We used female C57BL/6J mice, 2-5 months of age, 20-25 g of weight. Animals were kept in the animal facility of Palladin Institute of Biochemistry. They were housed in quiet, temperature-controlled rooms and provided with water and food pellets ad libitum. Before removing the liver or brain, mice were sacrificed by cervical dislocation. All procedures complied with the ARRIVE guidelines, were carried out in accordance with the Directive 2010/63/EU for animal experiments and were approved by the Animal Care and Use Committee of Palladin Institute of Biochemistry (Protocol 1/7-421).

Mitochondria purification and treatment. Mitochondria were isolated from either mouse liver or brain by differential ultracentrifugation according to standard published procedures [10] and their purity was assessed by ELISA using the antibodies against nuclear-specific lamin B1, mitochondria-specific voltage-dependent anion channel (VDAC1) or endoplasmic reticulum-specific IRE-1α as described [11]. The purified live mitochondria were resuspended and aliquoted in the incubation medium containing 10 mM HEPES, 125 mM KCl, 25 mM NaCl, 5 mM sodium succinate, and 0.1 mM Pi(K), pH 7 at room temperature.

In one set of experiments, the liver mitochondria were pre-incubated with either 100 nM PNU282987 or 10 nM conotoxin PeIA for 7 min at RT and then either treated or not with 0.9 μM $Ca(Cl)_2$ or 1 μM wortmannin for additional 5-7 min and pelleted by centrifugation (10 min, 7,000x g at 4°C).

In the other set of experiments, the liver mitochondria were pre-incubated with various doses of PNU282987, choline, phosphocholine (PC), L-α-glycerophosphocholine (G-PC) or 1-palmitoyl-sn-glycero-3-phosphocholine (P-GPC) for 5 min and then either treated or not with 0.9 μM $Ca(Cl)_2$ or 1 μM wortmannin for additional 5-7 min and pelleted by centrifugation.

The brain mitochondria were pre-incubated with 100 nM PNU282987 for 5 min and then either treated or not with 0.9μM $Ca(Cl)_2$ for additional 5-7 min and pelleted by centrifugation.

The mitochondria supernatants were collected and tested for the presence of Cyto *c* by sandwich assay as described (see below). The pellets were frozen at -20°C, thawed and treated with lysing buffer (0.01 M Tris-HCl, pH 8.0; 0.14 NaCl; 0.025% NaN_3; 1% Tween-20 and protease inhibitors cocktail) for 2 h on ice upon intensive stirring. The resulting lysates were cleared by centrifugation (20 min at 20,000 g). The protein concentration was established with the BCA Protein Assay kit.

Sandwich ELISA. To evaluate the level of the nAChR-Bax, nAChR-Bcl-2 or nAChR-VDAC1 complexes, the immunoplates (Nunc, MaxiSorp) were coated with either α7(179-190)-specific or α9(11-23)-specific antibody (10 μg/ml), blocked with 1% BSA, and the detergent lysates of the mitochondria were applied into the wells (1 μg of protein per 0.05 ml per well) for 2 h at 37°C. The plates were washed with water and the second biotinylated Bax- (5 μg/ml), Bcl-2- (5 μg/ml) or VDAC1-specific (3 μg/ml) antibodies were applied for additional 2 h at 37°C (the antibody concentrations were used according to manufacturer instructions).

To measure Cyto *c* released from mitochondria, the immunoplates were coated with rabbit polyclonal Cyto *c*-specific antibody (10 μg/ml) obtained and characterized by us previously. After blockade with 1% BSA, the mitochondria supernatants were applied for 2 h at 37°C. The plates were washed with water and the biotinylated Cyto *c*-specific antibody was applied for additional 2 h at 37°C. The use of similar coating and revealing Cyto *c*-specific antibody in Sandwich assay was justified due to its polyclonal nature and the presence of multiple epitopes on the Cyto *c* molecule.

The bound biotinylated antibodies were revealed with Neutravidin-peroxidase conjugate and

o-phenylendiamine-containing substrate solution. The optical density was read at 490 nm using StatFax 2000 ELISA Reader (Awareness Technologies, USA).

Statistical analysis. ELISA experiments have been performed in triplicates and mean values were used for statistical analysis using one-way ANOVA test and Origin 9.0 software. The data are presented as mean ± SD.

Results

The effects of choline and its derivatives on Cyto c release from mouse liver mitochondria. We compared the effects of choline and three its derivatives (PC, G-PC and P-GPC) on Cyto *c* release from isolated mouse liver mitochondria either by themselves or in the presence of 0.9 μM Ca^{2+} or 1 μM wortmannin. As shown in Fig. 1, choline and PC did not affect mitochondria by themselves, while GPC and, especially, P-GPC stimulated significant Cyto *c* release in the absence of any other apoptogenic stimuli.

When tested in the presence of either 0.9 μM Ca^{2+} or 1 μM wortmannin (Fig. 2), choline behaved very similarly to PNU282987: it attenuated Ca^{2+}-stimulated Cyto *c* release, and had a much weaker effect on wortmannin-stimulated Cyto *c* release, but was 10 times less efficient compared to PNU282987 (IC_{50} for PNU282987 was 5 nM; IC_{50} for choline – 50 nM) that is in accord with its published characteristics [12]. In contrast, PC taken within the same dose range (up to 1.0 μM) attenuated wortmannin-induced Cyto *c* release only. G-PC and P-GPC were tested in a smaller dose range (up to 0.1 μM) when their own Cyto *c*-stimulating effect was minimal. As shown in Fig. 2D-E, G-PC inhibited both Ca^{2+}- and wortmannin-stimulated Cyto *c* release while P-GPC was mainly efficient against Ca^{2+}. Therefore, in spite of their own apoptogenic activity, G-PC and P-GPC could be protective when present in low doses. Further experiments were performed with choline and PC, which did not stimulate Cyto *c* release by themselves.

PC was shown to activate nAChRs containing α7, α9 and α10 subunits in monocytes [6]. The presence of both α7 and α9 nAChRs in mouse liver mitochondria has been documented [13]. To understand which nAChR subtypes are triggered by choline or PC in mitochondria we compared the effects of synthetic α7 nAChR agonist PNU282987 and α9-specific conotoxin PeIA on Cyto *c* release

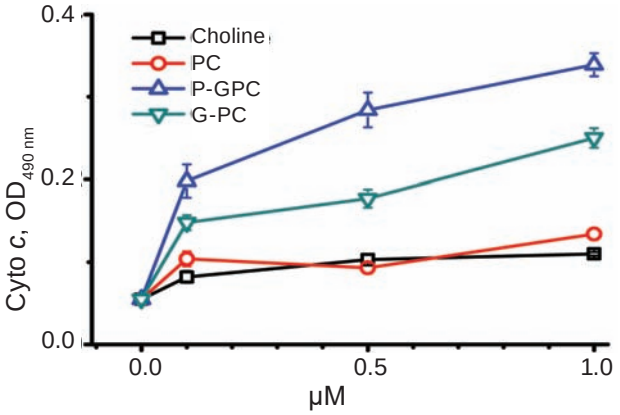

Fig. 1. The effects of choline, PC, GPC or P-GPC on Cyto c release from isolated mouse liver mitochondria in the absence of any apoptogenic agent. Each point corresponds to mean ± SD, n = 3

from mitochondria stimulated by either Ca^{2+} or wortmannin. Ca^{2+} affects mitochondrial Ca-calmodulin-dependent kinase CaKMII, while wortmannin inhibits PI3-kinase; both effects were shown to be sufficient to stimulate Cyto *c* release from mitochondria [5]. As shown in Fig. 3, PNU282987 attenuated Ca^{2+}-stimulated and, less, wortmannin-stimulated Cyto *c* release, while conotoxin PeAI was efficient against wortmannin only. This data indicated that stimulating α7 nAChRs influences mainly CaKMII-dependent pathway, while ligating/inhibiting α9 nAChRs is not efficient against Ca^{2+} but affects PI_3-kinase-dependent pathway in mitochondria. Correspondingly, regarding the data presented in Fig. 2, choline triggered mainly α7 nAChRs, while PC was mostly specific to α9 nAChRs.

The effects of choline and PC on the interaction of α7 and α9 nAChRs with Bcl2 family proteins and VDAC1. Previously we reported that mitochondrial α7 nAChRs of astrocytoma U373 cells interact with voltage-dependent anion channel (VDAC1) and pro-apoptotic protein Bax; PNU282987 prevented α7-Bax and favored α7-VDAC1 interaction [14]. Here we asked if it is the case for mouse mitochondria, if anti-apoptotic Bcl-2 protein and α9 nAChRs are involved and if choline and PC can modulate such interactions.

The experiment performed with mitochondria isolated from mouse brain demonstrated that α7 nAChRs were detected together with VDAC1, Bax, as well as Bcl-2. Similarly to mitochondria of U373 cells, Ca^{2+} stimulated interaction of α7 nAChRs with Bax and Bcl-2 but prevented its interaction with

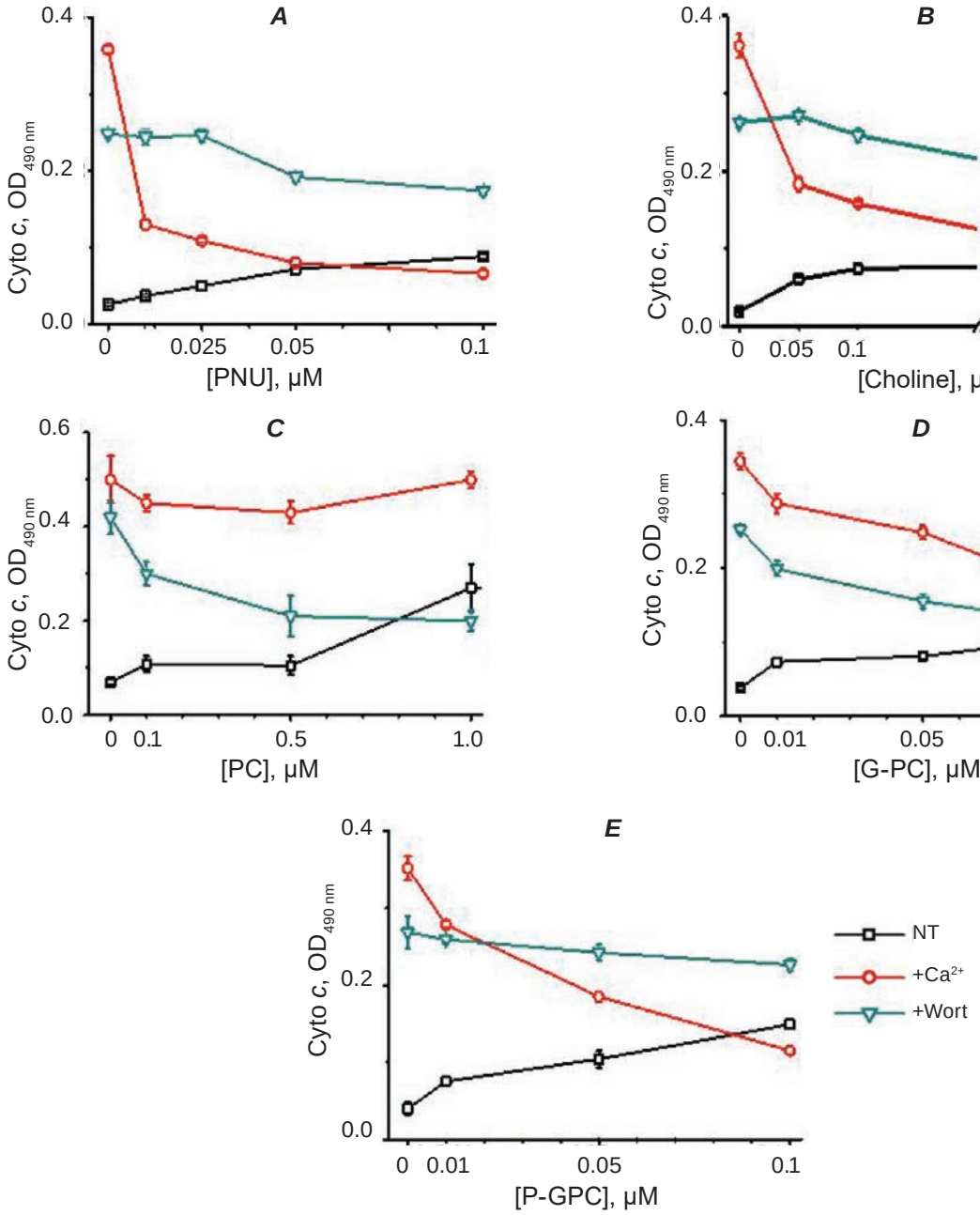

*Fig 2. The effects of PNU282987 (**A**), choline (**B**), PC (**C**), G-PC (**D**) or P-GPC (**E**) on Cyto c release from isolated mouse liver mitochondria in the presence or absence of 0.9 μM Ca^{2+} or 1 μM wortmannin (Wort). Each point corresponds to mean ± SD, n = 3. The dose ranges of each derivative have been selected not to exceed the dose, which stimulated Cyto c release by itself. NT – non-treated mitochondria*

VDAC1, while PNU282987 inhibited formation of α7-Bax and α7-Bcl-2 complexes and supported α7-VDAC1 interaction (Fig. 4).

In contrast, incubation of mouse liver mitochondria with PNU282987 (even without Ca^{2+}) decreased the level of all α7-containing complexes. Choline and PC exerted similar effect but less efficiently (Fig. 5, *A*). The initial level of potential complexes detected with α9-specific antibody was quite low and it was not affected, or weakly affected with PNU282987. In contrast, choline and PC significantly increased the level of α9-containing complexes (Fig. 5, *B*).

Ca^{2+} stimulated additional formation of α7 nAChRs complexes with Bax, Bcl-2 and VDAC1 and α9 nAChR complexes with Bcl-2. Either choline

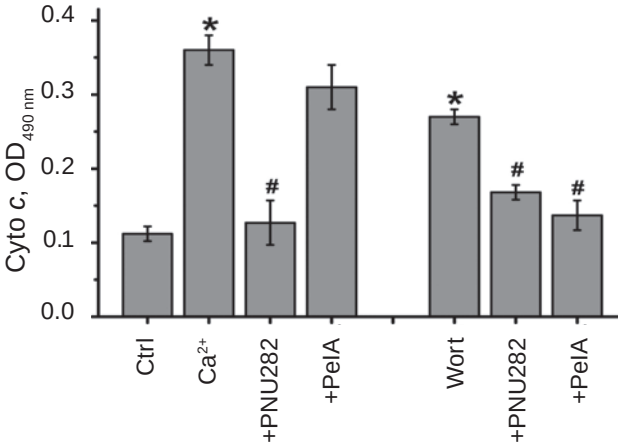

*Fig. 3. Cyto c found by sandwich ELISA (Methods) in supernatants of isolated mouse liver mitochondria treated with either 0.9 μM Ca^{2+} or 1 μM wortmannin (Wort) in the presence or absence of either 100 nM PNU282987 (PNU) or 10 nM conotoxin PeIA. Each column corresponds to mean ± SD, n = 3. *P < 0.05 compared to non-treated mitochondria (Ctrl); #P < 0.05 compared to Ca^{2+} or Wort*

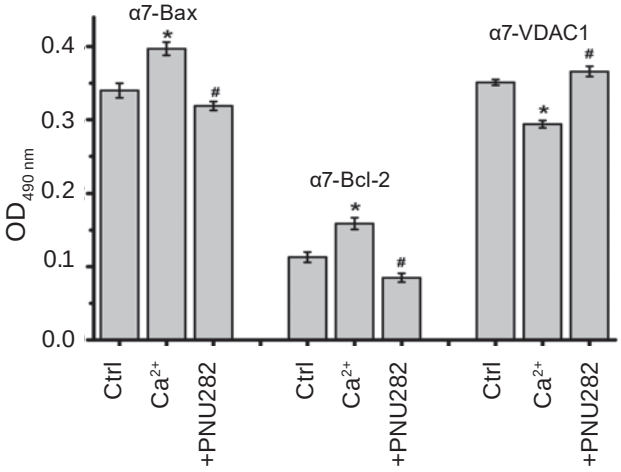

*Fig. 4. The level of α7 nAChR complexes with Bax, Bcl-2 or VDAC1 in mouse brain mitochondria: untreated (Ctrl), treated with 0.9 μM Ca^{2+} or with 0.9 μM Ca^{2+} and PNU282987 (+PNU282). Each column corresponds to mean ± SD, n = 3. *P < 0.05 compared to Ctrl; #P < 0.05 compared to Ca^{2+}-treated mitochondria*

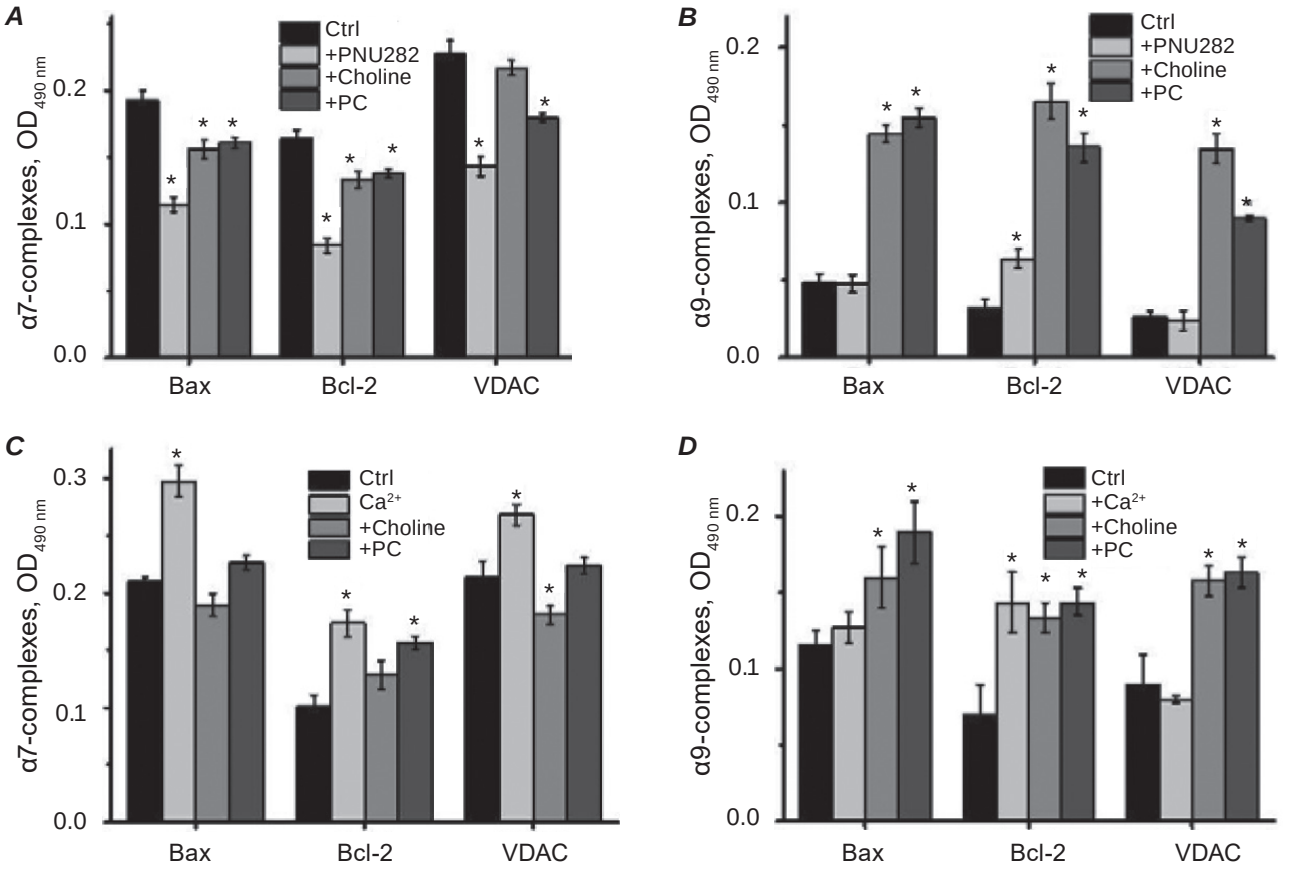

*Fig. 5. The level of α7 (**A**, **C**) and α9 (**B**, **D**) complexes with Bax, Bcl-2 or VDAC1 in isolated mouse liver mitochondria treated with PNU282987, choline or PC in the absence (**A**-**B**) or presence of 0.9 μM Ca^{2+} (**C**-**D**). Each column corresponds to mean ± SD, n = 3; *P < 0.05 compared to non-treated mitochondria (Ctrl)*

or PC prevented α7-containing complexes formation, choline being more potent, favored α9-Bax and α9-VDAC1 interaction, but did not further affect α9-Bcl-2 interaction. (Fig. 5, *C-D*).

These data indicated that Bax, Bcl-2 and VDAC1 interact with both α7 and α9 nAChRs in mouse liver mitochondria and such interaction can be modulated by choline and PC.

Discussion

Previously we reported that choline supported the viability of cultured cells of lymphoid origin upon mitochondria-stimulated apoptosis [15]. The data presented here demonstrate that choline and its derivatives (PC, G-PC and P-GPC) are able to interact with mitochondrial nAChRs and influence Cyto *c* release from mouse liver mitochondria. Choline, similarly to PNU282987, inhibited Cyto *c* release stimulated by Ca^{2+} and, therefore, affected mainly CaKMII-dependent pathway, while PC was efficient against wortmannin and, therefore, influenced PI_3-kinase.

Both CaKMII and PI_3-kinase are found in the cytoplasm of neurons and mediate signaling of α7 nAChRs expressed on the plasma membrane to regulate cell proliferation or survival [16-17]. The role of mitochondrial kinases is much less studied, although their connection to Cyto *c* release in response to apoptogenic stimuli has been demonstrated [18]. Previously we showed that inhibition of CaKMII diminished the amount of Cyto *c* released from mitochondria upon Ca^{2+} addition; therefore, active CaKMII was required for mitochondrial apoptosis-related pore opening. In contrast, inhibition of PI_3-kinase by wortmannin induced Cyto *c* release from mitochondria by itself and this effect was attenuated by α7 nAChR agonist PNU282987 [5].

In the cytoplasm, PI_3-kinase interacts with protein kinase B (Akt) to enable its phosphorylation by mTORC2 and phosphoinositide-dependent kinase 1 (PDPK1). Expression of active Akt targeted to mitochondria was found to be sufficient to significantly reduce the release of Cyto *c* from mitochondria [19]. We showed that the level of mitochondrial Akt phosphorylation was significantly decreased by either Ca^{2+} or wortmannin, while PNU282987 restored it [5]. This data indicated that the signal from α7 nAChR restored PI_3-kinase activity and, subsequently, Akt phosphorylation.

PC is formed upon phosphorylation of choline by α-choline kinase. It was shown that inhibiting PI_3-kinase down-regulates α-choline kinase and decreases PC levels [20]. Therefore, addition of PC could prevent PI_3-kinase inhibition by wortmannin and in such a way attenuate Cyto *c* release from mitochondria independently of nAChRs. However, the PC influence on the nAChR complexes with Bax, Bcl-2 and VDAC1 suggests that its anti-apoptotic effect is mediated by nAChRs. Application of α7- or α9-selective ligands (PNU282987 or α-conotoxin PeIA) allowed us to suggest that choline affected α7 nAChRs, while PC was specific for α9 nAChRs. However, either choline or PC prevented α7 nAChR interaction with Bax, Bcl-2 and VDAC1. This agrees with the data of Zakrzewicz et al. [6], where PC was classified as an agonist of both α7 and α9-containing nAChRs.

G-PC and P-GPC were shown to induce Cyto *c* release by themselves; however, when taken in lower doses, they could protect mitochondria from the effects of either Ca^{2+} or wortmannin. Cyto *c* release from mitochondria is the critical step in initiation of mitochondria apoptosis pathway. Experiment performed in isolated mitochondria is a model system; nevertheless, the data obtained allow suggesting that G-PC and P-GPC can be protective against mitochondrial apoptosis. However, when accumulated in high doses, they become pro-apoptotic. G-PC is a product of phosphatidylcholine breakdown. It can be formed along with cell membranes damage thus providing a signal for the cell to enter the apoptosis pathway. These data indicate that mitochondrial nAChR is a “double-edged sword”, which can both favor and attenuate apoptosis.

The involvement of choline and its derivatives in regulating apoptosis is supported by several observations concerning tumor cells, which are strongly viable and less sensitive to apoptosis compared to normal cells. Activated choline metabolism is a hallmark of carcinogenesis and tumor progression, which leads to elevated levels of PC and G-PC in all types of cancer [21]. Choline was shown to increase glioblastoma cell proliferation by binding and activating α7- and α9-containing nicotinic receptors [22]. Choline metabolites derived from its synthesis and catabolism were shown to contribute to both proliferative growth and apoptosis [23]. High PC levels are characteristic for high-grade gliomas, while G-PC is found in low-grade gliomas and normal cells [24]. This is in accord with our data demonstrating pro-apoptotic activity of high G-PC doses, which should help eliminating tumor cells. Most

cancer cells and tissues highly express α9-containing receptors involved in stimulating proliferation, inhibition of apoptosis, and metastasis [25]. Respectively, α9-specific PC can be an instrument supporting cancer cells viability. PC binding to α9α10 nAChRs was shown not to evoke transmembrane ion currents and, therefore, induced only metabotropic signaling [26]. Similarly, we observed Cyto *c*-attenuating effect of α-conotoxin PeIA, which does not induce ion current but can stimulate conformational changes in α9 nAChR subunit necessary for metabotropic signaling.

The different effects of choline derivatives found in this work are probably due to their molecular structures. Choline possesses positively charged quaternary nitrogen underlying its binding to α7 nAChR [27]. Modification by phosphoric acid radical in PC adds a negative charge that can weaken interaction with α7, but may be favorable for interaction with α9. Glycerol radical in G-PC and P-GPC partly neutralizes phosphoric acid influence, while palmitoyl radical in P-GPC can provide certain conformational constraints for the interaction with the nAChR ligand-binding site. As a result, G-PC and P-GPC demonstrate pro-apoptotic effect, possibly by preventing the correct α7-α9 interplay necessary for attenuating apoptosis.

Previously we reported that mitochondrial α7 nAChRs can interact with pro-apoptotic protein Bax and VDAC1. Stimulating apoptosis with H_2O_2 in cultured astrocytoma U373 cells favored α7-Bax interaction and disrupted α7-VDAC1 interaction, while α7-specific agonist (PNU282987) or positive allosteric modulator (PNU120596) prevented α7-Bax interaction and supported α7-VDAC1 interaction [14]. Here we show that α7 nAChRs can also interact with anti-apoptotic Bcl-2 protein. Similarly to human astrocytoma cells, incubation of the mouse brain mitochondria with Ca^{2+} favored both α7-Bax and α7-Bcl-2 interaction and disrupted α7-VDAC1 interaction; PNU282987 prevented α7-Bax and α7-Bcl-2 interaction and maintained α7-VDAC1. In contrast, in the liver mitochondria, Ca^{2+} stimulated interaction of α7 nAChRs with all three proteins: Bax, Bcl-2 and VDAC1, while choline and its derivatives prevented such interactions. This means that α7 nAChRs interact with both pro- and anti-apoptotic Bcl-2 family proteins (Bax and Bcl-2) affecting their involvement in mitochondria apoptosis channel formation; while the involvement of VDAC1 is tissue-specific. Possibly, this is due to different expression patterns of nAChR subtypes in the brain and liver. The involvement of α9 nAChRs adds even more complexity to such interactions in liver mitochondria. The data presented indicate that disruption of α7-containing complexes of Bax, Bcl-2 and VDAC-1 by choline or PC, but not PNU282987, is accompanied by α9-containing complexes formation. Since choline and PC are natural substances (in contrast to synthetic agonist PNU282987), it is reasonable to suggest that this is also the case in physiological situation and is, possibly, due to the ability of choline and PC to interact with both α7 and α9 nAChRs [22].

The idea of collaboration between α7 and α9 nAChRs has already been discussed in relation to cancer cells response to nicotine and choline: it was suggested that sufficient target cell activation can be ensured by the presence of both α7 and α9 nAChR subunits, either in neighboring receptors or even in heteropentameric α7α9-containing nAChR [25]. Functional interaction between α7-, α9-, and α10-containing nAChRs was suggested to explain the response of the rat mast/basophil cell line to nanomolar concentrations of nicotine [28]. Our data suggests that both mitochondrial α7 and α9 nAChRs are involved in the interplay of pro- and anti-apoptotic trends regulating cell survival: interaction of Bcl-2 family proteins with α7 nAChR facilitates mitochondria apoptosis channel opening, whereas choline or PC displace α7 subunits and transfer Bcl-2 proteins to α9 subunit (within either the neighbor receptor or the same heteromeric α7α9 receptor) that prevents mitochondria channel opening/formation.

The α7- and α9-specific antibodies used in this study were obtained in our laboratory and were used in multiple previous experiments; their specificity has been confirmed by using tissue preparations from α7-/- and α9-/- mice [13, 29]. Nevertheless, interactions of α7 and α9 nAChRs with Bax, Bcl-2 and VDAC1 observed in sandwich-ELISA should obviously be confirmed by alternative approaches, eg. mitochondrial nAChRs interactome analysis, that will be a subject of our future studies.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. This research was performed using the budget funding of Palladin Institute of Biochemistry.

ПОХІДНІ ХОЛІНУ ЯК ПРИРОДНІ ЛІГАНДИ МІТОХОНДРІАЛЬНИХ НІКОТИНОВИХ АЦЕТИЛХОЛІНОВИХ РЕЦЕПТОРІВ

О. Ю. Лихмус, М. Р. Ізмайлов, М. В. Скок✉

Відділ молекулярної імунології, Інститут біохімії ім. О. В. Палладіна НАН України, Київ;
✉e-mail: skok@biochem.kiev.ua

Нікотинові ацетилхолінові рецептори (нАХР) регулюють мітохондрійний шлях апоптозу, однак їх внутрішньоклітинні ліганди невідомі. В цій статті ми показуємо, що холін та його похідні: фосфохолін (PC), L-α-гліцерофосфохолін (G-PC) і 1-пальмітоїл-sn-гліцеро-3-фосфохолін (P-GPC), – дозозалежно впливають на вивільнення цитохрому *c* із мітохондрій печінки миші. Холін пригнічував вивільнення цитохрому *c* стимульоване Ca^{2+}, а PC – вивільнення цитохрому *c* стимульоване вортманіном. Малі дози G-PC і P-GPC (до 0,1 мкМ) захищали як проти Ca^{2+}, так і проти вортманіну, а більші дози (до 1 мкМ) самі стимулювали вивільнення цитохрому *c*. Холін і PC запобігали взаємодії VDAC1, Bax і Bcl-2 з мітохондрійними α7 нАХР і сприяли їх взаємодії з α9 субодиницями нАХР. Зроблено висновок, що метаболіти холіну можуть регулювати апоптоз, впливаючи на мітохондрійні нАХР.

Ключові слова: нікотиновий ацетилхоліновий рецептор, мітохондрії, апоптоз, холін, фосфохолін.

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