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Involvement of SIRT1 and SIRT3 in Effects of Hypoxic Hypobaric Preconditioning on Hippocampal Damage Induced by Chronic Cerebral Hypoperfusion in C57/6j and a7nAchRs(-/-) Mice

Participación de SIRT1 y SIRT3 en los Efectos del Precondicionamiento Hipobárico Hipóxico sobre el Daño del Hipocampo Inducido por Hipoperfusión Cerebral Crónica en Ratones C57/6j y a7nAchRs (-/-)

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SUMMARY: Hypoxic preconditioning is known to induce neuroprotection, but its effects and pathways in chronic brain pathology still unknown. The aim was to establish an involvement of a7 subunit of nicotinic acetylcholine receptors (a7nAchRs), and sirtuins of 1 (SIRT1) and 3 (SIRT3) types in the effects of hypoxic hypobaric preconditioning on brain damage in mice with chronic cerebral hypoperfusion caused by the left common carotid artery occlusion. The male C57/6j (C57, wild type) and a7nAchRs(-/-) mice were divided to six experimental groups (10 mice per group): sham-operated C57, C57 with chronic cerebral hypoperfusion, C57 with hypoxic hypobaric preconditioning and chronic cerebral hypoperfusion, sham-operated a7nAchRs(-/-) mice, a7nAchRs(-/-) with chronic cerebral hypoperfusion, a7nAchRs(-/-) with hypoxic hypobaric preconditioning and chronic cerebral hypoperfusion. For preconditioning, mice were exposed to hypoxia by "lifting" in barochamber to simulated altitude of 5600 m a.s.l. for 1 h/day on 3 consecutive days before surgical manipulation. Expressions of SIRT1, SIRT3 in brain tissue, and histopathological changes of the hippocampi were examined. It was shown that 8-week chronic hypoperfusion of the brain, caused by unilateral occlusion of the common carotid artery, was accompanied by injury to the neurons of the hippocampi of both hemispheres, which was more pronounced on the side of the occlusion. This damage, as well as the mechanisms of neuroprotection induced by hypoxic preconditioning, were maintained for at least 8 weeks by mechanisms mediated through a7nAChRs. Deficite of a7nAChRs was accompanied with reduction of neuronal damage caused CCH in 8 weeks, as well as preconditioning effects, and lead to compensatory activation of regulatory and protective mechanisms mediated by SIRT1, in normal conditions and in CCH. In wild-type (C57) mice, protective mechanisms in CCH were realized to a greater extent by increased expression of SIRT3 in both hemispheres of the brain.

KEY WORDS: Hypoxic hypobaric preconditioning; Unilateral common carotid artery occlusion, Chronic cerebral hypoperfusion; a7nAchRs(-/-) mice; SIRT1; SIRT3

INTRODUCTION

The major pathogenic factor associated with the development of cerebrovascular disease is chronic cerebral hypoperfusion. Inadequate blood supply to the brain manifests itself in the form of its hypoxia and metabolic disorders, which have various manifestations ranging from cognitive deficits to the development of acute ischemic stroke (He *et al*., 2023). Intermittent hypoxic training or hypoxic preconditioning aims to activate protective mechanisms by improving energy metabolism and antioxidant effects (Portnychenko *et al*., 2019; Ehrenreich *et al*., 2023), but effects and pathways of different modes of hypoxia influence in various diseases, including cerebrovascular, are still being investigated.

Silencing regulatory proteins 1 (SIRT1) and 3 (SIRT3) types are members of silencing information regulatory 2 proteins (Sirtuins, SIR2) that carry out epigenetic control of many biological processes from cell

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metabolism to response on cellular stress in pathological conditions (Li *et al*., 2018). SIRT1 is mainly expressed in the nucleus, and a small amount in the mitochondria. SIRT3 is primarily localized in mitochondrial matrix (Michishita *et al*., 2005). This feature of sirtuins location suggests their involvement in the mechanisms of diseases of the central nervous system (Jiao & Gong, 2020; Park *et al*., 2020). Despite the intensive study of sirtuins in recent years, their role in the mechanisms of the effect of hypoxic hypobaric preconditioning on brain damage in chronic cerebral hypoperfusion, which is caused by occlusion of the common carotid artery, is still unclear and needs to be clarified.

a7 subunits of nicotinic acetylcholine receptors (a7nAchRs), expressed in neurons and glial cells, are known as negative regulators of neuroinflammation and oxidative stress (Patel *et al*., 2017). Outside of the central nervous system (CNS), they have a role in the cholinergic antiinflammatory pathway (Kelso *et al*., 2012). The involvement of a7nAChRs in CCH pathways has been investigated in only a few works, in particular, a decrease in the expression of these receptors in hippocampus and thalamus after 12 months of CCH has been shown (Shang *et al*., 2016; Divanbeigi *et al*., 2020). Therefore, a7nAChRs may be interesting as a therapeutic target in CCH, and its role needs to be elucidated.

The aim of this study was to investigate the effect of hypoxic hypobaric preconditioning on brain damage, particularly in the hippocampus, in chronic cerebral hypoperfusion induced by unilateral common carotid artery occlusion in C57/6j (wild type) and a7nAchRs(-/-) mice. The next goal was to evaluate the possible role of SIRT1 and SIRT3 in the mechanisms of hypoxic hypobaric preconditioning under these conditions.

MATERIAL AND METHOD

Experimental animals and experimental design. The animal studies were performed in accordance with the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines 2.0 (Percie du Sert *et al*., 2020) and were approved by The Local Ethics Committee at Bogomoletz Institute of Physiology (Kyiv, Ukraine), as the investigations conducted accoding to requirements as European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the current legislations of Ukraine on protection of experimental animals (N3447-IV, 21.02.2006).

Experiments were performed on 30 male C57/6j (C57) mice and 30 male a7nAchRs(-/-) mice 6 weeks old (body weight 15-18 g) obtained from Palladin Institute of Biochemistry NAS of Ukraine. The animals were randomized into the following groups: 1) sham operated C57 mice (control C57, n=10); 2) C57 mice with chronic cerebral hypoperfusion (CCH) (n=10); 3) C57 mice with hypoxic hypobaric preconditioning+chronic cerebral hypoperfusion (HHP+CCH) (n=10); 4) sham operated a7nAchRs(-/-) mice (control a7nAchRs(-/-)); 5) a7nAchRs(-/-) mice with chronic cerebral hypoperfusion (n=10); 6) a7nAchRs(-/-) mice with hypoxic hypobaric preconditioning+ chronic cerebral hypoperfusion (n=10). The animals were kept under standard vivarium conditions on a 12-h light/dark cycle for a period of 8 weeks of the experiment with free access to standard mouse chow and water. At the end of the experiment, the animals were anesthetized with ketamine (60 mg/kg, i.p.), and tissue samples were removed for further examination.

Surgical model of chronic cerebral hypoperfusion. Mice underwent a left common carotid artery occlusion surgery, as previously described, to elicit chronic cerebral hypoperfusion (Zuloaga *et al*., 2016). Briefly, under ketamine (60 mg/kg, i.p.) anesthesia, the left common carotid artery was ligated with two 6-0 silk sutures. The sham surgery consisted of exposing the carotid artery without ligation.

Hypoxic hypobaric preconditioning (HHP). Mice were exposed to HHP by "lifting" in barochamber to simulated altitude of 5600 m a.s.l. with atmospheric pressure of 380 Torr (50.66 hPa) for 1 h/day (10:00 to 11:00) in 3 consecutive days. Mice in the control groups were placed in the same conditions but with sea level atmospheric pressure. Twenty-four hours after the last session of HHP, the animals were enrolled in the experiment.

Tissue sample preparation and histological examination. In anesthetized mice, the brain was quickly removed after decapitation and washed three times with a cooled PBS solution (NaCl 8.0 g/L, KCl 0,2 g/L, $Na_2HPO_4 \cdot 12H_2O$ 2,8 g/L, KH_2PO_4 0,2 g/L, pH 7,4, 4 °C). Then brain tissues were fixed with 10 % neutral-buffered formalin solution for 24 h. The paraffin-embedded brain blocks were sliced at a thickness of 2 μm to the brain sections including the hippocampus. The obtained sections were stained with hematoxylin and eosin solutions (H&E) for histopathological examination using XSP-139-TP microscope (×400) (China, 2018) equipped with digital camera Levenhuk M1400 PLUS (USA, 2020). Three images were used in each area of interest and separated into ipsilateral and contralateral hemispheres. The viable and dead cells were counted using ImageJ software (USA, 2022).

Western blot analysis. Protein expression was assayed in frozen brain hemisphere samples by immunoblotting as previously described (Portnychenko *et al*., 2023). Briefly,

tissue lysates (100 mcg of protein) were subjected to SDS-PAGE and transferred onto PVDF membrane (Sigma). Blots were blocked with Pierce™ Clear Milk Blocking Buffer (37587, ThermoScientific), and incubated overnight with primary antibodies (anti-SIRT1, PA5-87383, Invitrogen, 1:1000; anti-SIRT3, PA5-13220, Invitrogen, 1:1000; and anti-GAPDH, G9545, Sigma, 1:5000). After the washing, blots were treated with peroxidase-conjugated anti-rabbit IgG (A0545, Sigma; 1:2500) and stained using 1-Step™ TMB-Blotting Substrate Solution (34018, Thermo Scientific). Densitometric values were evaluated with ImageJ (NIH Image, USA, 2022) and normalized to expression of house-keeping GAPDH protein.

Statistical analysis. All data are presented as x ± SE (means ± standard error). Data were analyzed using GraphPad Prism version 8.1.0.325 for Windows (GraphPad Software, USA, No GPS-1461670-TEQH-6AC22). Shapiro-Wilk test was used to evaluate the distribution normality of data. To detect the homogeneity of variance among groups Levine's test was used. Two-way ANOVA with Tukey's post hoc test was used to determine the significant differences between the groups. $P < 0.05$ was considered as statistically significant difference.

RESULTS

Effect of chronic cerebral hypoperfusion on neuronal damage. In C57 mice, chronic cerebral hypoperfusion caused changes in the cell viability of neurons in the CA1 area of the hippocampi, the number of damaged neurons were increased by almost 2 times (Fig. 1). In a7nAchR (-/-) mice, there was no significant difference between groups with or without chronic cerebral hypoperfusion (Fig. 2).

Effect of hypoxic hypobaric preconditioning on neuronal damage in chronic cerebral hypoperfusion. In the C57 mice, a positive effect of preconditioning was observed, which was more pronounced in the left hippocampus. The number of damaged neurons in the left hippocampus decreased by 11.6 % ($P<0.05$) in HHP+CCH compared to CCH. In a7nAchR (-/-) mice, there was no significant difference between groups, and scores were also not different from those of C57 mice after preconditioning (Fig. 2).

Effect of chronic cerebral hypoperfusion and hypoxic hypobaric preconditioning on SIRT1 and SIRT3 expression. In C57 mice, there was no significant difference in SIRT1 protein expression between groups with or without

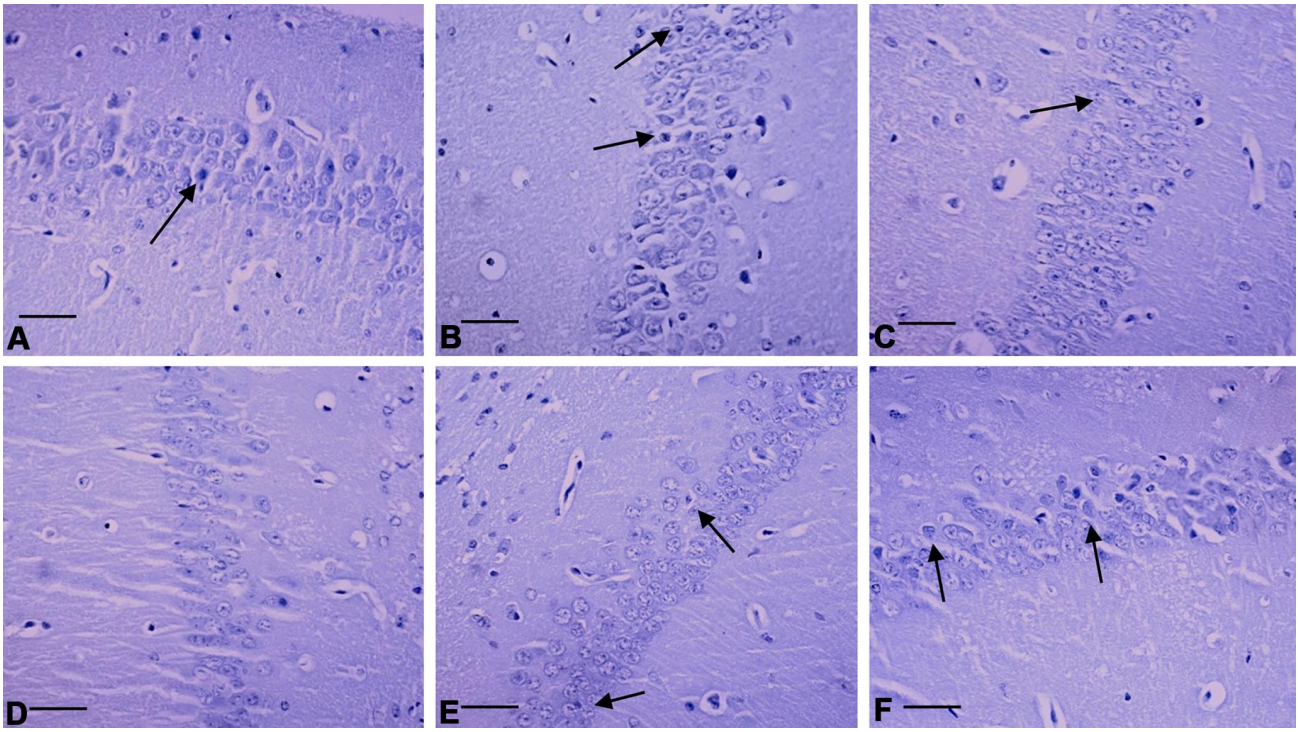

Fig. 1. Photomicrographs of hippocampus at bregma with hematoxylin-eosin staining in 8 weeks after left common carotid artery occlusion in mice; a-f - C1 area of the hippocampi in control C57 mice (a), in C57 mice with chronic cerebral hypoperfusion (b), in C57 mice with hypoxic hypobaric preconditioning and chronic cerebral hypoperfusion (c), in control a7nAchRs (-/-) mice (d), in a7nAchRs (-/-) mice with chronic cerebral hypoperfusion (e), in a7nAchRs (-/-) mice with hypoxic hypobaric preconditioning and chronic cerebral hypoperfusion (f); 10 mice per group; scale bar indicated the distance of 100 μm; arrows indicated injured neurons of the C1 area of the hippocampus.

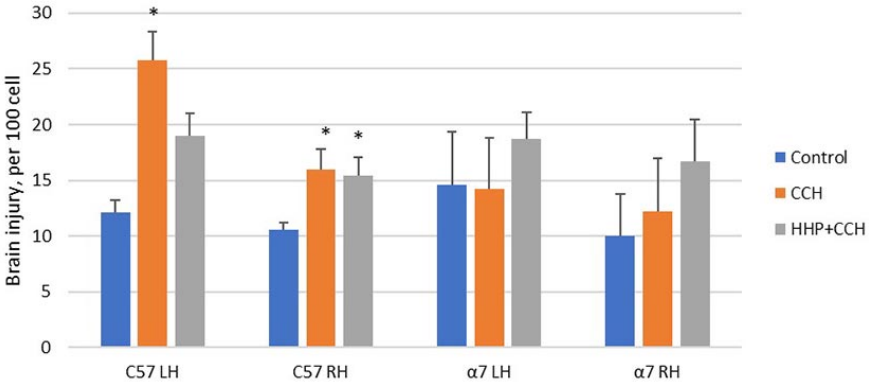

Fig. 2. Neuronal damage in the C1 area of the hippocampi of C57 and a7nAchR (-/-) mice after 8 weeks chronic cerebral hypoperfusion with or without hypoxic hypobaric preconditioning; each bar represents the number of injured neurons per 100 cells in the C1 area of the hippocampi of 10 mice per experimental group; CCH - chronic cerebral hypoperfusion; HHP - hypoxic hypobaric preconditioning; x ± SE. *P<0.05 compared to control; #P<0.05 compared to CCH.

CCH or HHP, as well as between both brain hemispheres (Fig. 3). In a7nAchRs(-/-) mice, there was a trend toward an increase in SIRT1 protein expression compared to C57 mice, without significant difference between the hemispheres (Fig.3). However, in contrast to C57, in a7nAchRs(-/-) mice, the protein expression level was significantly reduced in the RH after CCH, although it was maintained in the affected LH. After HHP+CCH, a significant decrease was observed in both brain hemispheres, which was more pronounced in the LH (Fig. 3).

The expression of SIRT3 protein had not significant interhemispheric difference in C57 mice, but its level was significantly increased in the affected LH, and tended to increase in the RH in 8 weeks after CCH (Fig. 3). In contrast, HHP+CCH resulted in a decrease in protein expression, significantly in LH (Fig. 3). In a7nAchRs(-/-) mice, SIRT3 expression levels were higher in the RH compared to C57 mice, and there was a difference between the hemispheres. CCH had no effect on protein expression in LH, in contrast to RH, where there was a significant decrease in protein expression. After HHP+CCH, a significant decrease was observed in both brain hemispheres, which was more pronounced in the LH (Fig. 3).

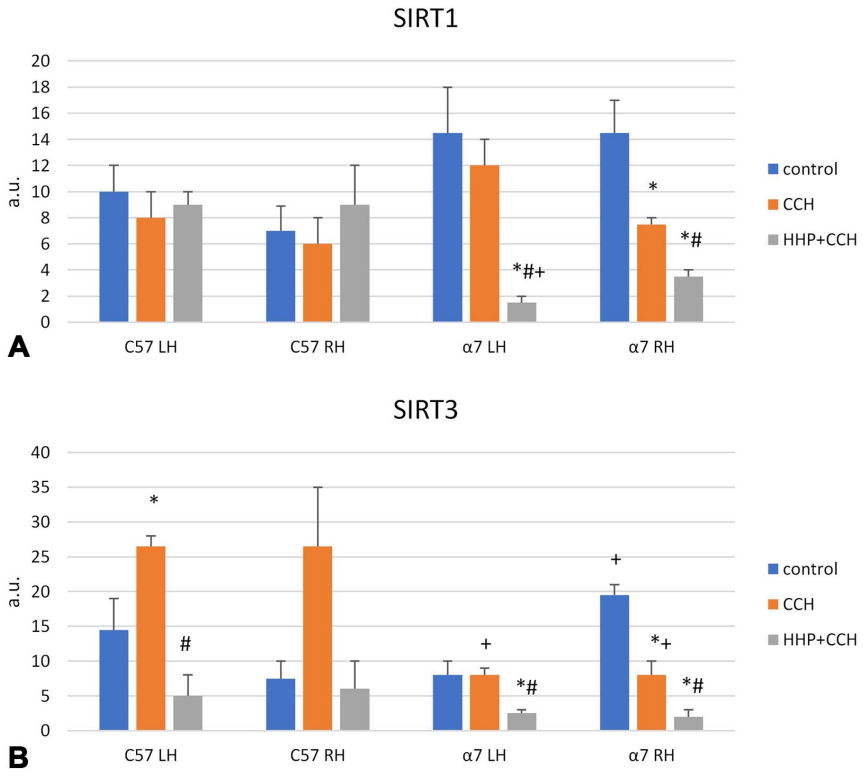

Fig. 3. Expressions of sirtuins in the brain hemispheres of a7nAchRs(-/-) mice after 8 weeks chronic cerebral hypoperfusion with or without hypoxic hypobaric preconditioning; each bar represents the expressions of SIRTs protein in the temporal lobe of the brain, expressed in arbitrary units, normalized to GAPDH in brain tissue; 10 mice per experimental group; SIRT1 - sirtuin of type 1 (A); SIRT3 - sirtuin of type 3 (B); RH - right hemisphere; LH - left hemisphere CCH - chronic cerebral hypoperfusion; HHP - hypoxic hypobaric preconditioning; x ± SE. *P<0.05 compared to control; #P<0.05 compared to CCH; +P<0.05 compared to C57.

DISCUSSION

In the present study, we demonstrated that HHP may be neuroprotective in mice after experimental chronic cerebral hypoperfusion, the mechanisms of which is mediated, at least in part, via involvement of SIRT1 and SIRT3 proteins, and a7nAchRs. In a7nAchR (-/-) mice, the neurons were protected from the harmful effects of CCH at the time of the study, or the damaged neurons were already eliminated, and the structure of the C1 zone was restored. Thus, the protective effect of preconditioning should not have been observed at the time of the study. However, in C57 mice, the processes of cell damage, as well as neuroprotection caused by preconditioning, continued 8 weeks after the onset of CCH. Hence, it can be asserted that the presence of a7nAchR supports the damage processes and, as a result, the activity of protective mechanisms in the brain during CCH. This assumption is also supported by the observation that pathological and compensatory processes were more active in the damaged brain hemisphere of C57 mice, while no hemispheric difference was observed in a7nAchR (-/-) mice. When comparing the expression values of sirtuins, it can also be assumed that the lack of a7nAchR leads to an increase in the role of SIRT1 in the cellular protection of brain neurons in normal and in CSN, while in wild-type mice a SIRT3-dependent response prevails.

Previously, different modes of hypobaric hypoxia were shown to have ambiguous acute effects on the brain. Hypobaric hypoxia in rat hippocampal neurons induces mitochondrial damage and its dysfunction, decreases membrane potential and respiration rate, increases NO production, and up-regulate expression of mitochondrial membrane-associated eNOS and nNOS (La Padula *et al*., 2023). It is known, that hypoxic preconditioning regimens protect some tissues in acute experiments, but this has not been investigated in long-term observation (Portnychenko *et al*., 2019). It was shown that three sessions of moderate hypobaric hypoxia were found to have a preventive neuroprotective effect in response to severe hypobaric hypoxia. This effect was demonstrated through decreased DNA methylation, as well as changes in the content of epigenetic factors such as increased acNOS24 and decreased meNOS9 and meDNA (Zhang *et al*., 2023). Modulation of the neurotrophin BDNF (Zhang *et al*., 2023), and increased expression and activation of KATP channels (Zhang *et al*., 2016) are also involved in the mechanisms of adaptation to intermittent hypoxia.

Moderate hypoxia is investigated in recent years in cerebrovascular research to study neuroprotective mechanisms, with the goal of activating protective mechanisms by improving energy metabolism and antioxidant effects (Portnychenko *et al*., 2019; Ehrenreich *et al*., 2023). Our results showed that in the mouse model of chronic cerebral hypoperfusion, in C57 mice, a positive effect of preconditioning was found, more pronounced in the left hippocampus, which may indicate a better neuronal resistance to hypoxia in these animals. In addition, the moderate chromatolysis we observed may be a manifestation of long-term hypermetabolic activity of neurons and may indicate their functional overload.

Alpha7nAChRs have been implicated in neurogenic and neuroprotective and are antiapoptotic processes (Egea *et al*., 2015; Shen *et al*., 2021). Activation of a7nAChRs in the early stages of ischemia has a protective effect by enhancing the function of endothelial cell precursors involved in angiogenesis and endothelial repair after ischemic injury, partly through the JAK2/STAT3 signaling pathway (Zhang *et al*., 2020), activating autophagy mediated by mTOR signaling, and restoring blood-brain barrier function (Su *et al*., 2022a).

Now, there are only a few data on the involvement of alpha7nAChR in the mechanisms of hypoxia effects, in particular, under chronic intermittent hypoxia (Shen *et al*., 2021). Our data also suggest its involvement in HHP mechanisms in the brain, as wild-type mice showed preconditioning effects. That is, the role of a7nAChRs in the effects of hypoxia may depend on the previous physiological state of the organism, and the regimen of hypoxic exposure.

Sirtuins are a cautious family of NAD-dependent deacetylases. SIRT1 is mainly located in the nucleus, and a small amount in the mitochondria. SIRT3 is predominantly expressed in mitochondria. It is worth noting that in a7nAChRs(-/-) mice we found a significant decrease in baseline sirtuin expression levels in both hippocampi compared to mice in the other groups, which may indicate a stimulatory role of alpha7nAChRs in sirtuin-mediated neuroprotection, and the role of alpha7nAChRs/SIRT-1,-3 mechanism in the long-term neuroprotective effects of hypoxic preconditioning in CCH.

We showed that in C57 mice, among the sirtuins studied, SIRT3 is more sensitive to hypoxic effects (CCH and HHP+CCH), with changes in its expression occurring mainly at the level of protein translation. At 8 weeks after CCH, SIRT3 activation was detected in these mice, whereas preliminary HHP reduced its expression. This may reflect a faster course of neuroprotective responses in this group, as indicated by the corresponding indicators of neuronal damage. These changes were not observed in the SIRT1-mediated cytoprotective response.

It is known that a7nAChRs may exert its beneficial effect on blood vessels by activating SIRT1 and inhibiting the action of angiotensin II (Tsai *et al*., 2016). This may have several interpretations in line with our data that in a7nAchRs(-/-) mice, SIRT1-mediated mechanisms are more active than in wild-type mice (and more active than SIRT3-mediated mechanisms), and may indicate a compensatory stimulation of SIRT1 in other ways. At the same time, down-regulation of the activity of sirtuin-dependent neuroprotective mechanisms in these mice, as well as in preconditioned C57 mice, may be associated with correspondent reduction in neuronal damage.

Intermittent hypoxia may lessen mitochondrial injury by activating the AMPK/PGC-1a/Sirt3 axis, which enhances mitochondrial biogenesis and improves mitochondrial function (Su *et al*., 2022b). Besides, the protective effect of a7nAChRs may occur through the effect on mitochondria by activating SIRT3, which prevents vascular smooth muscle cell migration by inhibiting platelet-derived growth factor (Li *et al*., 2019). A violation of this mechanism can be evidenced by the absence of response of SIRT3 to damage in a7nAChRs mice.

Thus, we have shown for the first time that 8-week chronic hypoperfusion of the brain, caused by unilateral occlusion of the common carotid artery, is accompanied by injury to the neurons of the hippocampi of both hemispheres, which is more pronounced on the side of the occlusion. This damage, as well as the mechanisms of neuroprotection induced by hypoxic preconditioning, are maintained for at least 8 weeks by mechanisms mediated through a7nAChRs. Absence of a7nAChRs leads to compensatory activation of regulatory and protective mechanisms mediated by SIRT1, in normal conditions and in CCH. In wild-type (C57) mice, protective mechanisms in CCH are realized to a greater extent by increased expression of SIRT3 in both hemispheres of the brain.

HARMATINA, O. Y.; ROZOVA, K. V.; VASYLENKO, M. I.; LAPIKOVA-BRYHINSKA, T. Y.; BELIKOVA, M. V. & PORTNYCHENKO, A. G. Participación de SIRT1 y SIRT3 en los efectos del precondicionamiento hipobárico hipóxico sobre el daño del hipocampo inducido por hipoperfusión cerebral crónica en ratones C57/6j y a7nAchRs(-/-). *Int. J. Morphol., 42(3)*:594-600, 2024.

RESUMEN: Se sabe que el precondicionamiento hipóxico induce neuroprotección, pero aún se desconocen sus efectos y vías en la patología cerebral crónica. El objetivo fue establecer la participación de la subunidad a7 de los receptores nicotínicos de acetilcolina (a7nAchR) y las sirtuinas de tipo 1 (SIRT1) y 3 (SIRT3) en los efectos del precondicionamiento hipóxico hipobárico sobre el daño cerebral en ratones con hipoperfusión cerebral crónica causada por la oclusión de la arteria carótida común izquierda. Los ratones macho C57/6j (C57, tipo salvaje) y a7nAchRs(-/-) se dividieron en seis grupos experimentales (10 ratones por grupo): C57 con operación simulada, C57 con hipoperfusión cerebral crónica, C57 con precondicionamiento hipobárico hipóxico y crónica. hipoperfusión cerebral, ratones a7nAchRs(-/-) operados de forma simulada, a7nAchRs(-/-) con hipoperfusión cerebral crónica, a7nAchRs(-/-) con precondicionamiento hipobárico hipóxico e hipoperfusión cerebral crónica. Para el preacondicionamiento, los ratones fueron expuestos a hipoxia "levantándolos" en una cámara de barro a una altitud simulada de 5600 m s.n.m. durante 1 h/día durante 3 días consecutivos antes de la manipulación quirúrgica. Se examinaron las expresiones de SIRT1, SIRT3 en tejido cerebral y los cambios histopatológicos de los hipocampos. Se demostró que la hipoperfusión cerebral crónica de 8 semanas, causada por la oclusión unilateral de la arteria carótida común, se acompañaba de lesión de las neuronas del hipocampo de ambos hemisferios y que era más pronunciada en el lado de la oclusión. Este daño, así como los mecanismos de neuroprotección inducidos por el precondicionamiento hipóxico, se mantuvieron durante al menos 8 semanas mediante mecanismos mediados por a7nAChR. El déficit de a7nAChR se acompañó de una reducción del daño neuronal causado por CCH en 8 semanas, así como de efectos de precondicionamiento, y condujo a una activación compensatoria de mecanismos reguladores y protectores mediados por SIRT1, en condiciones normales y en CCH. En ratones de tipo salvaje (C57), los mecanismos de protección en CCH se realizaron en mayor medida mediante una mayor expresión de SIRT3 en ambos hemisferios del cerebro.

PALABRAS CLAVE: Precondicionamiento hipobárico hipóxico; Oclusión unilateral de la arteria carótida común; Hipoperfusión cerebral crónica; Ratones a7nAchRs(-/-); SIRT1; SIRT3.

REFERENCES

Divanbeigi, A.; Nasehi, M.; Vaseghi, S.; Amiri, S. & Zarrindast, M. R. Tropisetron but not granisetron ameliorates spatial memory impairment induced by chronic cerebral hypoperfusion. *Neurochem. Res., 45(11)*:2631-40, 2020.

Egea, J.; Buendia, I.; Parada, E.; Navarro, E.; León, R. & Lopez, M. G. Anti-inflammatory role of microglial alpha7 nAChRs and its role in neuroprotection. *Biochem. Pharmacol., 97(4)*:463-72, 2015.

Ehrenreich, H.; Gassmann, M.; Poustka, L.; Burtscher, M.; Hammermann, P.; Sirén, A. L.; Nave, K. A. & Miskowiak, K. Exploiting moderate hypoxia to benefit patients with brain disease: Molecular mechanisms and translational research in progress. *Neuroprotection, 1(1)*:9-19, 2023.

Jiao, F. & Gong, Z. The beneficial roles of SIRT1 in neuroinflammation-related diseases. *Oxid. Med. Cell. Longev., 2020*:6782872, 2020.

Kelso, M. L. & Oestreich, J. H. Traumatic brain injury: central and peripheral role of a7 nicotinic acetylcholine receptors. *Curr. Drug Targets, 13(5)*:631-6, 2012.

La Padula, P.; Costa, L. E.; Karadayian, A.; Lores-Arnaiz, S. & Czerniczyniec, A. Differences in mitochondrial function between brain and heart of senile rats exposed to acute hypobaric hypoxia. Role of nitric oxide. *Exp. Gerontol, 173*:112100, 2023.

Li, D. J.; Tong, J.; Zeng, F. Y.; Guo, M.; Li, Y. H.; Wang, H. & Wang, P. Nicotinic ACh receptor a7 inhibits PDGF-induced migration of vascular smooth muscle cells by activating mitochondrial deacetylase sirtuin 3. *Br. J. Pharmacol, 176(22)*:4388-401, 2019.

Li, P.; Stetler, R. A.; Leak, R. K.; Shi, Y.; Li, Y.; Yu, W.; Bennett, M. V. L. & Chen, J. Oxidative stress and DNA damage after cerebral ischemia: Potential therapeutic targets to repair the genome and improve stroke recovery. *Neuropharmacology, 134(Pt. B)*:208-17, 2018.

Michishita, E.; Park, J. Y.; Burneskis, J. M.; Barrett, J. C. & Horikawa, I. Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. *Mol. Biol. Cell, 16(10)*:4623-35, 2005.

Park, J. H.; Burgess, J. D.; Faroqi, A. H.; DeMeo, N. N.; Fiesel, F. C.; Springer, W.; Delenclos, M. & McLean, P. J. Alpha-synuclein-induced mitochondrial dysfunction is mediated via a sirtuin 3-dependent pathway. *Mol. Neurodegener., 15(1)*:5, 2020.

Patel, H.; McIntire, J.; Ryan, S.; Dunah, A. & Loring, R. Anti-inflammatory effects of astroglial a7 nicotinic acetylcholine receptors are mediated by inhibition of the NF-kB pathway and activation of the Nrf2 pathway. *J. Neuroinflammation, 14(1)*:192, 2017.

Percie du Sert, N.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M. T.; Baker, M.; Browne, W. J.; Clark, A.; Cuthill, I. C.; Dirnagl, U. *et al*. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol., 18(7)*:e3000410, 2020.

Portnychenko, A. G.; Vasylenko, M. I.; Aliiev, R. B.; Kozlovska, M. G.; Zavhorodnii, M. O.; Tsapenko, P. K.; Rozova, K. V. & Portnichenko, V. I. The prerequisites for the development of type 2 diabetes or prediabetes in rats fed a high-fat diet. *Regul. Mech. Biosyst., 14(1)*:16-22, 2023.

Portnychenko, A. G.; Vasylenko, M. I.; Harmatina, O. Y.; Drevytska, T. I. & Portnichenko, V. I. *Myocardial Preconditioning: New Approaches and Molecular Mechanisms.* Kyiv, Znannia Ukrainy, 2019.

Shang, J.; Yamashita, T.; Zhai, Y.; Nakano, Y.; Morihara, R.; Fukui, Y.; Hishikawa, N.; Ohta, Y. & Abe, K. Strong impact of chronic cerebral hypoperfusion on neurovascular unit, cerebrovascular remodeling, and neurovascular trophic coupling in Alzheimer's disease model mouse. *J. Alzheimers Dis., 52(1)*:113-26, 2016.

Shen, H.; Meng, Y.; Liu, D.; Qin, Z.; Huang, H.; Pan, L.; Wang, W. & Kang, J. a7 nicotinic acetylcholine receptor agonist PNU-282987 ameliorates cognitive impairment induced by chronic intermittent hypoxia. *Nat. Sci. Sleep, 13*:579-90, 2021.

Su, Y.; Ke, C.; Li, C.; Huang, C. & Wan, C. Intermittent hypoxia promotes the recovery of motor function in rats with cerebral ischemia by regulating mitochondrial function. *Exp. Biol. Med. (Maywood), 247(15)*:1364-78, 2022a.

Su, Y.; Zhang, W.; Zhang, R.; Yuan, Q.; Wu, R.; Liu, X.; Wuri, J.; Li, R. & Yan, T. Activation of cholinergic anti-inflammatory pathway ameliorates cerebral and cardiac dysfunction after intracerebral hemorrhage through autophagy. *Front. Immunol., 13*:870174, 2022b.

Tsai, I. C.; Pan, Z. C.; Cheng, H. P.; Liu, C. H.; Lin, B. T. & Jiang, M. J. Reactive oxygen species derived from NADPH oxidase 1 and mitochondria mediate angiotensin II-induced smooth muscle cell senescence. *J. Mol. Cell. Cardiol., 98*:18-27, 2016.

Zhang, S.; Fu, W.; Jia, X.; Bade, R.; Liu, X.; Xie, Y.; Xie, W.; Jiang, S. & Shao, G. Hypoxic preconditioning modulates BDNF and its signaling through DNA methylation to promote learning and memory in Mice. *ACS Chem. Neurosci., 14(12)*:2320-32, 2023.

Zhang, S.; Guo, Z.; Yang, S.; Ma, H.; Fu, C.; Wang, S.; Zhang, Y.; Liu, Y. & Hu, J. Chronic intermittent hybobaric hypoxia protects against cerebral ischemia via modulation of mitoKATP. *Neurosci. Lett., 635*:8-16, 2016.

Zhang, X.; Mao, G.; Zhang, Z.; Zhang, Y.; Guo, Z.; Chen, J. & Ding, W. Activating a7nAChRs enhances endothelial progenitor cell function partially through the JAK2/STAT3 signaling pathway. *Microvasc. Res., 129*:103975, 2020.

Zuloaga, K. L.; Johnson, L. A.; Roese, N. E.; Marzulla, T.; Zhang, W.; Nie, X.; Alkayed, F. N.; Hong, C.; Grafe, M. R.; Pike, M. M.; *et al*. High fat diet-induced diabetes in mice exacerbates cognitive deficit due to chronic hypoperfusion. *J. Cereb. Blood Flow Metab., 36(7)*:1257-70, 2016.

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