

Мы полагаем, что полученные данные могут стать основой для разработки терапевтических препаратов на основе L-карнозина для применения его в качестве нетоксичного эндогенного криопротектора для нервной ткани.

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СПИСОК ЛИТЕРАТУРЫ

- Митюшов М.И., Емельянов Н.А., Мокрушин А.А., Войнер И.А., Багаева Т.Р.* 1986. Переживающий срез мозга как объект нейрофизиологического и нейрохимического исследования. Л.: Наука. 127 с. (*Mityushov M.I., Emelyanov N.A., Mokrushin A.A., Voiner I.A., Bagaeva T.R.* 1986. The surviving slice of the brain as an object of neurophysiological and neurochemical research. L.: Nauka. 127 p.)
- Мокрушин А.А.* 1997. Пептид-зависимые механизмы нейрональной пластичности в обонятельной коре. Автореф. докт. дис. СПб. 40 с. (*Mokrushin A.A.* 1997. Peptide-dependent mechanisms of neuronal plasticity in the olfactory cortex. Thesis Doct. Diss. St. Petersburg. 40 p.)
- Мокрушин А.А.* 2016. Эффекты глубокого замораживания и отогревания на ионотропные глутаматергические рецепторные механизмы *in vitro*. Бюлл. exper. биол. мед. Т. 161. С. 36. (*Mokrushin A.A.* 2016. Effects of deep freezing and rewarming on ionotropic glutamatergic receptor mechanisms *in vitro*. Bull. Exper. Biol. Med. V. 161. P. 36)
- Мокрушин А.А.* 2020. Влияние длительности криосохранения на активность ионотропных глутаматергических механизмов исследование *in vitro*. Известия РАН. серия биологическая. 2020. № 1. С. 1–10. (*Mokrushin A.A.* 2020. The influence of duration cryopreservation on activity of ionotropic glutamatergic mechanisms *in vitro* study. Proceedings of the Russian Academy of Sciences. Biological series. 2020. № 1. P. 1–10).
- Мокрушин А.А.* 2022. Улучшение кислотно-щелочного состава среды для длительного и обратимого криосохранения срезов мозга крыс. Цитология. Т. 64. № 1. С. 96. (*Mokrushin A.A.* 2022. Improvement of the acidbase composition of the environment for long-term and reversible cryopreservation of rat brain slices. Tsitologiya. V. 64. № 1. P. 96.)
- Мокрушин А.А., Боровиков С.Е.* 2017. Установка для изучения гипотермических эффектов на переживающих срезах мозга теплокровных. Междунар. журн. прикладных фундам. исследований. Т. 2. С. 214. (*Mokrushin A.A., Borovikov S.E.* 2017. Device for the study of hypothermic effects on the surviving brain slices of homeotherms. Int. J. Applied Basic Res. V. 2. P. 214.)
- Мокрушин А.А., Плеханов А.Ю.* 2001. Иммунологическая идентификация эндогенных пептидов, секретируемых клетками переживающих срезов обонятельной коры мозга крыс. Доклады Академии наук. Т. 378. № 4. С. 567. (*Mokrushin A., Plekhanov A.Yu.* 2001. Immunological identification of endogenous peptides secreted by surviving slices of rat olfactory cortex. Dokl. Biol. Sci. V. 378. P. 227.)
<https://doi.org/10.1023/a:1019206506211>
- Пичугин Ю.И.* 2013. Теоретические и практические аспекты современной криобиологии. М.: Научно-технический центр криобиологии и анабиоза. 60 с. (*Pichugin Y.I.* 2013. Theoretical and practical aspects of modern cryobiology. M.: Nauchno-Tekhn. Tsentr Kriobiol. Anabioz. 60 p.)
- Стволинский С.Л., Федорова Т.Н., Девятков А.А., Медведев О.С., Белоусова М.А., Рыжков И.Н., Тутельян В.А.* 2017. Нейропротективное действие карнозина в условиях экспериментальной фокальной ишемии-реперфузии головного мозга. Журн. неврологии и психиатрии. Т. 12. С. 60. (*Stvolinsky S.L., Fedorova T.N., Devyatov A.A., Medvedev O.S., Belousova M.A., Ryzhkov I.N., Tutelyan V.A.* 2017. Neuroprotective effect of carnosine in experimental focal ischemia-reperfusion of the brain. J. Neurol. Psychiatry. V. 12. P. 60.)
- Bae O., Majid A.* 2013. Role of histidine/histamine in carnosine-induced neuroprotection during ischemic brain damage. Brain Res. V. 1527. P. 246.
<https://doi.org/10.1016/j.brainres.2013.07.004>
- Berezhnoy D.S., Stvolinsky S.L., Lopachev A.V., Devyatov A.A., Lopacheva O.M., Kulikova O.I., Abaimov D.A., Fedorova T.N.* 2019. Carnosine as an effective neuroprotector in brain pathology and potential neuromodulator in normal conditions. Amino Acids. V. 51. P. 139.
- Boldyrev A.A., Aldini G., Derave W.* 2013. Physiology and pathophysiology of carnosine. Physiol. Rev. V. 93. P. 1803.
- Bonfanti L., Peretto P., De M.S., Fasolo A.* 1999. Carnosine-related dipeptides in the mammalian brain. Prog. Neurobiol. V. 59. P. 333.
- De Marchis S., Modena C., Peretto P., Migheli A., Margolis F.L., Fasolo A.* 2000. Carnosine-related dipeptides in neurons and glia. Biochemistry. V. 65. P. 824.
- Hipkiss A.R., Preston J.E., Himsworth D.T., Worthington V.C., Keown M., Michaelis J., Lawrence J., Mateen A., Allende L., Eagles P.A.* 1998. Pluripotent protective effects of carnosine, a naturally occurring dipeptide. Ann. N.Y. Acad. Sci. V. 854. P. 37.
- Ichikawa J., Yamada R.X., Muramatsu R., Ikegaya Y., Matsuki N., Koyama R.* 2007. Cryopreservation of granule cells from

- the postnatal rat hippocampus. *J. Pharmacol. Sci.* V. 104. P. 387.
- Khama-Murad A.X., Pavlinova L.I., Mokrushin A.A.* 2008. Neurotropic effect of exogenous L-carnosine in cultured slices of the olfactory cortex from rat brain. *Bull. Exp. Biol. Med.* V. 146. P. 1–3.
<https://doi.org/10.1007/s10517-008-0227-y>
- Khama-Murad A., Mokrushin A., Pavlinova L.* 2011. Neuroprotective properties of L-carnosine in the brain slices exposed to autolysis in the hemorrhagic stroke model in vitro. *Regul. Pept.* V. 167. P. 65.
<https://doi.org/10.1016/j.regpep.2010.11.007>. Epub. 2010 Dec. 9.
- Lopachev A.V., Lopacheva O.M., Akkuratov E.E., Stvolinskii S.L., Fedorova T.N.* 2017. Carnosine protects a primary cerebellar cell culture from acute NMDA toxicity. *Neurochem. J.* V. 11. P. 38.
- Lopachev A.V., Kazanskaya R.B., Khutorova A.V., Fedorova T.N.* 2020. An overview of the pathogenic mechanisms involved in severe cases of COVID-19 infection, and the proposal of salicyl-carnosine as a potential drug for its treatment. *Eur. J. Pharmacol.* V. 886. P. 173457.
<https://doi.org/10.1016/j.ejphar.2020.173457>
- Matsumura K., Hayashi F., Nagashima T.* 2021. Molecular mechanisms of cell cryopreservation with polyampholytes studied by solid-state NMR. *Commun. Materials.* V. 2. P. 116.
- Mokrushin A.A., Pavlinova L.I.* 2013. Effects of the blood components on the AMPA and NMDA synaptic responses in brain slices in the onset of hemorrhagic stroke. *Gen. Physiol. Biophys.* V. 32. P. 489.
- Mokrushin A.A., Pavlinova L.I., Borovikov S.E.* 2014. Influence of cooling rate on activity of ionotropic glutamate receptors in brain slices at hypothermia. *J. Therm. Biol.* V. 44. P. 5.
- Obrenovitch T.P., Urenjak J.* 1997. Altered glutamatergic transmission in neurological disorders: from high extracellular glutamate to excessive synaptic efficacy. *Progress Neurobiol.* V. 51. P. 39.
- Ouyang L., Tian Y., Bao Y., Xu H., Cheng J., Wang B., Shen Y., Chen Z., Lyu J.* 2016. Carnosine decreased neuronal cell death through targeting glutamate system and astrocyte mitochondrial bioenergetics in cultured neuron/astrocyte exposed to ogd/recovery. *Brain Res. Bull.* V. 124. P. 76.
- Pepper E.D., Farrell M.J., Nord G., Finkel S.E.* 2010. Antiglycation effects of carnosine and other compounds on the long-term survival of *Escherichia coli*. *Appl. Env. Microbiol.* V. 76. P. 7925.
- Rocha C.C., Kawai G.K.V., de Agostini L. J.D., Angrimani D., Rui B.R., de Cássia B.L., da Silva B., Alonso M.A., Mendes C.M.* 2018. Carnosine as malondialdehyde scavenger in stallion seminal plasma and its role in sperm function and oxidative status. *Theriogenol.* V. 119. P. 10.
- Sarkar P.K., Egusa A., Matsuzaki M., Sasanami T.* 2021. Effect of anserine and carnosine on sperm motility in the Japanese quail. *J. Poult. Sci.* V. 58. P. 186.
- Sassoe-Pognetto M., Cantino D., Panzanelli P., Verdandi C.L., Giustetto M., Margolis F.L., De B.S., Fasolo A.* 1993. Presynaptic co-localization of carnosine and glutamate in olfactory neurones. *Neuroreport.* V. 5. P. 7.
- Solana-Manrique C., Sanz F.J., Martínez-Carrión G., Paricio N.* 2022. Antioxidant and neuroprotective effects of carnosine: therapeutic implications in neurodegenerative diseases. *Antioxidants.* V. 11. P. 848.
- Teufel M., Saudek V., Ledig J.P., Bernhardt A., Boularand S., Carreau A., Cairns N.J., Carter C., Cowley D., Duverger D.* 2003. Sequence identification and characterization of human carnosinase and a closely related non-specific dipeptidase. *J. Biol. Chem.* V. 278. P. 6521.
- Tiedje K., Stevens K., Barnes S., Weaver D.* 2010. Beta-alanine as a small molecule neurotransmitter. *Neurochem. Int.* V. 57. P. 177.
- Warren D., Bickler P., Clark J., Gregersen M., Brosnan H., McKleroy W., Gabatto P.* 2012. Hypothermia and rewarming injury in hippocampal neurons involves intracellular Ca²⁺ and glutamate excitotoxicity. *Neurosci.* V. 207. P. 316.
<https://doi.org/10.1016/j.neuroscience.2011.12.034>. Epub. 2012 Jan. 12.
- Zhang X., Song L., Cheng X., Yang Y., Luan B., Jia L., Xu F., Zhang Z.* 2011. Carnosine pretreatment protects against hypoxia-ischemia brain damage in the neonatal rat model. *Eur. J. Pharm.* V. 667. P. 202.
- Zemke D., Krishnamurthy R., Majid A.* 2005. Carnosine is neuroprotective in a mouse model of stroke. *J. Cereb. Blood Flow Metab.* V. 25. P. S313.

Cryoprotective Characteristics of L-Carnosine Dipeptide (β -Alanyl-L-Histidine)

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The dipeptide (β -alanyl-L-histidine) is found in significant amounts in the muscles and brain of mammals, especially in the olfactory structures. L-Carnosine exhibits many protective effects when exposed to various cytotoxic factors on cells. We used slices of the rat olfactory cortex to study the cryoprotective characteristics of L-carnosine during cryopreservation (CP). Changes in the activity of N-methyl-D-aspartate receptors (NMDAR) were analyzed during registration of NMDA potentials induced by electrical stimulation of the lateral olfactory tract. Brain slices were preincubated with L-carnosine (20 mM) in solution, frozen (-10°C), and after a long CP (30 days) they were warmed up to 37°C and changes in the amplitudes of NMDA potentials were determined. It was found that the dipeptide optimized the pH of the freezing solution after CP and retained the activity of NMDAR, determined by the

amplitude of NMDA potentials. L-Carnosine after CP contributed to the dehydration of excess free water from the slices. The dipeptide inhibited the development of glutamate excitotoxicity in brain slices during CP and maintained normal NMDAR functioning. The data obtained prove that L-carnosine exhibits the properties of an endogenous cryoprotector in the nervous tissue.

Keywords: L-Carnosine, cortical slices, NMDA receptors, focal potentials, freezing/thawing, cryopreservation