PHYSIOLOGY

Encapsulation of rat brain slices in agar gel solution for long-term and reversible cryopreservation

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Abstract

Earlier there was found activity of glutamatergic ionotropic *α*-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPAR) and *N*-methyl-D-aspartate receptors (NMDAR) disturbed after prolonged cryopreservation of brain slices at temperature of –10 °C. To eliminate cryodamage of AMPAR and NMDAR, the slices were encapsulated in the special freezing solution (SFS). SFS consisted of agar at various concentrations (33, 44 and 50 %) and artificial cerebrospinal fluid (67, 56 and 50 %, respectively). This solution was used for long-term cryopreservation of slices (52 days, –10 °C). Alterations in amplitudes of AMPA and NMDA potentials in the slices after rewarming were studied. Recovery of AMPAR and NMDAR after cryopreservation of the slices in SFS was the most optimal when the agar concentration in SFS was 50 %. Cryopreservation of the slices in SFS with different agar concentrations predominantly promoted the development of long-term potentiation in 78 % of the tested slices. Thus, the encapsulation of brain slices in SFS contributes to the preservation of AMPAR and NMDAR activity during long-term and reversible cryopreservation.

Keywords: brain slices, cryopreservation, freezing/rewarming, agar

Introduction

Cryopreservation is the process by which cells or tissues are frozen at subzero temperatures to reduce cellular functions and keep life suspended. The study of the patterns and methods of reversible inhibition of the vital activity of mammalian cells and organs for long periods of time at subzero temperatures has practical importance for the creation of a cryobank of transplants (Ichikawa et al., 2007).

Currently, in medicine and veterinary science low-temperature storage of cells of the same type (oocytes, spermatozoa, blood cells, etc.) is successfully used with the subsequent restoration of their biological functions after rewarming. As for the low-temperature storage of tissues of warm blooded animals, a detailed cryopreservation protocol has not yet been developed. Particular difficulties arise during prolonged nerve tissue cryopreservation.

At the present time, the dominant direction of cryopreservation of nervous tissue is the use of low-temperature storage of primary neuronal culture (Parker et al., 2018; Pischedda et al., 2018). However, such structures lack synapses, that is, neural network is not formed. Obviously such cryopreserved neurons will have a limited clinical use only for the recovery of small structures of brain for transplantation. The clinic for recovery of large areas of the brain tissue of the recipient is necessary to use the nerve tissue of the relevant brain structures. It is these brain explants that are needed for cryobank for transplantation in neuropathologies such as stroke, epilepsy and trauma. Several reports indicate that brain explants cryopreserved as a whole structure (in toto) show comparable properties to the same tissues grafted immediately from the donor (Jensen, Sorensen, and Zimmer, 1987; Fang and Zhang, 1992; Collier, Gallagher, and Sladek, 1993; Petite and Calvet, 1997).

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We believe that for the development of protocols and methods for cryopreservation of nerve tissue, surviving brain slices are optimal experimental objects. They make it possible to study the recovery of activity not only of neurons, but also of key synaptic mechanisms. Previously it was found that at slow cooling rates of slices of the olfactory cortex (0.1–0.125 °C/min) to +16 °C, the activity of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPAR) and *N*-methyl-D-aspartate (NMDAR) receptors persisted (Mokrushin, Pavlinova, and Borovikov, 2014; Mokrushin, 2015).

In further studies, different resistance of AMPAR and NMDAR to freezing/rewarming of brain slices at low-temperature cryopreservation (–10 °C, 52 days) was revealed. AMPAR were preserved and enhanced, NMDA processes, on the contrary, were suppressed and blocked under these conditions, indicating that their functions were significantly impaired (Mokrushin and Borovikov, 2017; Mokrushin, 2020). These findings are important because NMDAR are key in the glutamatergic mediator system of the brain. They are adaptive mechanisms in the brain, which are involved in learning, the formation of memory traces, as well as in the development of various neuropathologies (epilepsy, stroke, trauma, etc.) (Obrenovitch and Urenjak, 1997).

Thus, the study of the features of cryodamage of AMPAR and NMDAR is of exceptional importance for maintaining the activity of these mechanisms during cryopreservation and to create a cryobank of transplants.

It can be assumed that cryoprotectors can be used to eliminate cryodamages of AMPAR and NMDAR. However, they have pronounced negative effects on neurons and block synaptic mechanisms, therefore their use is not appropriate (Pichugin, 2013).

Another promising approach to repair the cryodamage of AMPA and NMDA mechanisms is the use of special freezing media (Neurofreezing, CryoStor10 (CS10) Hibernate-A, Hibernate-E, HypoThermosol, etc). Indeed, recent studies showed the improvement of cryopreservation of primary neurons by using newly developed freezing media. However, the viability of cryopreserved neuronal cultures is reduced compared to freshly prepared neurons, indicating the reduced efficiency of these solutions (Day et al., 2017; Parker et al., 2018; Pischedda et al., 2018).

Alginate hydrogels are successfully used as the basis for freezing a cryoprotective medium (Zhang et al., 2018). For cells, cryopreservation based on using a variety of alginate hydrogels demonstrated an improvement in the survival rate and the retention of their normal functions (Schneider and Klein, 2011; Benson et al., 2018). However, the presence of bivalent Ba^{2+} and Sr^{2+} cations in the composition of such hydrogels will block synaptic activity in donor tissue and is not suitable for cryopreservation of brain explants. In addition, the use of such solutions is associated with the addition of a harmful cryoprotector (dimethylsulfoxide), which blocks synaptic transmission and, as a result, such brain explants are not suitable as donor ones.

In the present study, slices of the rat olfactory cortex were used to study long-term cryopreservation. The cryodamages of glutamatergic ionotropic AMPAR and NMDAR were studied. Before cryopreservation, slices were placed in agar gel and encapsulated in it during freezing.

Agar (a mixture of agarose and agaropectin polysaccharides) is used in biological and medical studies due to its biocompatibility and biodegradability properties. We hypothesized that the use of agar gel minimizes the damage of AMPAR and NMDAR during cryopreservation.

Material and methods

Animals and slice preparations

We used white male Wistar rats weighing 180–200 g for the study. All experiments were carried out on rats from the Collection of Laboratory Mammals with biocollections of different taxonomic affiliations of the Pavlov Institute of Physiology, Russian Academy of Sciences.

The studies were carried out on tangential slices of the olfactory cortex 400–500 μm thick, which were made from the brains of male rats (29 animals). The morphological features of the olfactory cortex are clearly distinguishable in a light microscope (MBS 9, Russia) (Fig. 1A).

The slices were maintained in an artificial cerebrospinal fluid (aCSF), which consisted of (in mM): 124 NaCl, 5 KCl, 2.6 $CaCl_2$, 1.24 KH_2PO_4, 1.2 $MgSO_4$, 3 $NaHCO_3$, 10 glucose, 23 Tris-HCl (Sigma, USA); equilibrated with O_2, the temperature was 37 °C, pH 7.2–7.3.

Electrophysiological recordings

To record the activity of AMPAR and NMDAR (hereafter, AMPA and NMDA potentials) extracellular field potentials (FP) were induced in slices by bipolar electrodes located on the lateral olfactory tract (LOT) (Fig. 1). The rectangular pulses with duration of 0.1 ms and the intensity of 1-3 μA were applied from stimulator (ESU-1, Russia) through platinum bipolar concentric electrode. FPs were recorded with a glass microelectrode filled with 1 M NaCl with a resistance of 1-5 MΩ. FPs recordings were performed using an NTO-2 amplifier (Pavlov Institute of Physiology, Russia). The reference silver electrode was located in a chamber floor.

We analyzed separate glutamatergic ionotropic mechanisms of FPs: AMPA and NMDA potentials. Pharmacological identification of these ones was pre-

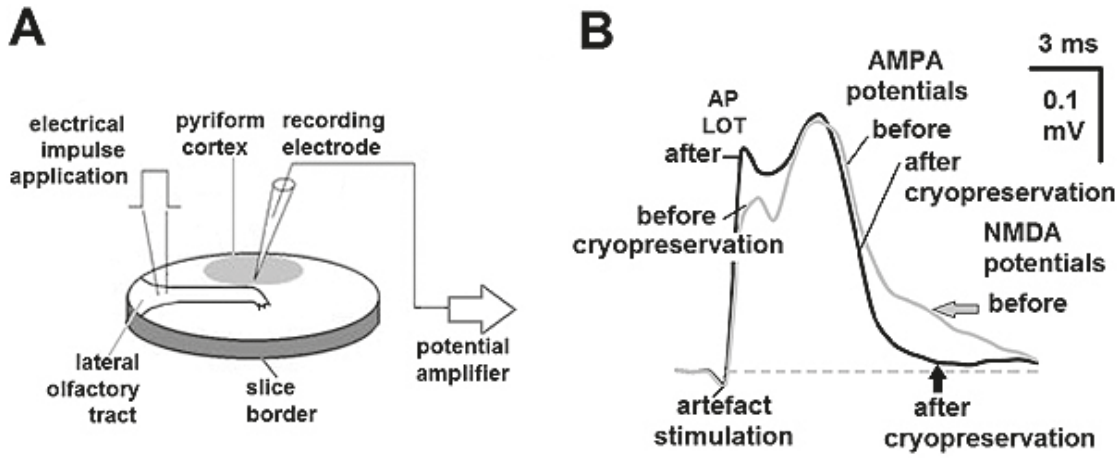

Fig. 1. Scheme of the slice of the rat olfactory cortex, view from pial surface, with the main morphological structures, localization of stimulating and recording electrodes (A). Potentials recorded in slices with indication of their individual components before and after cryopreservation. Horizontal gray dashed line corresponds to the potential recorded in the resting conditions. Calibration as indicated (B).

viously described in detail (Mokrushin and Pavlinova, 2013). FPs were recorded after stimulation with frequency 0.003 Hz during 15 min and referred as control values before cryopreservation (Fig. 1B).

Processing of AMPA and NMDA potentials

AMPA and NMDA potentials were processed in the online mode after amplifying (NTO-2, Russia), and then were digitized with the analog-digital converter E 20-10 (Russia) (sample rate 25 kHz) and transmitted to computer for registration and subsequent analysis using special homemade software (Pavlov Institute of Physiology, Russia).

FP is a multicomponent potential that consists of a presynaptic component that reflects the activity of the LOT conducting fibers (AP LOT) and postsynaptic components that reflect the activation of glutamatergic ionotropic mechanisms AMPA and NMDA potentials (Fig. 2).

Identification of individual components of the FP recorded in the slices of the olfactory cortex was carried out using specific blockers: CNQX (6-syano-7-nitroquinoxaline-2,3-dione) for AMPAR and D-APV (D-2-amino-5-phosphonovalerate) for NMDAR. FPs were recorded in response to LOT stimulation in the control aCSF at +37 °C (Fig. 2A) and during the incubation of slices with specific AMPAR and NMDAR antagonists (Fig. 2A–D).

To identify the AMPAR the slices were incubated with CNQX (25 μM) for 20 min; the study was carried out on 8 slices (Fig. 2B). As a result of CNQX action, all postsynaptic components of FP are reduced. In this case, the presynaptic component of the AP LOT is retained. Therefore, the early postsynaptic component of FP reflects the activation of AMPA receptors.

To identify the NMDA potentials, the slices were incubated with D-APV (50 μM); the study was carried out on 7 slices. To prevent the blockade of these receptors with Mg^{2+} ions, the slices were incubated in a medium with low concentration of these ions (200 μM) for 20 min. The obtained results demonstrated a reduction in the late synaptic component of the FP which indicates the activation by NMDAR (Fig. 2C). With the simultaneous action of these blockers (concentrations are the same, as in B, C) there is a complete blockade of AMPAR and NMDAR, but the amplitude of the presynaptic AP LOT component is retained (n = 5) (Fig. 2D).

The obtained data indicate that the early postsynaptic AMPA potential reflects the activation of AMPAR. Later postsynaptic NMDA potential reflects the activation of NMDAR (Fig. 2A).

We evaluated the amplitudes of AMPA and NMDA potentials from baseline to peak. The amplitudes of AMPA were assessed within a 2 ms window and centered at the peak of the response (n = 7). Peak NMDA potentials were assessed within an 8 ms window (n = 7).

The results of the pharmacological identification of AMPA and NMDA potentials, as well as the time intervals for measuring the amplitudes of these components, were used by us to study the cryopreservation of slices without and with the use of agar.

Cryopreservation protocol

The incubation solution for cryopreservation of slices was prepared in the following sequence (Fig. 3). Difco Bactor agar (USA) (3 g) was poured in 100 ml of 1 M NaCl and the solution was kept for 10 days in a thermostat at +32° … +35 °C. The resulting gel solution was centrifuged (Elecon P10-0.1, Russia) at a speed of 2000 rpm (revs) for 10 min. The light fraction was aspirated from the gel solution and used to prepare a medium for the cryopreservation of the slices. The heavy fraction of agar was not used.

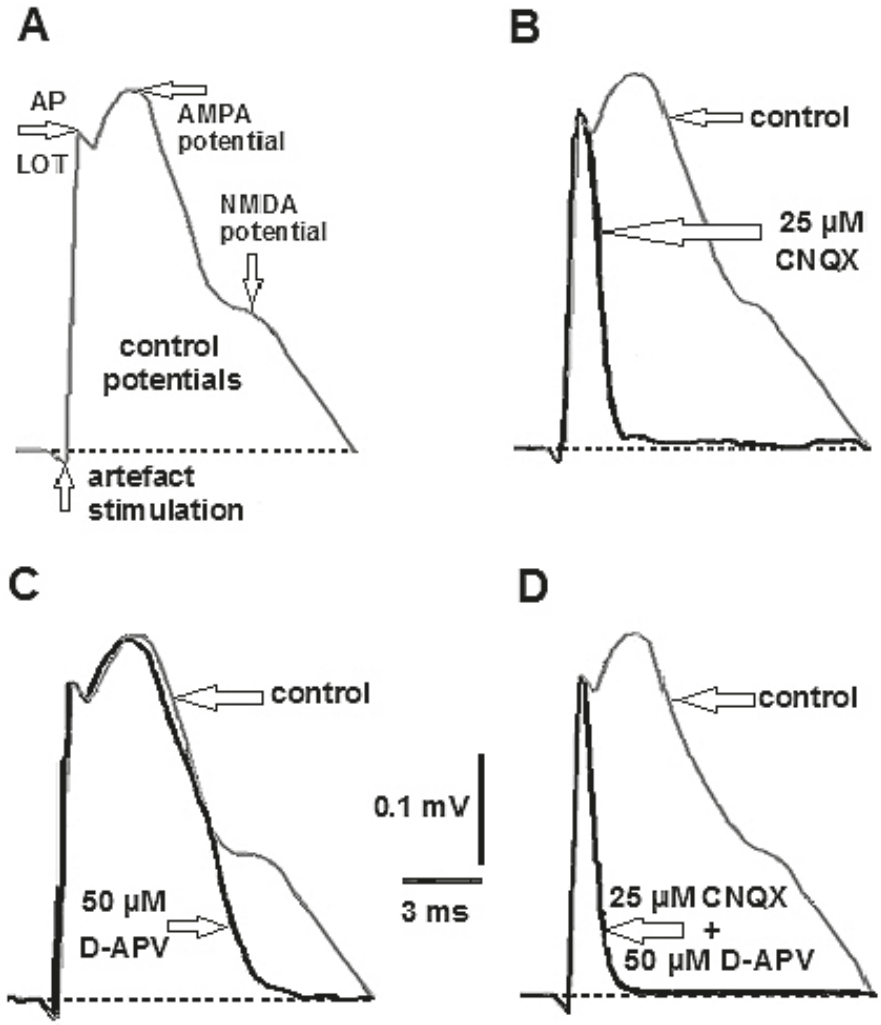

Fig. 2. Identification of pre- and postsynaptic components of field potentials (FPs) evoked by electrical stimulation of LOT. Averaged FPs specifying components in normal aCSF at +37 °C (n = 7) (A) and in the presence of the AMPAR blocker CNQX (n = 8) (B). Profiles in (C) are FPs in the presence of the NMDAR blocker D-APV in the medium with a reduced content of Mg^{2+} (200 μM) (n = 6). D — application CNQX and D-APV (the same concentrations as in B and C) (n = 5). Calibration as indicated.

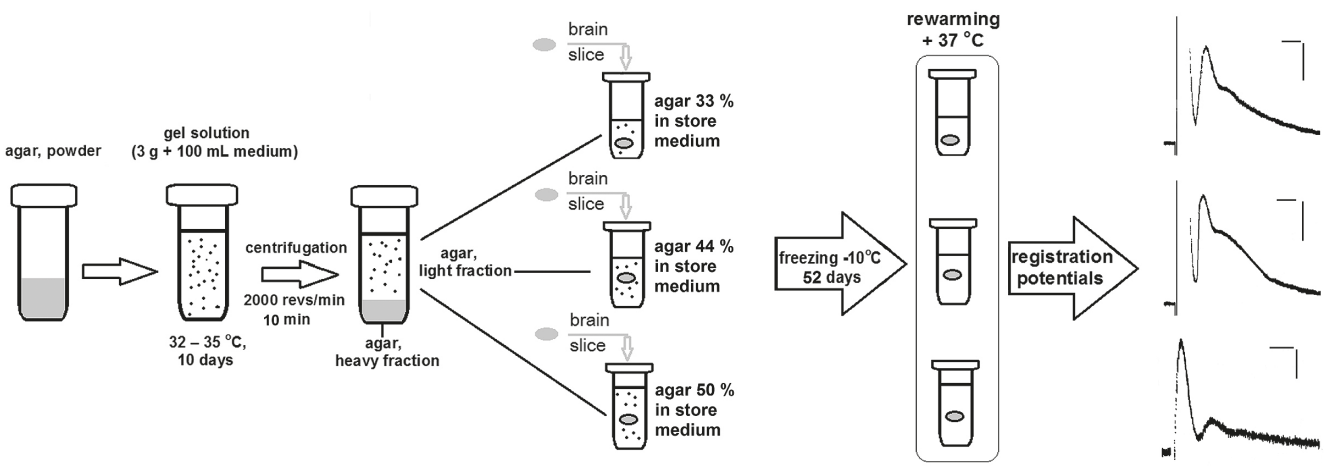

Fig. 3. Diagram of the protocol for preparing the incubation medium with different concentrations of the light fraction of agar in freezing medium and the sequence of experiment. The right panel shows records of potentials in slices after long-term cryopreservation (10 °C, 52 days) during warming (+37 °C). The potentials were obtained as photographs from an oscilloscope. Calibration: 5.0 ms; 0.1 mV (two upper potentials); 2.0 ms; 0.1 mV (lower potential).

Further, the incubation solution was prepared for cryopreservation of the slices. The composition of the solution was as follows: 0.5 mL of light fraction of agar and 1.0 mL of aCSF (final concentration of agar 33 %); 0.8 mL of light fraction of agar and 1.0 mL of aCSF (final concentration of agar 44 %); 1.0 mL of light fraction of agar and 1.0 mL of aCSF (final concentration of agar 50 %). Then, the efficiency of cryopreservation of the created solutions with different concentrations of agar was studied on 21 slices (7 slices for each agar concentration).

The process of encapsulation of the slices was as follows. Brain slices were placed in glass vials with solutions. Then the vials with the slices were gradually frozen to –10 °C at a slow speed (0.1 °C/min) in a ThermoStat plus thermostat freezer (Eppendorf, Germany) for 52 days. After 52 days, the slices vials were warmed to +37 °C at a slow rate of 0.1 °C/min, i. e., decapsulation of the slices occurred (Fig. 3).

For testing the slices viability, we used the method of assessing the electrical activity of AMPAR and NMDAR by the values of the amplitudes of the AMPA and NMDA potentials. For this, the slices one by one were removed from the vials and placed in the registration chamber of an electrophysiological setup for recording AMPA and NMDA potentials (Mokrushin and Borovikov, 2017). Stimulating and recording electrodes were inserted into the slices; their localization is indicated in Fig. 1A. For 15 min, the slices perfused by the aCSF medium without agar (the composition is indicated above). The AMPA and NMDA potentials were recorded in the slices and their amplitude values were considered as control ones and expressed as a percentage.

After the cryopreservation the slices were perfused with aCSF without agar for 15 min. During this time the slices were completely washed from agar. Each slice was visually assessed using a binocular microscope MBS-10 (Russia): LOT, piriform cortex, slices boundaries (Fig. 1A). The absence of destruction of these structures was evaluated as good. The main criterion for successful cryopreservation of the slices was to preserve AMPA and NMDA potentials.

In this study we did not use cryoprotectors, since they blocked the activity of neurons and synapses (Pichugin, 2013).

Reagents

All chemical reagents used for the incubation medium were from "ChimReactive" (Russia), Difco Bactor agar (USA).

Statistical analysis

Statistical comparisons were performed with nonparametric Wilcoxon — Mann — Whitney U-test. Numerical data were expressed as mean ± standard error of the mean (S. E. M.). The level of statistical significance was set at $p \le 0.05$. Detailed statistical processing of the research results is presented in the Results section, in Figs 5–7, as well as in their captions.

Results

The data on cryopreservation of AMPAR and NMDAR in gel solutions with different agar concentrations after warming up are shown in Fig. 4. First of all, it was found that the activity of AMPAR and NMDAR was 100 % preserved after cryopreservation of the slices for 52 days at –10 °C in the storage solution consisting of agar and aCSF. Second, it was found that the degree of recovery of AMPAR and NMDAR after cryopreservation depended on the concentration of agar in a solution.

During the cryopreservation of the slices in gel medium with a 33 % agar concentration, it was found that upon rewarming, the amplitude of AMPA potentials increased by an average of 54 % ($n = 7$), compared to the amplitude of AMPA potentials before cryopreservation (Fig. 4A). These data indicate hyperactivation of AMPAR after cryopreservation. The amplitude of the presynaptic component of the field potential (AP LOT) also increased above 55 % on average ($n = 7$).

At this concentration of agar in the medium, the amplitude of NMDA potentials recovered completely after cryopreservation. However, the decline curve of NMDA potential values was faster than before cryopreservation (Fig. 4A). Statistical analysis confirmed that after cryopreservation, the amplitude of AMPA potentials increased significantly, on average by 57 % ($U = 11$, $p \le 0.05$, $n = 7$). The amplitude of NMDA potentials increased on average by 3 % to the value before freezing ($U = 2$, $p \ge 0.05$, $n = 7$) (Fig. 4B).

Thus, the cryopreservation of slices in a gel solution with 33 % agar promotes the preservation of both glutamatergic ionotropic AMPAR and NMDAR, but the degree of their preservation is different. Based on the data obtained, we can assume that agar at a concentration of 33 % is not optimal for the cryopreservation of AMPAR and NMDAR.

To obtain the optimal effect of cryopreservation of AMPAR and NMDAR, the concentration of agar in the medium was increased to 44 %. Under these conditions, it was found that the amplitude of AMPA potentials increased by an average of 57 % ($n = 7$), compared to the amplitude of AMPA potentials before cryopreservation (Fig. 5).

These values indicate hyperactivation of AMPAR after cryopreservation. Notably, the amplitude of the presynaptic component AP LOT changed synchronously with the AMPA potentials and also increased by more than 140 %. The amplitude of NMDA potentials as well as AMPA potentials increased on average by 76 %

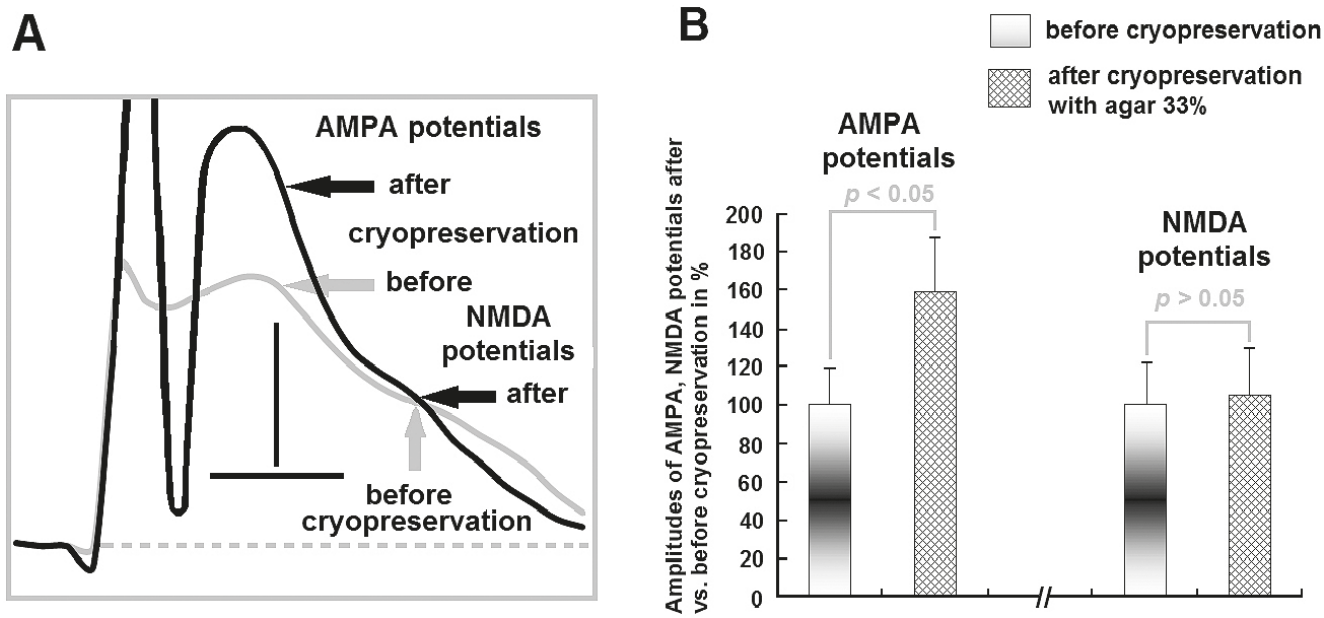

Fig. 4. Modifications of AMPA and NMDA potential profiles before and after cryopreservation of slices for 52 days at –10 °C in aCSF with agar concentrations of 33 %. The recordings made when the slices were warmed up to +37 °C. The potentials recorded in aCSF without agar before cryopreservation (control) are marked in gray. Gray dotted line, potential level recorded in slices without stimulation. Arrows indicate amplitudes of AMPA and NMDA potentials. Calibration: 3 ms; 0.1 mV (A). Average amplitudes of AMPA and NMDA potentials before and after cryopreservation in solution with 33 % agar. On the y-axis, the amplitudes of AMPA and NMDA potentials during rewarming, in % to the values before cryopreservation (control, 100 %), n = 7. Differences in the amplitudes of AMPA and NMDA potentials after cryopreservation compared to the values before cryopreservation (control) were carried out using the Wilcoxon — Mann — Whitney U-test. After cryopreservation, the amplitude of AMPA potentials increased significantly, on the contrary, the amplitude of NMDA potentials was equal to the value before freezing (B).

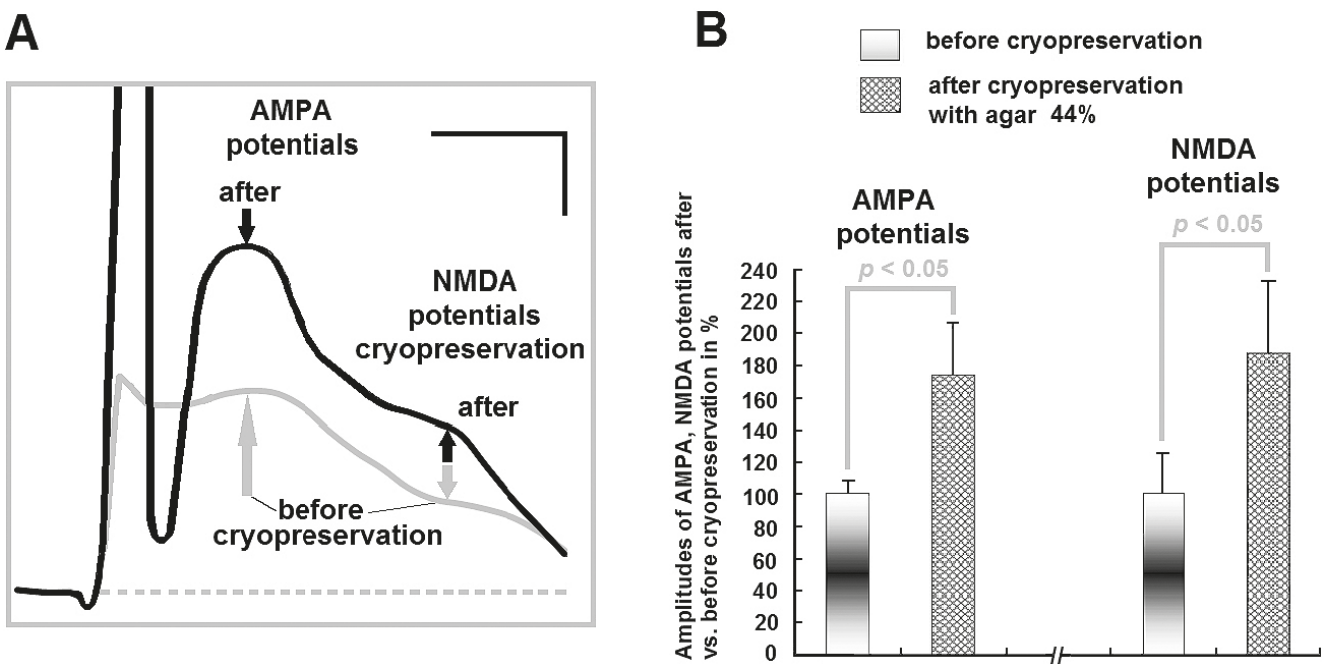

Fig. 5. Modifications of AMPA and NMDA potential profiles before and after cryopreservation of slices for 52 days at –10 °C in aCSF with agar concentrations of 44 %. The notations are the same as in Fig. 4.

(n = 7) compared to the value before cryopreservation, i. e. there was hyperactivation (Fig. 5).

Consequently, the cryopreservation of slices in gel solution with agar concentration of 44 % induces hyperactivation of glutamatergic ionotropic both AMPAR and NMDAR and is not optimal.

Further, the concentration of agar in the gel medium was increased to 50 %. Under these conditions of cryopreservation of slices the amplitude of AMPA potentials was restored, but the degree of recovery was lower than before cryopreservation, on average by 12 % (n = 9). The amplitude of the presynaptic component

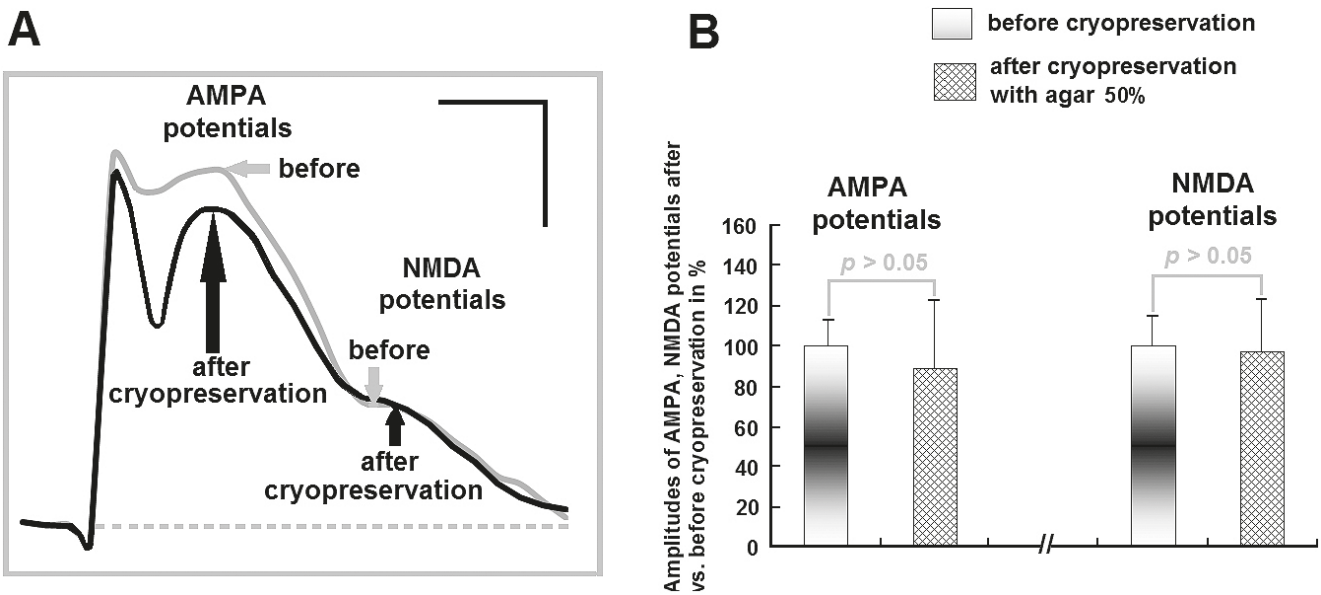

Fig. 6. Modifications of AMPA and NMDA potential profiles before and after cryopreservation of slices for 52 days at –10 °C in aCSF with agar concentrations of 50 %. The notations are the same as in Fig. 4.

AP LOT was restored to values before cryopreservation (Fig. 6).

It is noteworthy that the amplitude of NMDA potentials recovered completely after cryopreservation. Importantly, the decline curve of NMDA potentials was congruent with the curve before cryopreservation, which also indicates an improvement in cryopreservation of NMDAR (Fig. 6).

These results indicate that the cryopreservation of slices in gel medium with agar concentration of 50 % promotes complete recovery of both AMPAR and NMDAR. Consequently, the concentration of agar 50 % in medium does not induce cryodamages of both glutamatergic ionotropic mechanisms, determined by the electrophysiological testing and is optimal for their long-term cryopreservation.

Discussion

The study describes a cryopreservation protocol for brain slice, an important source of nerve tissue for application in therapy against such neuropathologies as epilepsy, stroke and trauma. The aim of our study was to achieve the preservation of AMPAR and NMDAR activity after cryopreservation. To preserve the activity of these mechanisms during long-term cryopreservation, a storage solution, consisting of agar and artificial cerebrospinal fluid was used. During freezing (–10 °C), the slices were encapsulated in agar solution. At the end of cryopreservation (52 days), the gel solution was warmed up (+37 °C) and turned into a liquid, that is, decapsulated.

Agar (a mixture of agarose and agaropectin polysaccharides) is used in biological studies due to its biocompatibility and biodegradability properties and is considered a suitable biomaterial to mimic the extracellular matrix scaffolds with a porous structure. We hypothesized the use of agar hydrogel would minimize the damage to AMPAR and NMDAR during the cryopreservation process. As indicators of the viability of the slices, we investigated the changes in the amplitudes of the AMPA and NMDA potentials caused by electrical stimulation of the LOT.

The data obtained indicate that the cryopreservation of slices in gel solution with agar contributes to 100 % preservation of AMPAR and NMDAR activity during cryopreservation (–10 °C, 52 days). It is important to note that during the cryopreservation of slices in aCSF solution (without agar) only AMPAR were preserved, NMDAR, on the contrary, were irreversibly blocked.

We used different concentrations of agar in the cryopreservation solution. It turned out that the degree of recovery of AMPAR and NMDAR after cryopreservation of slices in gel solution was the most optimal when the agar concentration was 50 % in freezing solution. At this concentration there was a complete recovery of AMPAR and NMDAR.

Note that the use of media with agar at concentrations of 33 and 44 % on AMPAR and NMDAR did not cause a pronounced protective effect compared with the cryopreservation without agar. AMPAR activities both with and without the use of agar were amplified in equal values. In interpreting the data obtained, the composition of AMPAR and NMDAR subunits should be taken into account. Stressors such as freezing/thawing have been found to alter the activity of these receptor subunits so that AMPAR is hyperactivated and NMDAR is blocked. Blockade of NMDAR activity probably occurs by inhibiting the main NR1 subunit of these receptors (Mokrushin,

2019). Hence, agar shows its properties as a cryoprotector for preserving the activity of NMDAR subunits.

The data obtained indicate the protective property of agar during cryopreservation of brain slices. It is known that there are two main types of cryoprotectants: penetrating and non-penetrating (Matsumura, Hayashi, and Nagashima, 2021). The first include cryoprotectants that penetrate into the cell. Their molecular weight does not exceed 100 Da (Pegg, 2010). The most common penetrating cryoprotectants are: dimethylsulfoxide; glycerin, propylene glycol, ethylene glycol et al. All of them are toxic when exposed to nerve cells and synapses (Pichugin, 2013) and were not used in our studies for this reason.

The second type includes cryoprotectants (for example, sucrose and trehalose) and high molecular weight compounds (ficoll, albumin and other) that do not penetrate into cells with molecular weights of tens to hundreds of kDa. They are less toxic than penetrating cryoprotectants at equal concentrations (Stiff et al., 1983; Stolzing, Naaldjik, Fedorova, and Sethe, 2012; Matsumura, Hayashi, and Nagashima, 2021). Obviously, in terms of molecular weight (32–200 kDa), agar should be classified as a non-penetrating type of a cryoprotectant. By its chemical nature, agar is a biopolymer, the macromolecules of which are formed by two polysaccharides agarose and agaropectin.

Agarose, which is part of agar (50–80 %), is a linear polysaccharide and is regarded as a less high molecular weight fraction of the polymer, while agaropectin has a network structure with a higher molecular weight (Delattre,Fenoradosoa, and Michaud, 2011; Usov, 2011; Shipunov, Koptev, and Markin, 2018).

It has been established that agar in aqueous solutions exhibits a high ability to hydrate even at its low concentrations (Usov, 1998; Shipunov, Koptev, and Markin, 2018). Due to the large surface of agar gel, when it comes into contact with an aqueous solution, rapid diffusion into the polymer structure of both water molecules and substances dissolved in it occurs (Antsiferov, Kudryavtseva, and Soboleva, 2010). Obviously, these properties of the agar gel cause the absorption of water molecules from the intercellular space of the slices. Therefore, we believe that agar contributes to the protection of nerve cell membranes from osmotic stress, formation of ice crystals and their destructive effect on membranes during cryopreservation. It was revealed that such (protective) properties of agar as water absorption depend significantly on its concentration in an aqueous solution (Antsiferov, Kudryavtseva, and Markin, 2018), which was confirmed in our studies. We found that to maintain the activity of AMPAR and NMDAR, the optimal concentration was 50 % agar in freezing solution.

It should be taken into account that the processes of formation of the structure of agar and its interaction with AMPAR and NMDAR will change in different phases of the cryopreservation process: freezing-storage-warming. These physical effects on AMPAR and NMDAR are likely to affect their function depending on the concentration of agar used.

Conclusions

We believe that our protocol for cryopreservation of brain slices in a capsule consisting of agar and cerebrospinal fluid contributes to the preservation of glutamatergic ionotropic AMPA and NMDA mechanisms. The presented data prove that the agar capsule for cryopreservation of slices is a biomaterial to mimic the extracellular matrix that protects the key vulnerable structures of neurons from cryodamage. The protocol we developed for cryopreservation of brain explants in an agar capsule will contribute to the creation of a biobank of nervous tissue for clinical use in neuropathology therapy.

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