Improvement of the Acid-Alkaline Composition of the Medium for Prolonged and Reversible Cryopreservation of Rat Brain Slices

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Previously, it was found that the activity of glutamatergic ionotropic *N*-methyl-D-aspartate receptors (NMDAR) after long-term cryopreservation of brain slices at a temperature of –10°C decreased or blocked. The slices of the olfactory cortex of rats were used to study the mechanisms of NMDAR cryodestruction. To recover after cryopreservation NMDAR activity examined the effects of pH changes in extracellular freezing media during registration NMDA-potentials induced by electrical stimulation of the lateral olfactory tract. It was found that, after cryopreservation, the freezing medium was acidified to pH 6.5, while the amplitude of the NMDA potentials decreased in comparison with the amplitudes before cryopreservation. To preserve the NMDAR activity after cryopreservation, the buffer capacity of the freezing medium was increased by using a hybrid buffer system: carbonate ($NaHCO_3$), phosphate (KH_2PO_4) tris(hydroxymethyl)aminomethane ($(HOCH_2)_3CNH_2$), the salt composition did not change when using such a buffer system. After cryopreservation of the slices in such a medium, the amplitude of the NMDA potentials was 22 ± 7% compared to the amplitude before cryopreservation. Additionally, a method was used to increase the pH of the medium to 7.6–7.7 in the process of freezing the slices in the temperature range 20–22°C. This was followed by recovery NMDAR activity. Thus, the use of a hybrid buffer system in a freezing medium and a simultaneous increase in its pH to 7.6–7.7 promoted the recovery of NMDAR after cryopreservation.

Keywords: brain slices, NMDA potentials, cryopreservation, buffer systems