ELSEVIER

Brain Research Protocols 3 (1998) 76–82

BRAIN RESEARCH PROTOCOLS

Protocol

Cryopreservation of rat cortical synaptosomes and analysis of glucose and glutamate transporter activities, and mitochondrial function

James G. Begley [a], D. Allan Butterfield [b,c], Jeffrey N. Keller [a,d], Tanuja Koppal [b], Jennifer Drake [b], Mark P. Mattson [a,c,e,*]

[a] *Sanders-Brown Research Center on Aging, University of Kentucky, Lexington, KY 40536, USA*
[b] *Department of Chemistry, University of Kentucky, Lexington, KY 40536, USA*
[c] *Center of Membrane Sciences, University of Kentucky, Lexington, KY 40536, USA*
[d] *Department of Biological Sciences, University of Kentucky, Lexington, KY 40536, USA*
[e] *Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536, USA*

Accepted 29 April 1998

Abstract

Direct comparisons of synaptic functional parameters in brain tissues from different groups of experimental animals and different samples from post mortem human brain are often hindered by the inability to perform assays at the same time. To circumvent these difficulties we developed methods for cryopreservation and long-term storage of neocortical synaptosomes. The synaptosomes are suspended in a cryopreservation medium containing 10% dimethylsulfoxide and 10% fetal bovine serum, and are slowly cooled to −80°C and then stored in liquid nitrogen. The function of plasma membrane glucose and glutamate transporters, and mitochondrial electron transport activity and membrane potential were measured in fresh, cryopreserved (CP), and non-cryopreserved freeze–thawed (NC) synaptosomes. Glucose and glutamate transporter activities, and mitochondrial functional parameters in CP synaptosomes were essentially identical to those in fresh unfrozen synaptosomes. Glucose and glutamate transport were severely compromised in NC synaptosomes, whereas mitochondrial function and cellular esterase activity were largely maintained. Electron paramagnetic resonance studies in conjunction with a protein-specific spin label indicated that cryopreservation did not alter the physical state of synaptosomal membrane proteins. These methods provide the opportunity to generate stocks of functional synaptosomes from different experiments or post mortem samples collected over large time intervals.

Themes: Excitable membranes and synaptic transmission

Topics: Presynaptic mechanisms

Keywords: Cerebral cortex; Electron paramagnetic resonance spectroscopy; Esterase; Excitatory amino acid; Mitochondrial respiration

1. Type of research

- Establishment of ‘synaptosome banks’ for studies of synaptic physiology and pathophysiology.
- Simultaneous analyses of synaptic functional parameters in post mortem brain tissues from adult rats and human patients collected at different times.
- Quantification of glucose and glutamate transport in synaptosomes.
- Quantification of mitochondrial function in synaptosomes.

2. Time required

- Synaptosome preparation. The time period from removal of brain tissue until pure synaptosomes are obtained is 5–6 h.
- Glucose and glutamate transport assays. Each assay can be completed within 2–3 h.
- Assay of mitochondrial function. The MTT assay requires 4–5 h.
- Analysis of membrane protein structure. Electron para-

* Corresponding author. 211 Sanders-Brown Building, University of Kentucky, Lexington, KY 40536-0230, USA. Fax: +1-606-323-2866; E-mail: mmattson@aging.coa.uky.edu

1385-299X/98/$ - see front matter
PII: S1385-299X(98)00024-5

magnetic resonance spectroscopy (EPRS) analysis of protein conformation using the MAL-6 spin label requires 3–4 h.
- Assay of membrane integrity. The protocol for assessing the ability of synaptosomes to accumulate the fluorescent probe calcein-AM takes approximately 2 h.

3. Materials

3.1. Animals

Adult male Sprague–Dawley rats (250–300 g) were purchased from Harlan (Indianapolis, IN).

3.2. Chemicals, reagents and kits

- Homogenization buffer. 0.32 M sucrose, 4 μg/ml pepstatin, 5 μg/ml aprotinin, 20 μg/ml trypsin inhibitor, 4 μg/ml leupeptin, 0.2 mM PMSF, 2 mM EDTA, 2 mM EGTA, and 20 mM HEPES (all from Sigma, St. Louis, MO).
- Locke's buffer. NaCl, 154 mM; KCl, 5.6 mM; $CaCl_2$, 2.3 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 3.6 mM; glucose, 5 mM; HEPES, 5 mM (reagents from Sigma).
- Cryovials (1.0 ml capacity) were purchased from Fisher Scientific (Pittsburgh, PA).
- [^{3}H]-2-deoxy-glucose and [^{3}H]glutamate were purchased from Amersham (Chicago, IL).
- Whatman GF/C glass microfibre filters with 1.2-μm retention (Whatman #1822-024) were purchased from Fisher Scientific.
- Scintillation vials and Scintiverse solution were purchased from Fisher Scientific.
- Calcein-AM was purchased from Molecular Probes (Eugene, OR).
- 2,2,6,6-tetramethyl-4-maleimidopiperidine-1-oxyl (MAL-6) was purchased from Sigma.
- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma.
- 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolocarbocyanine iodide (JC-1) was purchased from Molecular Probes.
- Other reagents. Sucrose, dimethylsulfoxide (cell culture grade), and all other chemicals were purchased from Sigma.

3.3. Special equipment

- A Millipore Cytofluor 2350 fluorescence plate reader was employed to quantify calcein-AM fluorescence in synaptosomes.
- A Bruker 300 EPR spectrometer (Bruker, Billerica, MA) was used to acquire EPR spectra.
- A CERES 900 visible light plate reader was used to quantify levels of MTT reduction.
- A Millipore Cytofluor 2350 fluorescence plate reader was used to quantify JC-1 fluorescence.

4. Detailed procedure

4.1. Synaptosome preparation and cryopreservation

Adult male Sprague–Dawley rats (250–300 g) were anesthetized with halothane and decapitated. The brain was removed and the cerebral cortex cut into small fragments, and homogenized in a solution (cortex from 2 brains/10 ml buffer) containing 0.32 M sucrose, 4 μg/ml pepstatin, 5 μg/ml aprotinin, 20 μg/ml trypsin inhibitor, 4 μg/ml leupeptin, 0.2 mM PMSF, 2 mM EDTA, 2 mM EGTA, and 20 mM HEPES (pH 7.4). The homogenate was centrifuged for 10 min at $300 \times g$ at 4°C in a Sorvall RT6000B centrifuge with a swinging bucket rotor. The supernatant was then centrifuged for 10 min at $12{,}400 \times g$ at 4°C in a Beckman Model J2-21 centrifuge using a JA-20 fixed angle rotor; the pellet was resuspended in homogenization buffer and re-centrifuged at $20{,}250 \times g$. The pellet was collected and resuspended in 1.5 ml of 0.32 M sucrose and placed atop a sucrose gradient (7 ml 1.18 M sucrose, pH 8.5; 7 ml 1 M sucrose, pH 8.0; 7 ml 0.85 M sucrose, pH 8) and centrifuged at $87{,}275 \times g$ for 2 h at 4°C in a Beckman model L-80 ultracentrifuge using a SW-28 swinging bucket rotor. Synaptosomes in the 1 M sucrose/1.18 M sucrose interface were removed, resuspended in PBS, and centrifuged at 10,000 rpm for 10 min at 4°C. The pellet was resuspended in Locke's buffer (NaCl, 154 mM; KCl, 5.6 mM; $CaCl_2$, 2.3 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 3.6 mM; glucose, 5 mM; HEPES, 5 mM; pH 7.2) at a concentration of 1 mg protein/ml. Protein concentration was quantified using a BCA kit (Pierce). Frozen non-cryopreserved synaptosomes were prepared by placing tubes containing synaptosomes in a −80°C freezer for 24–48 h and then thawing to room temperature. For cryopreservation, synaptosomes were suspended in Locke's buffer containing 10% dimethylsulfoxide (v/v) and 10% fetal bovine serum (v/v), transferred to cryovials (1 ml/vial), and the vials were cooled to −80°C at a rate of approximately −1°C/min and were then stored in liquid nitrogen for 48 h–2 months. The cooling rate was controlled by enclosing the cryovials in a styrofoam 'sandwich' consisting of two sheets of styrofoam, each 3 cm thick. Synaptosomes were then rapidly thawed by placing the cryovials in a water bath at 37°C.

4.2. Glucose and glutamate transport assays

These methods were similar to those described previously [14,22]. Briefly, synaptosomes (200 μg protein/tube) were washed three times with either normal Locke's solution (glutamate uptake assay) or Locke's solu-

tion lacking glucose, and the assay was started by the addition of either [^{3}H]-2-deoxy-glucose (1.5 μCi) or [^{3}H]glutamate (0.1 μCi/ml; specific activity 0.2 μCi/ml). Seven minutes later the assay was stopped by placing the synaptosomes on Whatman filters in a vacuum filtration apparatus and rapidly washing three times with Locke's solution (3 ml/wash). The filters were then placed in scintillation vials containing Scintiverse and radioactivity determined by scintillation spectroscopy.

4.3. Assays for membrane integrity / esterase activity and protein conformation

In order to evaluate the integrity of synaptic membranes and, at the same time, determine whether the synaptosomes retain functional cytoplasmic enzymes, we employed the acetoxymethyl ester form of the fluorescent dye calcein (calcein-AM; Molecular Probes, Eugene, OR). Synaptosomes were incubated for 30 min in the presence of 5 μM calcein-AM. The synaptosomes were then washed twice with Locke's buffer and fluorescence was quantified using a UV plate reader (488 nm excitation and 510 nm emission). Protein conformation was assessed using an electron paramagnetic resonance (EPR) spin labeling method that employs the protein-specific spin label 2,2,6,6-tetramethyl-4-maleimidopiperidine-1-oxyl (MAL-6) which forms covalent bonds with cysteine SH groups [1,2]. Spin labeling of synaptosomal membrane proteins was performed as described previously [10]. After incubation with MAL-6 (20 μg MAL-6/mg protein) for 16–18 h, synaptosomes were washed six times with lysing buffer (10 mM HEPES, 2 mM EDTA, 2 mM EGTA; pH 7.4) to remove excess spin label. The pellet was then resuspended in approximately 400 μl lysing buffer and allowed to come to room temperature. EPR spectra were acquired on a Bruker 300 EPR spectrometer (Bruker, Billerica, MA) operating at the following conditions: incident microwave power of 18 mW; modulation amplitude of 0.4 G; time constant of 1.28 ms; a conversion time of 10 ms; and sweep width of 40 G.

4.4. Assays of mitochondrial function and transmembrane potential

The conversion of the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan crystals in cells has been shown to be related to function of mitochondrial respiratory chain reactions [20]. MTT was dissolved in D-PBS at a concentration of 2.5 mg/ml. The MTT solution was mixed with synaptosomes (1:10 MTT:synaptosomes, v:v) and allowed to incubate 3–4 h. The synaptosomes were then pelleted by centrifugation, the pellet was suspended in solubilization buffer (100% dimethylsulfoxide), and absorbance (592 nm) of each sample was quantified using a plate reader. The fluorescent probe JC-1 was used as a measure of mitochondrial transmembrane potential, using methods similar to those described previously [26]. Briefly, synaptosomes were incubated for 30 min in the presence of 20 μM JC-1 and were then washed twice with Locke's solution. Synaptosomes were resuspended in Locke's solution and fluorescence was measured using a Millipore Cytofluor 2350 fluorometric plate reader. Relative mitochondrial transmembrane potential was determined by taking the ratio of the fluorescence emission intensity at 530 and 490 nm, with excitation at 500 nm.

5. Results

5.1. Glucose and glutamate transport in control and cryopreserved synaptosomes

Levels of [^{3}H]glucose uptake in fresh and cryopreserved (CP) synaptosomes were not significantly different (Fig. 1A). In contrast, levels of glucose uptake in NC synaptosomes were significantly reduced to less than 50% of control levels. Exposure of CP synaptosomes to phloretin, a selective inhibitor of the glucose transporter, reduced the level of glucose uptake by more than 80% (Fig. 1A) indicating that the vast majority of glucose uptake into the synaptosomes was mediated by the plasma membrane glucose transporter. Levels of [^{3}H]glutamate uptake in fresh and cryopreserved (CP) synaptosomes were not significantly different (Fig. 1B). In contrast, levels of glutamate uptake in NC synaptosomes were significantly reduced to approximately 10% of control levels. Exposure of CP synaptosomes to excess unlabeled glutamate reduced the level of [^{3}H]glutamate uptake to approximately 12% of the control level (Fig. 1B), and the glutamate transport inhibitor L-*trans*-2,4-PDC inhibited greater than 90% of the [^{3}H]glutamate uptake (data not shown), indicating that the vast majority of glutamate uptake into the synaptosomes was mediated by a specific transport mechanism.

5.2. Membrane protein conformation in cryopreserved synaptosomes

In order to determine whether membrane protein structure was modified by the CP procedure, we performed EPR spectroscopy analyses using the spin label MAL-6 [1,2,10,25]. This protein-specific spin label selectively binds to thiol moieties on protein cysteine residues located in two different types of environments. Sulfhydryls located on the surface of protein molecules only weakly restrict the rotational motion of the spin label, allowing essentially free rotations of MAL-6, which produce relatively narrow resonance lines in the EPR spectrum of labeled proteins. In contrast, the environment of sulfhydryls, buried in a deep cleft in the protein, hinders the rotational motion of the bound spin label, due to steric effects, yielding broadened resonance lines in the EPR spectrum of labeled proteins.

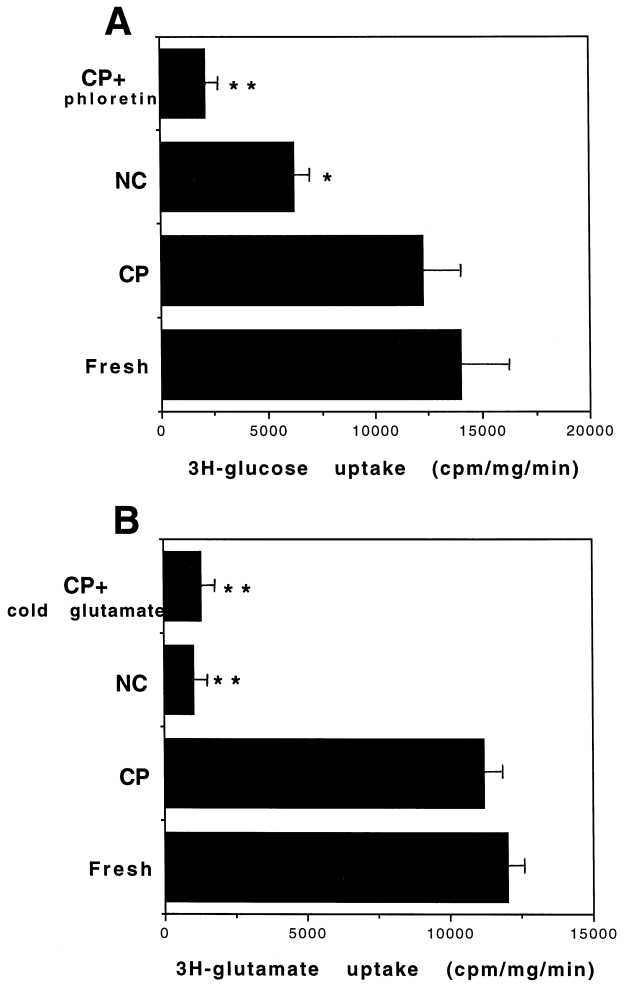

Fig. 1. Glucose and glutamate transport activities are retained in cryopreserved synaptosomes. [^{3}H]glucose uptake (A) and [^{3}H]glutamate uptake (B) were quantified in freshly prepared (unfrozen) synaptosomes, cyropreserved synaptosomes, and non-cryopreserved (frozen) synaptosomes (see Section 4). Phloretin (50 μM; a specific inhibitor of high-affinity glucose transport) or unlabeled glutamate (100 μM) were included in some samples of cryopreserved synaptosomes. Values are the mean and S.E.M. of determinations made in 4–8 synaptosome preparations. $*\ p < 0.01$, $**\ p < 0.001$ compared to values for fresh and cryopreserved synaptosomes (ANOVA with Scheffe's post-hoc tests).

The resulting composite EPR spectrum reflects both environments (Fig. 2A). The ratio of the EPR spectral amplitudes of the weakly-immobilized line (W) to that of the strongly-immobilized line (S) in the low-field region of the EPR spectrum of MAL-6-labeled synaptosomal membranes, the W/S ratio, is highly sensitive to protein conformational changes and protein–protein interactions [1,4,13]. The W/S ratio of MAL-6 was not significantly different in CP synaptosomes compared to fresh control synaptosomes (Fig. 2B) indicating no change in the physical state of membrane proteins. In contrast, NC synaptosomes exhibited a significantly decreased W/S ratio ($p < 0.005$), suggesting an altered conformation of membrane proteins (Fig. 2B).

5.3. Membrane integrity and esterase activity in cryopreserved synaptosomes

We next incubated fresh, CP and NC synaptosomes in the presence of the acetyoxymethyl ester form of the fluorescent dye calcein (calcein-AM). This lipophilic dye passes into cells wherein endogenous esterases cleave the ester bond thus releasing the methyl group, which renders the calcein molecule hydrophilic and traps it inside the cell. Thus, the level of calcein fluorescence is an indicator of membrane integrity and esterase activity. Levels of calcein fluorescence were decreased by approximately 20% in CP synaptosomes and by approximately 40% in NC

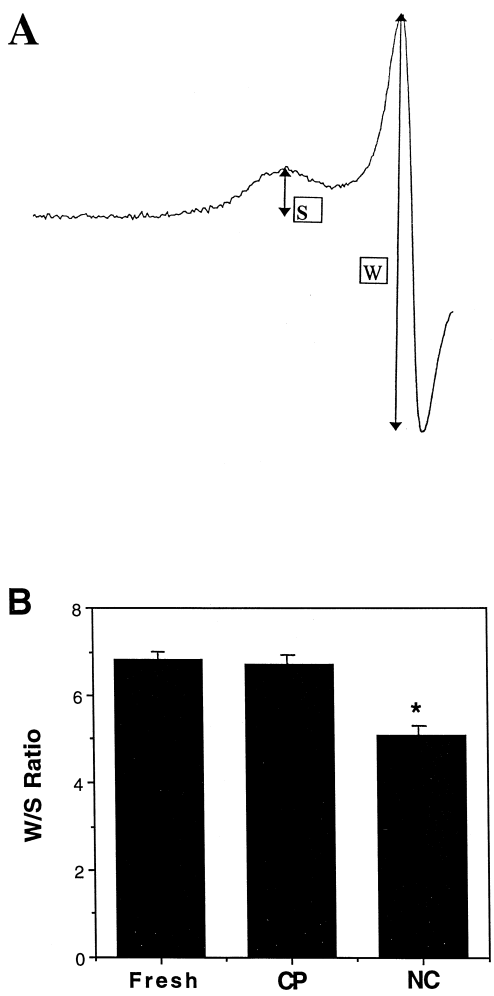

Fig. 2. Synaptosomal membrane protein structure is unaffected by cryopreservation. (A) Typical EPR spectrum of the lo-field resonance lines of MAL-6 covalently attached to membrane proteins in cortical synaptosomal membranes. The W and S spectral components, from which the W/S ratio is calculated, are indicated. (B) Synaptosomal membrane protein infrastructure is unaffected by cryoprotection. Fresh, CP and NC synaptosomes (4 mg protein/ml lysing buffer) were spin labeled with MAL-6, and the W/S ratio of this protein-specific spin label was quantified. Values are the mean and S.E.M. ($n = 4$). $*\ p < 0.005$ compared to the values for fresh and CP synaptosomes.

synaptosomes compared to the fluorescence level in fresh synaptosomes (Fig. 3A).

5.4. Mitochondrial function and transmembrane potential in cryopreserved synaptosomes

Mitochondria have been reported to be relatively resistant to freeze–thaw [17]. We therefore quantified levels of MTT reduction, a measure of mitochondrial energy charge and redox status [20,23] in fresh, CP and NC synaptosomes. Levels of MTT reduction were essentially identical in fresh, CP and NC synaptosomes (Fig. 3B), indicating that a freeze–thaw cycle in the absence of cryopreservation does not adversely affect mitochondrial respiration in synaptosomes. In an additional experiment, we assessed

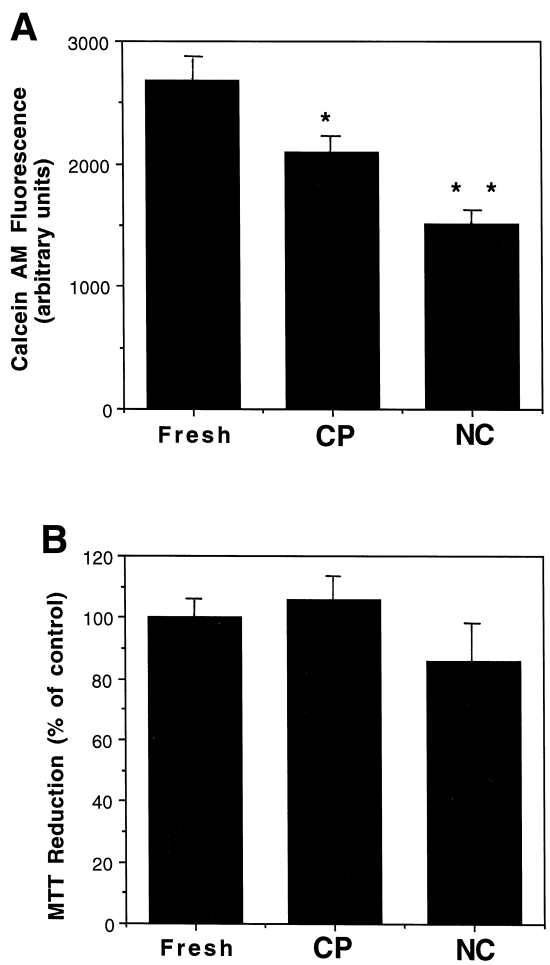

Fig. 3. Plasma membrane integrity, esterase activity and mitochondrial function are maintained in cyropreserved synaptosomes. (A) Calcein fluorescence was quantified in fresh, CP and NC synaptosomes (see Section 4). Values are the mean and S.E.M. of six synaptosome samples. $* p < 0.05$ compared to the value for fresh synaptosomes; $* * p < 0.01$ compared to the value for fresh synaptosomes and $p < 0.05$ compared to the value for CP synaptosomes. ANOVA with Scheffe's post-hoc tests. (B) Levels of MTT reduction (a measure of mitochondrial energy charge and redox state) were quantified in fresh, CP and NC synaptosomes. Values are the mean and S.E.M. of determinations made in three separate preparations.

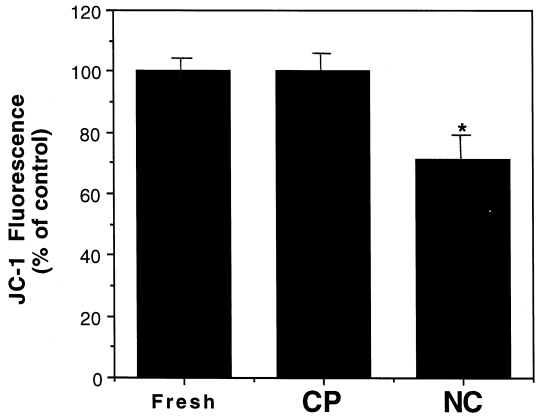

Fig. 4. Mitochondrial transmembrane potential is maintained in cryopreserved synaptosomes. Levels of JC-1 fluorescence, a measure of mitochondrial transmembrane potential, were quantified in fresh, CP and NC synaptosomes. Values are the mean and S.E.M. of determinations made in three separate preparations. $* p < 0.05$ compared to values for fresh and CP synaptosomes.

mitochondrial transmembrane potential using the fluorescent probe JC-1 [26]. Levels of JC-1 fluorescence were similar in fresh and CP, but were significantly decreased in NC synaptosomes (Fig. 4), indicating that mitochondrial transmembrane is not adversely affected by cryopreservation.

6. Troubleshooting

6.1. Tissue preparation

Ideally, synaptosomes should be prepared as soon as possible following death. However, we have successfully prepared 'viable' synaptosomes from rat and mouse brains that had been kept at 4°C for up to 18 h, and from human hippocampal and cortical tissues with up to a 12 h post mortem interval. In order to obtain a high yield of synaptosomes, homogenization must be thorough with at least 15–20 strokes in a Dounce homogenizer. It is important to limit the amount of cortical tissue loaded into each sucrose gradient to that obtained from two brains. Increasing the amount of tissue results in poorer separation of synaptosomes and a lower yield of synaptosomes/brain.

6.2. Sucrose gradient

Sucrose solutions should be swirled before use to ensure the suspension is thoroughly mixed. Failure to mix the solution before pouring the gradient may result in indistinct boundaries in the gradient. Sucrose solutions should be added slowly to prevent mixing of the layers—this can be accomplished by adding 1 ml of solution at a time with a pipette. Following separation of the cortical tissue in the gradient, there will often be one band of 'debris' at the top of the gradient. If bands other than that at the 1 M

sucrose/1.18 M sucrose interface are present, then it is likely that the sucrose solutions were improperly prepared. Finally, there are alternative methods for preparing synaptosomes which are less time-consuming including the use of a Percoll gradient; the effectiveness of such methods in preserving synaptosomal functions following cryopreservation remains to be determined.

7. Discussion

Synaptosomes, which are prepared by centrifugation of nervous tissue homogenates through a density gradient, consist of pre- and post-synaptic elements of neurons, and associated astrocytic end feet [5,11,19]. Studies of synaptosomal preparations have provided insight into regulation of a variety of synaptic functions including neurotransmitter release and reuptake [21,27], energy metabolism [6] and ion transport systems [7]. In addition, mechanisms of synaptic dysfunction and degeneration that may occur in neurological and neurodegenerative disorders such as Parkinson's disease [18] schizophrenia [24] and Alzheimer's disease [14,15,25] may be studied in synaptosomal preparations. Unfortunately, analyses of many functional parameters in synaptosomes require that the synaptosomes be freshly prepared because, as with intact cells, a freeze–thaw cycle will disrupt membranes and damage the synaptosomes and render them dysfunctional. In many circumstances (e.g., studies of synaptosomes from patients with neurodegenerative disorders) the requirement for fresh synaptosomes precludes direct comparisons of synaptosomes among patients.

Adapting procedures previously employed for cryopreservation of primary rat hippocampal neurons [16], we have developed a protocol for cryopreservation of synaptosomes prepared from neocortex of adult rats. The present findings demonstrate that plasma membrane glutamate and glucose transport systems, and mitochondrial transmembrane potential and electron transport activities, are maintained in CP synaptosomes at levels essentially identical to freshly prepared synaptosomes. Additionally, magnetic resonance studies showed no alteration in the physical state of synaptosomal membrane proteins in cryopreserved samples. The slight reduction in the ability of CP synaptosomes to accumulate calcein-AM may be due to reduced esterase activity, because the data from glucose and glutamate transport studies make it unlikely that the membranes were disrupted in the CP synaptosomes. The altered membrane protein conformation detected by EPR analysis of MAL-6 in NC synaptosomes, and the reduced ability of NC synaptosomes to retain calcein-AM strongly suggest that freeze–thaw causes severe damage to the plasma membranes. Increased protein–protein interactions, decreased segmental motion, and/or conformational changes in spin-labeled proteins leads to decreased molecular motion, resulting in a lowering of the W/S ratio. Such changes in membrane protein conformation can be caused by a variety of perturbations including exposure of cells to oxidative stress [3,4,10,13,25]. The membrane damage was correlated with severe compromise of glucose and glutamate transport systems in NC synaptosomes. These results suggest that failed membrane transport systems were probably not secondary to energy failure.

Previous studies have shown that neurotransmitter release and uptake can be quantified in cyropreserved synaptosomes prepared from post mortem human and rodent brains [8,9,12,24]. We have found that the same methods for cryopreservation of rat cortical synaptosomes described in the present study can be successfully applied to synaptosomes prepared from post mortem human brain tissues (M.P.M. and J.N.K., unpublished data). The ability to cryopreserve synaptosomes therefore provides the opportunity to establish 'banks' of synaptosomes from patients with different neurological disorders (and controls), and then to simultaneously perform various functional assays on synaptosomes from different cases. Synaptosomal cryopreservation can also be employed as a time-saving approach that allows the maintenance of large stocks of synaptosomes, thereby reducing the need for repeated preparation of fresh synaptosomes.

8. Essential literature references

Original papers: Refs. [2,5,15,20,27].

Acknowledgements

This work was supported by grants to M.P.M. from the NIH (AG14554, AG05119 and AG05144) and to D.A.B. from the NIH (AG10836 and AG05119).

References

[1] D.A. Butterfield, Spin labeling in disease, Biol. Magn. Resonance 4 (1982) 1–78.
[2] D.A. Butterfield, Principles of biological membrane structure: alteration in the physical state of one side of the plasma membrane by modulation of the physical state of the opposite side, J. Membr. Sci. 53 (1990) 3–17.
[3] D.A. Butterfield, β-amyloid-associated free radicals, oxidative stress and neurotoxicity: implications for Alzheimer's disease, Chem. Res. Toxicol. 10 (1997) 495–506.
[4] D.A. Butterfield, B.J. Howard, S. Yatin, K.Z. Allen, J.M. Carney, Free radical oxidation of brain proteins in accelerated senescence and its modulation by *N-tert*-butyl-α-phenylnitrone, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 674–678.
[5] C.W. Cotman, Isolation of synaptosomal and synaptic plasma membrane fractions, Methods Enzymol. 31 (1974) 445–452.
[6] M. Erecinska, D. Nelson, Effects of 3-nitropropionic acid on synaptosomal energy and transmitter metabolism: relevance to neurodegenerative brain diseases, J. Neurochem. 63 (1994) 1033–1041.
[7] G. Fontana, R.S. Rogowski, M.P. Blaustein, Kinetic properties of the sodium–calcium exchanger in rat brain synaptosomes, J. Physiol. 485 (1995) 349–364.

[8] J. Gleitz, A. Beile, B. Vilffert, F. Tegtmeier, Cryopreservation of freshly isolated synaptosomes prepared from the cerebral cortex of rats, J. Neurosci. Methods 47 (1993) 191–197.
[9] N. Haberland, L. Hetey, Studies in post mortem dopamine uptake: I. Kinetic characterization of the synaptosomal dopamine uptake in rat and human brain after post mortem storage and cryopreservation. Comparison with noradrenaline and serotonin uptake, J. Neural Transm. 68 (1987) 289–301.
[10] K. Hensley, J.M. Carney, N.C. Hall, D.A. Butterfield, Electron paramagnetic resonance investigations of free radical induced alterations in neocortical synaptosomal membrane protein infrastructure, Free Radic. Biol. Med. 17 (1994) 321–333.
[11] L. Hertz, Functional interactions between neurons and astrocytes: I. Turnover and metabolism of putative amino acid transmitters, Prog. Neurobiol. 13 (1979) 277–323.
[12] L. Hetey, R. Schwizkosky, T. Ott, H. Barz, Diminished synaptosomal dopamine (DA) release and DA autoreceptor supersensitivity in schizophrenia, J. Neural Transm. 83 (1991) 25–35.
[13] B.J. Howard, S. Yatin, K. Hensley, K.L. Allen, J.P. Kelly, J.M. Carney, D.A. Butterfield, Prevention of hyperoxia-induced alterations in synaptosomal membrane-associated proteins by *N-tert*-butyl-α-phenylnitrone and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (tempol), J. Neurochem. 67 (1996) 2045–2050.
[14] J.N. Keller, R.J. Mark, A.J. Bruce, E.M. Blanc, J.D. Rothstein, K. Uchida, M.P. Mattson, 4-hydroxynonenal, an aldehydic product of membrane lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes, Neuroscience 80 (1997) 685–696.
[15] J.N. Keller, Z. Pang, J.W. Geddes, J.G. Begley, A. Germeyer, G. Waeg, M.P. Mattson, Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid β-peptide: role of the lipid peroxidation product 4-hydroxynonenal, J. Neurochem. 69 (1997) 273–284.
[16] M.P. Mattson, S.B. Kater, Isolated hippocampal neurons in cryopreserved long-term cultures: development of neuroarchitecture and sensitivity to NMDA, Int. J. Dev. Neurosci. 6 (1988) 439–452.
[17] L.E. McGann, H.Y. Yang, M. Walterson, Manifestations of cell damage after freezing and thawing, Cryobiology 25 (1988) 178–185.
[18] K.S. McNaught, U. Thull, P.A. Carrupt, C. Altomare, S. Cellamare, A. Carotti, B. Testa, P. Jenner, C.D. Marsden, Inhibition of [^{3}H]dopamine uptake into striatal synaptosomes by isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Biochem. Pharmacol. 52 (1996) 29–34.
[19] E.E. Mena, C.A. Hoeser, B.W. Moore, An improved method of preparing rat brain synaptic membranes. Elimination of a contaminating membrane containing 2′,3′-cyclic nucleotide 3′-phosphohydrolase activity, Brain Res. 188 (1980) 207–231.
[20] D.A. Musser, A.R. Oseroff, The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells following in vitro photodynamic therapy with Photofrin II, Photochem. Photobiol. 59 (1994) 621–626.
[21] D.G. Nicholls, Release of glutamate, aspartate, and gamma-aminobutyric acid from isolated nerve terminals, J. Neurochem. 52 (1989) 331–341.
[22] S. Pogun, V. Dawson, M.J. Kuhar, Nitric oxide inhibits [^{3}H]-glutamate transport in synaptosomes, Synapse 18 (1994) 21–26.
[23] M. Shearman, S. Hawtin, V. Taylor, The intracellular component of cellular 3-(4:5-dimethylthiazol-2-yl)-2:5-diphenyltetrazolium bromide (MTT) reduction is specifically inhibited by β-amyloid peptides, J. Neurochem. 65 (1995) 218–227.
[24] A.D. Sherman, T.S. Hegwood, S. Baruah, R. Waziri, Deficient NMDA-mediated glutamate release from synaptosomes of schizophrenics, Biol. Psychiatry 30 (1991) 1191–1198.
[25] R. Subramaniam, F. Roediger, M.P. Mattson, J.N. Keller, G. Waeg, D.A. Butterfield, The lipid peroxidation product 4-hydroxy-2-*trans*-nonenal alters conformation of cortical synaptosomal membrane proteins, J. Neurochem. 69 (1997) 1161–1169.
[26] J.R. White, I.J. Reynolds, Mitochondrial depolarization in glutamate stimulated neurons: an early signal specific to excitotoxin exposure, J. Neurosci. 16 (1996) 5688–5697.
[27] V.P. Whittaker, Thirty years of synaptosome research, J. Neurocytol. (1993) 735–742.