

Glucose/Oxygen Deprivation Induces Release of [³H]5-hydroxytryptamine Associated with Synapsin 1 Expression in Rat Hippocampal Slices

EunMi Park, Sang Hui Chu, and KyungEun Lee

Department of Pharmacology, College of Medicine, Ewha Womans University, Seoul 158–710, Korea

It has been well documented that a massive release of not only glutamate but also other neurotransmitters may modulate the final responses of nerve cells to the ischemic neuronal injury. But there is no information regarding whether the release of monoamines is directly associated with synaptic vesicular proteins under ischemia. In the present study, it was investigated whether synapsin 1, syntaxin and SNAP-25 are involved in the release of 5-hydroxytryptamine ([³H]5-HT) in glucose/oxygen deprived (GOD) rat hippocampal slices. And, the effect of NMDA receptor using DL-2-amino-5-phosphonovaleric acid (APV) on ischemia-induced release of 5-HT and the changes of the above proteins were also investigated. GOD for 20 minutes enhanced release of [³H]5-HT, which was in part blocked by the NMDA receptor antagonist, APV. The augmented expression of synapsin 1 during GOD for 20 minutes, which was also in part prevented by APV. In contrast, the expression of syntaxin and SNAP-25 were not altered during GOD. These results suggest that ischemic insult induces release of [³H]5-HT associated with synapsin 1, synaptic vesicular protein, via activation of NMDA receptor in part.

Key Words: Hippocampal slices, Glucose/oxygen deprivation, 5-Hydroxytryptamine, Synapsin, Syntaxin, SNAP-25

INTRODUCTION

Brain tissues have high energy demand requiring a constant and unimpeded supply of oxygen and substrate (Siesjö, 1984). Ischemia is conveniently defined as a reduction in cerebral blood flow of sufficient severity to cause functional or metabolic deficits, or overt brain damage (Siesjö et al, 1998). The primary effects of ischemia are reduced supply of substrate for energy metabolism (oxygen and glucose) and cause neuronal and glial death directly. These primary abnormalities and after reperfusion can also trigger secondary processes leading to cell death in regions of incomplete ischemia (Sharp et al, 1998). Secondary effects of ischemia in the brain include release of neurotransmitters, calcium influx into cells, activation

of protease and lipases, production of free radicals and proinflammatory molecules, activation of intracellular second messengers, and induction of genes that promote cell death via apoptosis (Sharp et al, 1998).

It is well-known that glutamate is released during ischemia (Benveniste et al, 1984; Globus et al, 1988a) and several other neurotransmitters appear simultaneously in the extracellular space; γ -aminobutyric acid (GABA) (Globus et al, 1988b; Phillis et al, 1994), dopamine (Globus et al, 1989; Phebus & Clements, 1989), 5-hydroxytryptamine (5-HT) (Baker et al, 1991; Prehen et al, 1991), glycine (Korf et al, 1988; Hillered et al, 1989), norepinephrine (Globus et al, 1989) and acetylcholine (Bertrand et al, 1993; Ishimura et al, 1994). It has been well documented that a massive release of not only glutamate but also other neurotransmitters may modulate the final responses of nerve cells to the ischemic neuronal injury.

Synaptic transmission is based on the rapid exocytosis of neurotransmitters from synaptic vesicles

Corresponding to: KyungEun Lee, Department of Pharmacology, College of Medicine, Ewha Womans University, 911-1 Mok-dong, Yangcheon-gu, Seoul 158-710, Korea. (Tel) 82-2-650-5744, (Fax) 82-2-653-5076, (E-mail) kelee@mm.ewha.ac.kr

(Katz, 1969). The SNARE hypothesis states that each class of transport vesicle contain a specific targeting protein (v- or vesicle SNARE) that is capable of associating only with a receptor protein (t- or target SNARE) specific to the appropriate acceptor membrane. The proteins hypothesized to serve as SNAREs at the synapse include the synaptic vesicle protein VAMP (vesicle-associated membrane protein, also called synaptobrevin) (Trimble et al, 1988; Baumert et al, 1989) and two plasma membrane proteins, syntaxin (also HPC-1) (Bennet et al, 1992; Inoue et al, 1992) and SNAP-25 (synapse-associated protein of 25 kDa) (Oyler et al, 1989). Syntaxin, SNAP-25 and synaptobrevin form a trimeric complex referred to as the core synaptic complex. The complex is very stable (Hayashi et al, 1994) and it serves as a binding site for N-ethylmaleimide-sensitive factor (NSF) and soluble NSF-attachment protein (SNAP) which dissociate the complex by using energy from ATP hydrolysis (Hanson et al, 1992; Söller et al, 1993). Synapsin I, a vesicle protein, may be to regulate vesicle availability, placing the liberation of vesicles from the cytoskeleton more tightly under the control of activated Ca^{2+} -calmodulin-dependent (CaM) kinase II. So critical synapsin I function occurs at a step between docking and fusion (Hirokawa et al, 1989; Jessell & Kendal, 1993; Hicks et al, 1997).

Evidence has been found that anoxia/ischemia causes the release of various neurotransmitters by a Ca^{2+} -dependent and a Ca^{2+} -independent mechanism (Drejer et al, 1985; Kauppinen et al, 1988; Ikeda et al, 1989). But there is no information regarding whether the release of monoamines is directly associated with synaptic vesicular proteins under ischemic conditions.

Therefore this study was aimed to clarify the relationship between the release of 5-hydroxytryptamine (5-HT) and synaptic vesicular protein under glucose and oxygen deprivation in rat hippocampal slices. Additionally, we tested that the release of 5-HT and expression of vesicular proteins were mediated by the action of the glutamate receptor.

METHODS

Preparation and maintenance of rat hippocampal slices

Male Sprague-Dawley rats (180~200 g) were

used. Rat was decapitated and its brain was rapidly removed and dissected on ice (Paschen & Djuricic, 1995). Each hippocampus isolated from both sides of the brain was sliced transversely at 400 μ m with a tissue slicer (Stoelting Co., Wood Dale, IL, USA) on filter paper soaked in cold normal buffer (2~4°C, 95% O_2 /5% CO_2). The normal buffer solution consisted of the following (mM): NaCl 124, KCl 4, $CaCl_2$ 2, KH_2PO_4 1.25, $NaHCO_3$, 25, Glucose 10. 12.5 μ M Nialamide (Sigma Chemical Company, St. Louis, MO, USA), monoamine oxidase inhibitor, was supplemented to prevent metabolization of 5-HT.

Hippocampal slices were divided into four groups: slices in normal buffer, slices in glucose/oxygen deprived (GOD) buffer, slices in normal buffer with DL-2-amino-5-phosphonovaleric acid (APV 30 μ M, Research Biochemical Incorporated, Natick, MA, USA) and slices in GOD with APV.

Preparation of glucose and oxygen deprivation and uptake of [3H]5-HT

After preparation, hippocampal slices were transferred to warmed 8 ml normal buffer and slices were allowed to recover from preparation stress for 30 min in a shaking water bath. Temperature of buffer was set to 37°C throughout the experiments. After 30 min-preincubation period, normal buffer was changed to buffer with 0.1 μ M [3H]5-HT (74 μ Ci/8 ml, Amersham pharmacia biotech, Uppsala, Sweden) and slices were allowed to uptake [3H]5-HT in tissue sufficiently for 30 min. And then, buffer with [3H]5-HT was discarded and slices were washed with normal buffer. Washed slices were randomly divided into 4 vials with 2 ml buffer solution with zimelidine (10 μ M, Research Biochemical Incorporated, Natick, MA, USA), 5-HT reuptake inhibitor. Buffer solutions were changed every 10 min during 120 min incubation period. Glucose/oxygen deprivation (incubation of slices in glucose-free medium gassed with 95% N_2 /5% CO_2) was induced for 20 min at 50-min incubation step. 10 ml mannitol (Sigma Chemical Company, St. Louis, MO, USA) was added to glucose/oxygen deprived buffer instead of glucose for maintaining of same osmolarity. 30 μ M APV, a NMDA receptor antagonist, was administered simultaneously with GOD for 20 min.

Counting of radioactivity of [³H]5-HT release

500 μ l of medium drained from the vial every 10 min was mixed with 5 ml of liquid scintillation cocktail (READY SAFE, Beckman Instruments INC., Fullerton, CA, USA). The radioactivity of the mixture was counted with a liquid scintillation counter (Beckman Instruments INC., Fullerton, CA, USA). To measure the radioactivity of [³H]5-HT left in the hippocampal slices, the tissues were treated with 1 ml of tissue solubilizer (Lumasolve, Lumac LSC, Groningen, Netherlands) and incubated for 60 min. Then 100 μ l of the completely solubilized sample was mixed with 5 ml of liquid scintillation cocktail for counting. Total radioactivity of [³H]5-HT was sum of the radioactivity of [³H]5-HT released into the media collected every 10 min from the given time fraction to the end of the observation period and the radioactivity of [³H]5-HT remaining in hippocampal slices after 120-min observation period. All the results of radioactivity released into the media collected during the given 10-min time fraction were expressed as fractional release to total radioactivity. After 50-min period, the fraction release was stabilized and then percent of the [³H]5-HT release at 50-min was assigned as 100%.

Data were expressed as mean \pm standard error. Statistical comparisons among multiple groups were carried out using analysis of variances (ANOVA) followed by Dunnett's test.

Western blot analysis of synapsin 1, syntaxin, and SNAP-25

Homogenates from hippocampal slices in each group were analysed using gel electrophoresis (Patano et al, 1997). Proteins (20 μ g for synapsin 1, 5 μ g for syntaxin and SNAP-25) were electrophoresed using 30% acrylamide gels/Bis solution (Bio-Rad Laboratories, Hercules, CA, USA) and total protein concentration was determined using the method of Bradford (1976). Proteins were transferred from the gels to nitrocellulose (Amersham Pharmacia Biotech, Uppsala, Sweden). After incubated overnight in blocking solution (5% skim milk in tris buffered saline, DIFCO Laboratories, Detroit, MI, USA), blots were allowed to react for 120 min with monoclonal primary antibodies (used at dilution of 1 : 500) as follows : synapsin 1 (Calbiochem, La Jolla, CA, USA), syntaxin (Sigma Chemical Company, St. Louis, MO,

USA) and SNAP-25 (Calbiochem, La Jolla, CA, USA). Immunoblots were washed three times in Tween-Tris buffered saline (Tris 20 mM, NaCl 500 mM, 0.05% Tween-20), and then incubated for 60 min with secondary antibodies (1 : 1000, anti-mouse IgG-POD, Fab fragments, Boeringer mannheim, Basel, Switzerland). After washing with tris buffered saline (Tris 20 mM, NaCl 500 ml), protein bands were visualized with Fuji medical X-ray film by Enhanced chemiluminescence method (Amersham Life Science, Uppsala, Sweden). Density of proteins on autoradiogram was measured by densitometer. Proteins of slices in normal buffer were assigned to 100%.

RESULTS

[³H]5-HT release in normal buffer

The hippocampal slices showed spontaneous 5-HT release throughout the experiment. 5-HT release decreased rapidly during the first 40 min, and then a steady release of 5-HT was observed up to 120 min. Therefore, 5-HT release at 5th 10-min period was used as a control and the changes of 5-HT release were expressed as percent values compared to the control (Fig. 1).

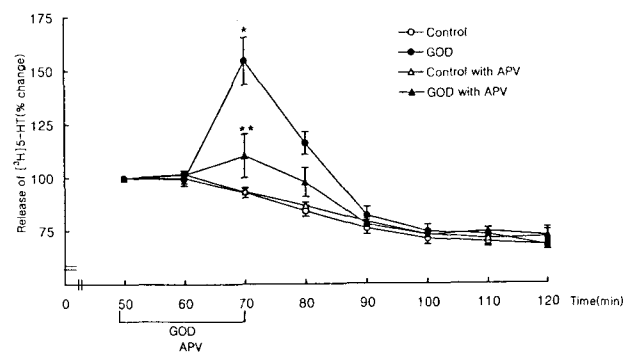


Fig. 1. Effects of glucose/oxygen deprivation (GOD) and DL-2-amino-5-phosphonovaleric acid (APV) on the spontaneous release of [³H]5-hydroxytryptamine (HT) in the rat hippocampal slices. GOD was induced for 20 min by changing glucose-free incubation medium aerated with 95% N₂/5% CO₂ gas. Values are mean \pm S.E. expressed as percent of the [³H]5-HT release at 50 minutes, which was assigned as 100%. **p* < 0.05 compared to control. ***p* < 0.05 compared to GOD with APV (Dunnett method was used).

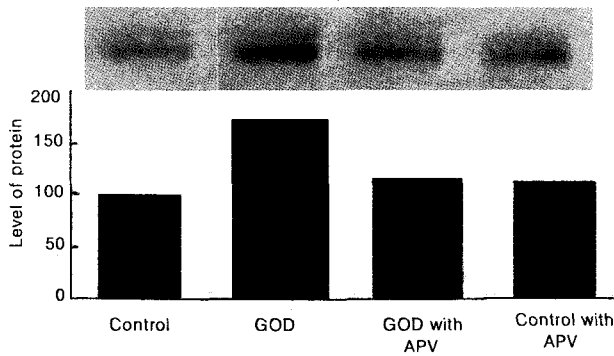


Fig. 2. Expression of synapsin 1 in glucose/oxygen deprived (GOD) rat hippocampal slices in the presence and absence of DL-2-amino-5-phosphonovaleric acid (APV). GOD was induced for 20 minutes by changing incubation medium depleted glucose and aerated with 95% N₂/5% CO₂ gas. Above: Representative Western blot analysis, Below: Densitometric quantification.

[³H]5-HT release in GOD

When exposed to GOD for 20 min from 5th 10 min-period, [³H]5-HT release was beginning to increase from 6th 10 min period and then showed peak increase at 7th 10 min period. After changing of normal buffer, [³H]5-HT release was not different with control (Fig. 1).

[³H]5-HT release by APV

When treated with APV for 20 min from 5th 10-min period, [³H]5-HT release was not different from control level (Fig. 1). When exposed to GOD with APV for 20 min, [³H]5-HT release was partially inhibited compared to [³H]5-HT release of GOD only at 7th 10-min period, and then values returned approximately to control level.

Western blot analysis of synapsin 1, syntaxin and SNAP-25

Amount of synapsin 1, which is a synaptic vesicular protein, increased in GOD and this increase was partially inhibited by APV (Fig. 2). But the amounts of syntaxin and SNAP-25, which are plasma membrane proteins, didn't show differences apparently between groups (Fig. 3, 4).

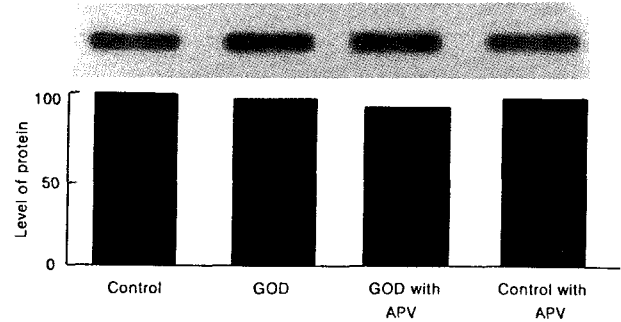


Fig. 3. Expression of syntaxin in glucose/oxygen deprived (GOD) rat hippocampal slices in the presence and absence of DL-2-amino-5-phosphonovaleric acid (APV). Other legends are the same as Fig. 2.

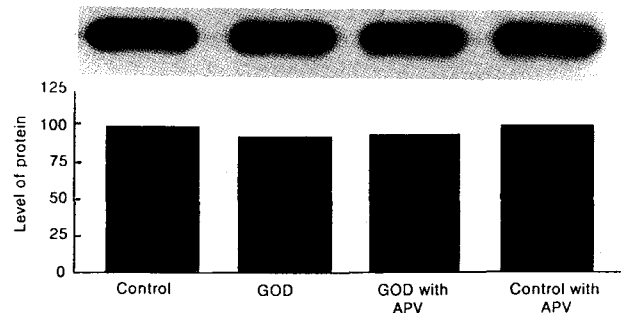


Fig. 4. Expression of SNAP-25 in glucose/oxygen deprived (GOD) rat hippocampal slices in the presence and absence of DL-2-amino-5-phosphonovaleric acid (APV). Other legends are the same as Fig. 2.

DISCUSSION

Because the ischemia is defined as a reduction in cerebral blood flow of sufficient severity to cause metabolic deficits (Siesjö, 1984), it seems (to be) that the condition of glucose and oxygen deprivation (GOD) is close to the meaning of ischemia in vivo.

In this study, [³H]5-HT release markedly increased by GOD and the increase of release was inhibited partially by NMDA receptor antagonist, APV. This indicates that activation of NMDA receptor may be involved in the mechanism of [³H]5-HT release during ischemia. The relationship between 5-HT release and glutamate receptor has been shown by some other reports. Ohta et al (1994) reported that presynaptic ionotropic glutamate receptors exist on 5-HT terminals in the rat striatum and their subtypes equally contributed to facilitating 5-HT release by experiment of microdialysis in vivo. Fink et al (1995) showed that activation of NMDA or non-NMDA

receptors elicited a release of 5-HT in rat brain cortex slices. So, uninhibited portion of 5-HT release in this study may be related to activation of non-NMDA receptor or Ca²⁺-independent release.

Our goal of the present study was to evaluate that the release of [³H]5-HT during GOD was related to synaptic vesicular protein. We have observed that expression of synapsin 1 increased with GOD and the increase was partially inhibited by APV but those of syntaxin and SNAP-25 didn't show apparent change.

Several studies show that neurotransmitter-related proteins are increased after ischemia in vivo. Increased expression of synaptophysin in rat striatum (Korematsu et al, 1993), SVP-38 (synapsin 1 and synaptophysin) in gerbil forebrain (Miyazawa et al, 1995) and SNAP-25 in the hippocampus of gerbil (Martí et al, 1998) after ischemia were showed by immunoreactivity. But their reports provide only the evidence of involvement of these proteins in tissue injury after ischemia but did not mentioned the relationship with release of neurotransmitter during ischemia.

In this study, the pattern of change of synapsin 1 on GOD was similar to that of [³H]5-HT release. Although exocytosis is inhibited after few minutes of ischemia because synaptic vesicle release is calcium and ATP dependent (Sanchez-Prieto & Gonçalez, 1988; Nicholls, 1989), these results suggest that synapsin 1 is involved in the release of neurotransmitter during ischemia. Synapsin 1 is believed to regulate the number of vesicles available for release by reversibly anchoring them to the actin-based cytoskeletal matrix of the presynaptic nerve terminals (Bähler et al, 1990; Linás et al, 1991; Greengard et al, 1993). This vesicle-anchoring property is regulated by the phosphorylation state of synapsin 1. The interaction of synapsin 1 both with vesicles and with actin are reduced upon its phosphorylation by CaM kinase II (Schiebler et al, 1986; Bähler & Greengard, 1987). So apparent increase of synapsin 1 other than syntaxin and SNAP-25 during GOD may be related with activation of CaM kinase II by Ca²⁺ influx. In addition (to), the synapsin constitute a high percentage of the synaptic vesicle membrane-bound protein fraction so relatively large amount of synapsin 1 may be an another factor.

Because the increased expression of synapsin 1 was partially inhibited by APV in GOD, expression of synapsin 1 may be regulated by NMDA receptor in part. Fukunaga et al (1992) reported that glutamate

can activate CaM kinase II through the ionotropic NMDA receptor, which in turn increase the phosphorylation of microtubule-associated protein 2 and synapsin 1. And Katchman & Hershkowitz (1997) suggested that NMDA receptor-activated nitric oxide (NO) production may enhance vesicular synaptic glutamate release, which would in turn contribute to excitotoxicity during hypometabolic states.

Evidences have been found that ischemia causes the release of various neurotransmitters by a Ca²⁺-independent mechanism (Sanchez-Prieto & Gonçalez, 1988; Ikeda et al, 1989). And several studies have shown that the Ca²⁺-independent component of the ischemia-induced release of neurotransmitters may be due to the reversal of Na⁺-dependent neurotransmitter carriers, as a result of the alteration in intracellular ions occurring during ATP depletion (Erecinska & Silver, 1994). So further investigation is required to test which mechanism is related to the release of neurotransmitter during ischemia by control of Ca²⁺ or CaM kinase II.

In summary, we showed that the release of [³H]5-HT and the expression of synapsin 1 increased significantly during GOD. These increases were inhibited partially by an NMDA receptor antagonist, so we suggested that the mechanism of [³H]5-HT release during GOD is associated with synapsin 1 via activation of NMDA receptor in part.

ACKNOWLEDGEMENT

This study was supported by Ewha Womans University Research Grant (1999) and Ministry of Health and Social Affairs in Korea (1999).

REFERENCES

- Bähler M, Greengard P. Synapsin 1 bundles F-actin in a phosphorylation-dependent manner. *Nature* 326: 704–707, 1987
- Bähler M, Benfenati F, Valtorta F, Greengard P. The synapsins and the regulation of synaptic function. *Bioassay* 12: 259–263, 1990
- Baker AJ, Zornow MH, Scheller MS, Yaksh TL, Skilling SR, Smullin DH, Larson AA, Kuczenski R. Changes in extracellular concentrations of glutamate, aspartate, glycine, dopamine, serotonin, and dopamine metabolites after transient global ischemia in the rabbit brain. *J Neurochem* 57: 1370–1379, 1991

- Baumert M, Maycox PR, Navone F, De Camili P, Jahn R. Synaptobrevin: an integral membrane protein of 18,000 daltons present in small synaptic vesicles of rat brain. *EMBO J* 8: 379–384, 1989
- Bennet MK, Calakos N, Scheller RH. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. *Science* 257: 255–259, 1992
- Bennet MK, Scheller RH. Molecular correlates of synaptic vesicle docking and fusion. *Curr Opin Neurobiol* 4: 324–329, 1994
- Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem* 43: 1369–1374, 1984
- Bertrand N, Ishii H, Beley A, Spatz M. Biphasic striatal acetylcholine release during and after transient cerebral ischemia in gerbils. *J Cereb Blood Flow Metab* 13: 789–795, 1993
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254, 1976
- Drejer J, Benveniste H, Diemer NH, Schousboe A. Cellular origin of ischemia-induced glutamate release from brain tissue in vivo and in vitro. *J Neurochem* 45: 145–151, 1985
- Erecinska M, Silver IA. Ions and energy in mammalian brain. *Prog Neurobiol* 43: 37–71, 1994
- Fink K, Schmitz V, Boing C, Gothert M. Stimulation of serotonin release in the rat brain cortex by activation of ionotropic glutamate receptors and its modulation via alpha-2-heteroreceptors. *Naunyn Schmiedeberg Arch Pharmacol* 352: 394–401, 1995
- Fukunaga K, Soderling TR, Miyamoto E. Activation of Ca^{2+} /calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons. *J Biol Chem* 267: 22527–22533, 1992
- Globus MYT, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Intra-ischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. *Neurosci Lett* 91: 36–41, 1988a
- Globus MYT, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Effect of ischemia on the in vivo release of striatal dopamine, glutamate, and γ -aminobutyric acid studied by intracerebral microdialysis. *J Neurochem* 51: 1455–1464, 1988b
- Globus MYT, Busto R, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Direct evidence for acute and massive norepinephrine release in the hippocampus during transient ischemia. *J Cereb Blood Flow Metab* 13: 789–795, 1989
- Greengard P, Valtrota F, Czernik AJ, Benfenati F. Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science* 259: 780–785, 1993
- Hanson PI, Schulman H. Neuronal Ca^{2+} /calmodulin-dependent protein kinase. *Annu Rev Biochem* 61: 559–601, 1992
- Hayashi T, McMahon H, Yamasaki S, Binz T, Hata Y, Südhof TC. Synaptic vesicle membrane fusion complex; action of clostridial neurotoxins on assembly. *EMBO J* 13: 5051–5061, 1994
- Hicks A, Davis S, Rodger J, Helme-Guizon A, Laroche S, Mallet J. Synapsin 1 and syntaxin Ib: key elements in the control of neurotransmitter release are regulated by neuronal activation and long-term potentiation in vivo. *Neuroscience* 79: 329–340, 1997
- Hillered L, Hallström A, Segersvärd S, Persson L, Ungerstedt U. Dynamics of extracellular metabolites in the striatum after middle cerebral artery occlusion in the rat monitored by intracerebral microdialysis. *J Cereb Blood Flow Metab* 9: 607–616, 1989
- Hirokawa N, Sobue K, Kanda K, Harada A, Yorifusi H. The cytoskeletal architecture of the presynaptic nerve terminal and molecular structure of synapsin 1. *J Cell Biol* 108: 111–126, 1989
- Ikeda M, Nakazawa T, Abe K, Kaneko T, Yamatsu K. Extracellular accumulation of glutamate in the hippocampus induced by ischemia is not calcium dependent in vitro and in vivo evidence. *Neurosci Lett* 96: 202–206, 1989
- Inoue A, Obata K, Akagawa K. Cloning and sequence analysis of cDNA for a neuronal cell membrane antigen, HPC-1. *J Bio Chem* 267: 10613–10619, 1992
- Ishimaru H, Takahashi A, Ikarashi Y, Marutama Y. Temporal changes in extracellular acetylcholine and CA1 pyramidal cells in gerbil hippocampus following transient cerebral ischemia. *Brain Res* 639: 66–72, 1994
- Jessell TM, Kandel ER. Synaptic transmission: a bidirectional and self-modifiable form of cell-cell communication. *Cell* 72(suppl 1): 1–30, 1993
- Katchman AN, Hershkowitz N. Nitric oxide modulates synaptic glutamate release during anoxia. *Neurosci Lett* 228: 50–54, 1997
- Katz B. *The release of neurotransmitter substances*. Liverpool Univ. Press, Liverpool UK, 1969
- Kauppinen RA, McMahon HT, Nicholls DG. Ca^{2+} -dependent and Ca^{2+} -independent glutamate release, energy status and cytosolic free Ca^{2+} concentration in isolated nerve terminals following metabolic inhibition: possible relevance to hypoglycemia and anoxia. *Neuroscience* 27: 175–182, 1988
- Korematsu K, Goto S, Nagahiro S, Ushio Y. Changes of immunoreactivity for synaptophysin ('protein p38') following a transient cerebral ischemia in the rat striatum. *Brain Res* 616: 320–324, 1993
- Korf J, Klein HC, Venema K, Postema F. Increases in

- striatal and hippocampal impedance and extracellular levels of amino acids by cardiac arrest in freely moving rats. *J Neurochem* 50: 1087–1096, 1988
- Llinás R, Gruner J, Sugimori M, McGuinness TL, Greengard P. Regulation by synapsin 1 and Ca²⁺-calmodulin-dependent protein kinase II of transmitter release in squid giant synapse. *J Physiol* 436: 257–282, 1991
- Martí E, Ferrer I, Ballabriga J, Blasi J. Increase in SNAP-25 immunoreactivity in the mossy fibers following transient forebrain ischemia in the gerbil. *Acta Neuropathol* 95: 254–260, 1998
- Miyazawa T, Sato K, Obata K. A synaptic vesicle-associated protein (SVP-38) as an early indicator of delayed neuronal death. *J Cereb Blood Flow Metab* 15: 462–466, 1995
- Nicholls DG. Release of glutamate, aspartate, and γ -aminobutyric acid from isolated nerve terminals. *J Neurochem* 52: 331–341, 1989
- Ohta K, Araki N, Shibata M, Komatsumoto S, Shimazu K, Fukuuchi Y. Presynaptic ionotropic glutamate receptors modulate in vivo release and metabolism of striatal dopamine, noradrenalin, and 5-hydroxytryptamine: involvement of both NMDA and AMPA/kainate subtype. *Neurosci Res* 21: 83–89, 1994
- Oyler GA, Higgins GA, Hart RA, Battenberg E. The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. *J Cell Biol* 109: 3039–3052, 1989
- Paschen W, Djuricic B. Comparison in vitro ischemia-induced disturbances in energy metabolism and protein synthesis in the hippocampus of rats and gerbils. *J Neurochem* 65: 1692–1697, 1995
- Patanow CM, Day JR, Billingsley ML. Alterations in hippocampal expression of SNAP-25, GAP-43, Stannin and glial fibrillary acidic protein following mechanical and trimethyltin-induced injury in the rat. *Neuroscience* 76: 187–202, 1997
- Phebus LA, Clements JA. Effects of transient ischemia on striatal extracellular dopamine, serotonin and their metabolites. *Life Sci* 44: 1335–1342, 1989
- Phillis JW, Smith-Barbour M, Perkins LM, O'Regan MH. Characterization of glutamate, aspartate, and GABA release from ischemic rat cerebral cortex. *Brain Res Bull* 34: 457–466, 1994
- Prehen JH, Backhaus C, Karkoutly C, Nuglish J, Peruche B, Roß-Berg C, Kriegstein J. Neuroprotective properties of 5-HT_{1A} receptor agonists in rodent models of focal and global cerebral ischemia. *Eur J Pharmacol* 203: 213–222, 1991
- Sanchez-Prieto J, González P. Occurrence of a large Ca²⁺-independent release of glutamate during anoxia in isolated nerve terminals (synaptosomes). *J Neurochem* 50: 1322–1324, 1988
- Schiebler W, Jahn R, Doucet JP, Rothlein J, Greenberg P. Characterization of synapsin 1 binding to small synaptic vesicles. *J Biol Chem* 261: 8383–8390, 1986
- Sharp FR, Swanson RA, Honkaniemi J, Kogure K, Massa SM. Neurochemistry and molecular biology. In *Stroke: pathophysiology, diagnosis, and management*, edited by Henry JM, Barnett JP, Yatsu FM ed. Pennsylvania Churchill Livingstone, 1998
- Siesjö BK. Cerebral circulation and metabolism. *J Neurosurg* 60: 883–908, 1984
- Siesjö BK, Kristián T, Katsura K. Overview of bioenergetic failure and metabolic cascades in brain ischemia. In *Cerebrovascular disease: pathophysiology, diagnosis, and management*, edited by Ginsberg M.D. & Bogousslavsky J. MA Blackwell Science, 1998
- Söllner T, Bennett MK, Whiteheart SW, Scheller RH, Rothman JE. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. *Cell* 75: 409–418, 1993
- Trimble WS, Cowan DM, Scheller RH. VAMP-1: a synaptic vesicle-associated integral membrane protein. *Proc Natl Acad Sci USA* 85: 4538–4542, 1998