Journal of Korean Medical Science
Vol. 9, No. 5, 394-401 October 1994

Effect of hypocapnia on extracellular glutamate and glycine concentrations during peri-ischemic period in the rabbit hippocampus

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Glutamate (GLU) is a neurotransmitter. Massive release of GLU and glycine (GLY) into the brain's extracellular space may be triggered by ischemia, and may result in acute neuronal lysis or delayed neuronal death. The aim of this study was to evaluate the possible relationship between hyperventilation and the level of GLU and GLY during brain ischemia.

Rabbits were anesthetized with halothane and oxygen. Group 1 was allowed to hyperventilate ($PaCO_2$ 25-35 mmHg). $PaCO_2$ was maintained throughout the study. Group 2 was a normal control group that maintained normocapnia. Two global cerebral ischemic episodes were produced. Microdialysate was collected during the peri-ischemic and reperfusion periods from the dorsal hippocampus. GLU and GLY concentrations were determined using high-performance liquid chromatography.

In the control group, GLU and GLY were significantly elevated during each episode of ischemia ; these levels returned to baseline within 10 minutes after reperfusion. In contrast, in the hyperventilation group GLU and GLY concentrations increased during ischemia, but they were not statistically significant.

We were able to demonstrate that hypocapnia during periischemic period lowered extracellular GLU and GLY concentrations. These results can explain a part of the protective action of hypocapnia during cerebral ischemia.

Key Words : *Hypocapnia, Glutamate, Glycine, Cerebral ischemia, Microdialysis.*

INTRODUCTION

The clinical management of patients in coma after cardiac arrest involves the restoration of adequate cardiopulmonary function to prevent further cerebral injury. Over the years, many therapeutic approaches have been devised to alleviate the dismal outcome of cerebral ischemia. One of these modalities is the idea of regulating $PaCO_2$ to reduce ischemic brain damage. Manipulation of the arterial carbon dioxide tension ($PaCO_2$) is a potent mean by which cerebral blood flow (CBF) can be changed. There are many reports that support the beneficial effect

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This study was supported by a research grant from the Keimyung Medical Science Institute, 1993.

of hyperventilation(Todd et al., 1985). One of the underlying mechanisms of the beneficial effect of hyperventilation is the improvement of intracellular acidic environment(Vanicky et al., 1992) However, numerous studies demonstrated hyperventilation after traumatic brain injury is deleterious. Possible disadvantages include cerebral vasoconstriction to such an extent that cerebral ischemia ensues and only transient effect on the cerebrospinal fluid(CSF) pH with loss of HCO_3^- buffer from the CSF. Prolonged hyperventilation also provided a relative ischemia in brain tissue and promoted production of brain lactate(Yoshida and Marmarou, 1991). Accordingly, as long as hyperventilation has a cerebral protective action, there should be other mechanisms in addition to the above-mentioned ones. Glutamate is a known neurotoxin when present in excessive concentrations and may make neuronal destruction. Under anoxic and or ischemic conditions, synaptically released excitatory transmitters, most likely glutamate, accumulate to neurotoxic level. Also neurotransmitter release is enhanced by anoxia. If we can demonstrate that hypocapnia during the peri-ischemic period lowers the extracellular glutamate concentration, we can explain a part of the protective mechanism to be the ability of hypocapnia to attenuate the increased release of glutamate. We used the well-established rabbit models of global ischemia and evaluated the changes produced by a 3-h period of hypocapnia.

MATERIALS AND METHODS

Six New Zealand white rabbits weighing 2.24± 0.21 (mean±SD) kg were anesthetized in a plexiglas box with 5% halothane in oxygen. After intubation of the trachea with a 3.5 mm uncuffed wire reinforced endotracheal tube, anesthesia was maintained with 1.2% halothane in oxygen, the animals were mechanically ventilated with animal ventilator to maintain normocapnia (Harvard Apparatus, Animal ventilator, U.S.A. $PaCO_2$ = 35-45 mmHg). Body temperature was controlled and maintained at 37.5 ℃. After infiltration with 0.25% bupivacaine, a catheter (PE-90) was inserted into the femoral artery for measurement of arterial blood pressure and sampling for the arterial blood gases. The femoral vein was also cannulated for the administration of drugs during inflation of the neck tourniquet. An ear vein catheter was inserted for the administration of fluids (0.9% saline) and drugs. All rabbits were initially hydrated with 0.9% saline solution (40 $ml \cdot kg^{-1}$) administered over one hour period. This was followed by a maintenance infusion at 4 $ml \cdot kg^{-1} \cdot h^{-1}$ throughout the study. The rabbit's head was positioned in a stereotactic frame (David Kopf, USA), and a pneumatic tourniquet (8 inches in length, Zimmer, U.S.A) was secured loosely around the neck. After infiltration with 0.25% bupivacaine, the cranium was exposed and burr holes were made bilaterally over the dorsal hippocampus (4 mm posterior and 4 mm lateral to the bregma) for the insertion of microdialysis probes(CMA-10, Carnegie Medicin, Sweden). Biparietal needle electrodes were placed into the scalp for continuous recording of the EEG. Monitored variables included mean arterial pressure, heart rate, arterial blood gases, hematocrit, blood glucose concentration, esophageal temperature, and the electroencephalogram (EEG). Following the completion of these surgical preparations, the inspired halothane concentration was decreased to 1%.

Recovery rates for each microdialysis probe were determined using 10^{-2} M dextrose solution *in vitro* prior to their insertion into the brain. The dura over the dorsal hippocampus were then incised, and microdialysis probes of concentric design (fiber length=4 mm, diameter=0.25 mm) were inserted vertically to a depth of 6 mm using micromanipulators (David Kopf, U.S.A.). The probes were perfused with artificial cerebrospinal fluid (147 mM NaCl, 2.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, 4.0 mM KCl) at a rate of 2 $\mu L \cdot min^{-1}$. After implantation into the brain, the probes were perfused for at least 1 hour prior to collecting baseline samples of brain tissue microdialysate.

The rabbits were randomly assigned to one of two groups. Group 1 (n=3) was allowed to hyperventilate to the level of $PaCO_2$ 25-35 mmHg 60 min. prior to the onset of ischemia. $PaCO_2$ was maintained throughout the study. Group 2(n=3) was a normal control group that maintained normocapnia. Global cerebral ischemia was induced by lowering the mean arterial blood pressure to less than 50 mmHg by using bolus doses(10mg) of trimethapan, and the application of positive end-expiratory airway pressure. The neck tourniquet was then inflated to a pressure of 20 psi for 7.5 minutes. A tendency to hypertension during the first three minutes of ischemia was treated with additional doses of trimethapan as needed to keep mean arterial pressure below 50 mmHg. Global cerebral ischemia was veri-

fied in each rabbit by observation of an isoelectric EEG within 30 seconds after tourniquet inflation. Immediately after deflation of the tourniquet, a bolus dose and then infusion of phenylephrine was done to restore the mean arterial pressure to 75 mmHg. After one hour of recirculation, a second identical period of global ischemia was instituted.

At the end of the study period, the microdialysis probes were removed and the recovery rate for each microdialysis probe was again determined using 10^{-2} M dextrose solution *in vitro*. To verify the position of probes, 5 ml of Evans blue dye (2%) was administered intravenously. After the animal was euthanized, the brain was removed and sectioned coronally to see the staining of the dye along the tracks of the probes.

Samples of microdialysate were collected as follows from the dorsal hippocampus. Two baseline samples (each of 20 minute's duration) were collected 60 minutes after insertion into the brain. After collection of baseline samples manipulation of ventilation was started. Three samples (20 minute's duration) were collected in all groups before the onset of the first ischemia. Global cerebral ischemia was induced as described and every five minutes samples were taken (two ischemia samples, followed by two immediate reperfusion samples). Two samples (20 minute's duration) were collected before the onset of the second ischemia. For the second ischemia this process was repeated. Finally three reperfusion samples (each 20 minute's duration) were collected at 80, 120 and 160 minutes after the onset of the first ischemia.

All samples were collected on ice and immediately frozen and stored at −25℃ until their analysis for amino acid content by high-performance liquid chromatography(HPLC).

The dialysate from the dorsal hippocampus was analyzed for glutamate and glycine concentrations using HPLC with o-phthaldehyde derivatization on a reverse phase C-18 column. Derivatives were detected fluorometrically and peak areas were integrated and quantified based on linear calibration with known amino acid standards. This method has been shown to be sensitive to low picomolar range concentrations of glutamate.

The means and SEMs for the concentrations of glutamate and glycine were calculated for each time period. Data for amino acid concentrations were corrected using *in vitro* recovery rates for dextrose and analyzed by 2-way ANOVA (groups vs. time). Physiological data were tabulated and compared using ANOVA test at each point. Differences associated with $p<0.05$ were considered statistically significant.

RESULTS

Physiologic data are shown in Table 1. Other than the intended differences in $PaCO_2$ and pH, there were no other significant differences in physiologic variables between the groups. As intended, $PaCO_2$ decreased from the mean value of 39.7±3.2 mmHg to 31±2.2 mmHg in the hyperventilation group. These differences in $PaCO_2$ persisted throughout the study(Fig. 1). In vitro mean recovery rate of microdialysis catheters for dextrose was 28±5%.

Prior to the onset of ischemia, stable concentrations of extracellular glutamate and glycine were documented (from t=−95 to −25 minutes). In the control group the hippocampal extracellular concentrations of glutamate and glycine were significantly elevated during each episode of ischemia (Fig. 2 and 3) ; these levels returned to baseline within 10 minutes after reperfusion. In contrast, in the hyperventilation group hippocampal glutamate and glycine concentrations increased during ischemia, but they were not statistically significant. Two way ANOVA for the peri-ischemic periods (t=15,80) revealed lower glutamate values for the hyperventilated animals(p=

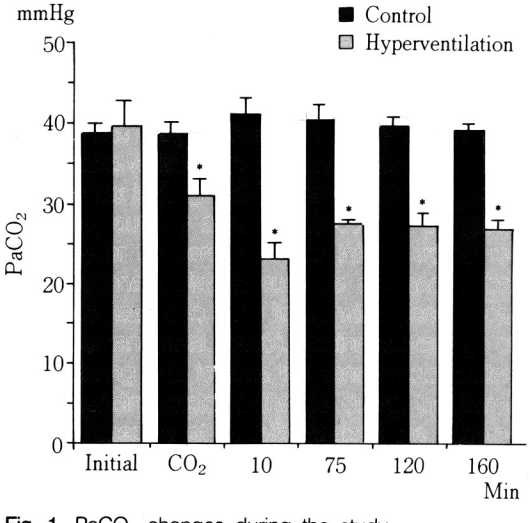

Fig. 1. $PaCO_2$ changes during the study.
* : $p<0.05$ between groups

Table 1. Summary of physiologic Data

Variable	Control (n=3)	Hyperventilation (n=3)
pH		
Initial	7.35±0.02	7.36±0.05
Hyperventilation	7.33±0.02	7.45±0.05*
10 min after ischemia	7.28±0.02	7.46±0.04*
75 min after ischemia	7.29±0.03	7.38±0.03*
120 min after ischemia	7.36±0.02	7.45±0.01*
160 min after ischemia	7.36±0.01	7.49±0.02*
$PaCO_2$		
Initial	38.8±1.3	39.7±3.2
Hyperventilation	38.6±1.6	31 ±2.2*
10 min after ischemia	41.1±2.2	23.3±1.9*
75 min after ischemia	40.5±2	27.6±0.5*
120 min after ischemia	39.7±1.2	27.1±1.6*
160 min after ischemia	39.3±0.8	27 ±1*
PaO_2		
Initial	371±49	429±23
Hyperventilation	444±22	467± 7
10 min after ischemia	446±32	490± 8
75 min after ischemia	468±40	506± 9
120 min after ischemia	460±37	501±23
160 min after ischemia	440±46	462±16
B.E		
Initial	−3 ±0.7	−2.1±2.9
Hyperventilation	−4 ±1.1	−0.7±1.5
10 min after ischemia	−6.2±1.1	−4.7±1.4
75 min after ischemia	−6.1±1.4	−6.5±1.8
120 min after ischemia	−2 ±1.4	−2.7±1.5
160 min after ischemia	−1.6±0.7	−0.8±1.7
Hematocrit		
Hyperventilation	30.2±1.2	32±2.1
160 min after ischemia	30.1±0.9	32±2.5

Variable	Control (n=3)	Hyperventilation (n=3)
Mean Arterial Pressure		
	69±3	72± 8
	70±3	72± 4
	86±9	80±13
	81±8	68±12
	67±3	80± 3
	67±2	76± 7
Heart Rate		
	235±10	257± 2
	255± 5	266± 6
	229± 6	223± 9
	219± 5	197± 3
	241± 8	217±12
	247± 8	235±20
Esophageal Temperature		
	38.5±0.1	37.6±0.4
	38.4±0.1	38.2±0.3
	38.4±0.1	37.8±0.2
	38.4±0.1	37.6±0.1
	38.4±0.1	37.7±0.4
	38.5±0.1	37.8±0.5
Blood Glucose		
	140±13	172±51
	129±17	130±24
	139±14	135±27
	130±14	99±11
	122±13	88±13
	113±13	85±10

* : $p<0.05$ from control

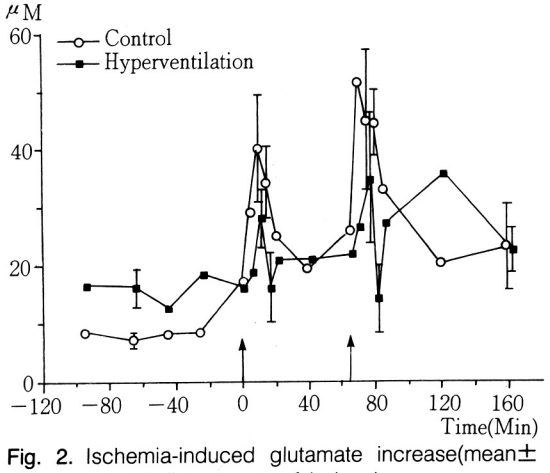

Fig. 2. Ischemia-induced glutamate increase(mean±SEM). Arrows indicate onset of ischemia.

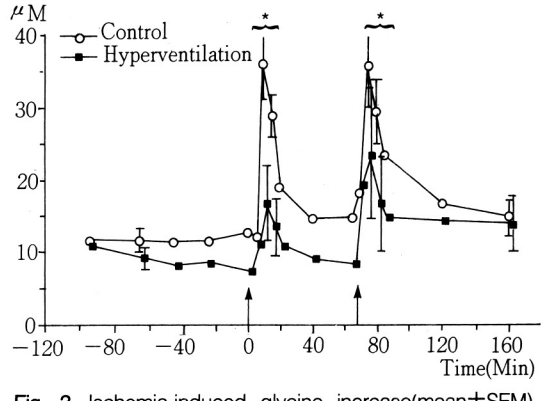

Fig. 3. Ischemia-induced glycine increase(mean±SEM). Arrows indicate ischemic episodes.
* : $p<0.05$ between groups

0.06). A similar analysis of peri-ischemic glycine concentrations revealed significantly lower values in the hyperventilated group($t=10,15,75,80$: $p=0.03$) as compared to normal controls.

DISCUSSION

Considerable evidences have now accumulated from both neuronal cultures and in vivo experiments that excitatory amino acids play an important role in the evolution of ischemic brain damage. It appears that these excitatory amino acids are released into the brain's extracellular space during normal neurotransmission as well as in response to a variety of insults including hypoxia and ischemia(Siejo, 1992a ; Siejo, 1992b ; Zivin and Choi, 1991 ; Benveniste et al., 1989). Acutely, glutamate, acting at post-synaptic NMDA receptors, activates associated ion channels resulting in a massive influx of Na^+ and Cl^- ions and H_2O(Choi et al., 1988). This influx of water can lead to an acute osmotic lysis of the neuron. Delayed neuronal damage is thought to be secondary to the glutamate induced calcium influx-(Rothman et al., 1987). An excessive rise in intracellular Ca^{++} results in overactivation of lipases, proteases, and endonucleases which may be detrimental to cellular homeostasis(Siejo, 1992a). Electron microscopy has demonstrated that the neuropathological state produced by glutamate is characterized by rapid cellular swelling and is most marked near dendrosomal components of the neurons that contain the excitatory amino acid receptors(Rothman and Olney, 1986).

Glycine has a facilitatory effect on glutamate's neurotoxic action. Activation of the NMDA receptor by glutamate has recently been shown to require the presence of glycine at strychnine-insensitive binding site(Foster and Kemp, 1989 ; Kloog et al., 1990). Thus, any intervention that results in reduction of such elevated levels may be expected to lessen the ischemic injury. To prevent further ischemic neuronal damage, it would be better to block the initial step in this cascade of events.

Glutamate is normally released by Ca^{++} dependent exocytosis(Nicholls, 1989), but ATP is also required and the release is therefore arrested within a few minutes after the start of ischemia(Siejo, 1988). Two transport pathways are integral to the ability to function as a neurotransmitter : first, a powerful uptake carrier located in both neurons and glia and capability of lowering the extracellular glutamate concentration to about 1 μM ; and, second, a more specific transporter capable of packaging glutamate into a subpopulation of synaptic vesicles for subsequent exocytosis.

Since there are several glutamate pools, the question arises from which compartment(s) glutamate is released during ischemia : (presynaptic) cytoplasm, vesicles, neuronal soma, astrocytes, etc. Because the vesicular release of glutamate is inhibited when ATP levels fall after a few minutes of anoxia, it seems likely that much of the glutamate released into the extracellular space during anoxia is Ca^{++}-independent, nonvesicular release(Sánchez-Prieto and González, 1988).

During ischemia, the extracellular K^+ concentration in the brain, $[K^+]_o$, increase steeply to 60-80 mM(Hansen, 1985 ; Hansen et al., 1982). A rise in $[K^+]_o$ will release more glutamate into the extracellular space in two ways, First, it will depolarize neurons, increasing vesicular release of glutamate(if this is not already inhibited by a fall in ATP levels : see below) ; secondly, it will promote the release of glutamate by reversal of the plasma membrane glutamate uptake carrier. Several factors lead to failure of glutamate uptake in the pathologic conditions described above. First, a rise in $[K^+]_o$ inhibits uptake directly because it hinders the loss of counter-transported K^+ from the carrier. Second, depolarization of the cell by the raised $[K^+]_o$ inhibits uptake. Third arachidonic acid released by the high glutamate concentration inhibits uptake. Finally, a decrease in $[Na^+]_o$, which can result from low ATP levels inhibiting the Na^+ pump or from a large K^+ influx through glutamate- and voltage-gated Na^+ channels, also inhibits uptake(Szatkowski et al., 1990). The combination of these factors is sufficient to reduce the rate of uptake less than 10% of its normal magnitude(Nicholls and Attwell, 1991). The resulting rise in glutamate concentration will depolarize neurons further and thus release more K^+ (Nicholls and Attwell, 1991). This is a positive feedback system that tends to lead to a large increase in extracellular glutamate concentration. Thus, blocking the increase of $[K^+]_o$ can be a part of the cerebral protective measures during ischemia. Hypocapnia causes respiratory alkalosis which in turn induces intracellular shift of K^+ to compensate the decreased concentration of H^+ in ECF, then $[K^+]_o$ decrease(Flemma and Young, 1964). If hyperventilation in peri-ischemic period can also attenuate the increase of $[K^+]_o$, the increased levels of gluta-

mate in extracellular fluid would be prevented. We did not measure $[K^+]_o$. However, It would be possible to assume that $[K^+]_o$ is already reduced by hyperventilation and that $[K^+]_o$ during the ischemia might be lower than $[K^+]_o$ without hyperventilation. The major finding of this study was that hypocapnia during the peri-ischemic period attenuated the increase of glutamate and glycine concentrations following transient global ischemia. Thus, our results might support such an assumption.

Apart from discussing about the absolute concentration of $[K^+]_o$ during ischemia, ion channels which modulates K^+ currents have gotten much attentions(Cook, 1988). Electrophysiological studies has shown that K^+-channel openers could protect neurons against ischemic injury(Abele and Miller, 1990 ; Hamilton et al., 1986). Intracellular recordings show that a brief anoxic episode in the CA_3 region of hippocampal slices induces a transient hyperpolarization due to the activation of K^+ currents. followed by a depolarization resulting glutamate release(Ben-Ari, 1990 ; Fujiwara et al., 1987). In the first minutes, the anoxic depolarization is sensitive to tetrodotoxin, reduced by the application of the K^+ -channel openers, and enhanced by the blocker. The release of glutamate evoked by a 6-8 min period of ischemia was reduced by 25-40% in the presence of K^+ -channel openers, lemakalim, RP52891, galanin. The hyperpolarization resulting from the opening of K^+ATP channel by the openers could temporarily protect neurons against the dramatic depolarization consecutive to ischemia and delay cytosolic glutamate release(Zini et al., 1993). It is uncertain that hyperventilation during ischemia is associated with activation fo the potassium channel. Hypercapnia leads to hyperpolarization of nerve cells. However, the hyperpolarization in hypercapnia is accompanied by increased membrane resistance indicating a mechanism of the hyperpolarization different from the one causing hyperpolarization during anoxia(which activates K^+ channel)(Caspers and Speckmann, 1972). This requires further investigation.

Clearly, there are other mechanisms by which hypocapnia may have a protective effect on neuronal injury. The potential beneficial effects of hyperventilation include a reduction in CBF with the consequent reduction in cerebral blood volume and intracranial pressure (ICP)(Maiese and Caronna, 1988 ; Soloway et al., 1968). Hypocapnia may induce a redistribution of CBF from normal areas(with preserved CO_2 reactivity) to injured areas with abnormal CO_2 reactivity ("inverse steal" phenomenon)(Artru and Merriman, 1989).

On the contrary, cerebral arteriolar diameter in experimental animals was found to be decreased shortly after institution of hyperventilation, but returned to baseline within 24 hours and increased slightly over baseline after that period(Muizelaar er al., 1988).

Thus, we can assume the prolonged hyperventilation can not guarantee persistent reduction of ICP. Severe hyperventilation can result in a reduction in CBF that is sufficient to produce cerebral ischemia, particularly if the $PaCO_2$ is rapidly decreased below 20 mmHg. If the $PaCO_2$ is allowed to go below that level, cerebral vasoconstriction may be so intense as to impede oxygen delivery, thus exacerbating the existing cerebral ischemia and its deleterious effects.

Recently, it has been reported that animals treated with sustained hyperventilation($PaCO_2$=20 mmHg) produced the highest lactate level(Yoshida and Marmarou, 1991). There was a report that moderate hypocapnia ($PaCO_2$=25-30 mmHg) contrary to severe hypocapnia ($PaCO_2$<25 mmHg) produces a better outcome in head-injured patients(Gordon and Rossanda, 1970 ; Muizelaar et al., 1991). According to clinical experiences, active hyperventilation during the recording of an EEG revealed enhanced epileptiform activity ; therefore, hypocapnia may influence seizure activity(Chater and Simpson, 1988).

Cerebral metabolic acidosis is often found after ischemia and is correlated with poor outcome(Ljungren et al., 1974). As CO_2 freely passes the blood-brain barrier, hyperventilation shoule diminish cerebral CO_2 content thereby increasing the pH of extracellular fluid and counteract acidosis(Maruki et al., 1993 ; Vanicky et al., 1992).

When respiratory alkalosis is induced by hyperventilation, normalization of CSF acid-base balance attained rapidly within 8-12 hr after deviation of normal pH(Christensen, 1974). This limits potential beneficial effect of hyperventilation.

It is apparent that the potential for both beneficial and detrimental effects exists. Therefore, the aim of this study was to elucidate the possible association of excitatory amino acid, GLU, GLY with the favorable action of hyperventilation in the brain ischemia.

In our study, hyperventilation attenuated the increase of glutamate level in peri-ischemic periods,

even if it did not show statistical significance (p= 0.06). Another interesting finding of this study was that periischemic glycine levels were lower in the hyperventilation group. Since glycine facilitates the actions of glutamate at the NMDA receptor(Forsythe et al., 1988 ; Foster and Kemp, 1989 ; Kleckner and Dingledine, 1988 ; Larson and Beitz, 1988), this provides a possible explanation for the hypocapnia's beneficial effect on neurologic outcome. Attenuation of glutamate's actions may prevent neuronal injury.

In summary, pre-existing hypocapnia was associated with decreased peri-ischemic concentrations of glutamate in brain tissue *in vivo* model of global cerebral ischemia. Glycine levels were decreased also during the peri-ischemic period and this may account, in part, for previous reports of cerebral protective action associated with hyperventilation.

REFERENCES

Abele AE, Miller RJ . *potassium channel activators abolish excitotoxicity in cultured hippocampal pyramidal neurons. Neurosci Lett 1990 ; 115 : 195-200.*

Artru AA, Merriman HG . *Hypocapnia added to hypertension to reverse EEG changes during carotid endarterectomy. Anesthesiology 1989 ; 70 : 1016-8.*

Ben-Ari Y . *Galanin and Glibenclamide modulate the anoxic release of glutamate in rat CA_3 hippocampal neurons. Eur J Neurosci 1990 ; 2 : 62-8.*

Benveniste H, Jorgensen MB, Sandberg M, Christensen T, Hagberg H, Diemer NH . *Ischemic damage in hippocampal CA_1 is dependent on glutamate release and intact innervation from CA_3. J Cereb Blood Flow Metab 1989 ; 9 : 629-39.*

Caspers H, Speckmann EJ . *Cerebral PO_2, PCO_2 and pH ; Changed during convulsive activity and their significance for spontaneous arrest of seizures. Epilepsia 1972 ; 13 : 699-725.*

Chater SN, Simpson KH . *Effect of passive hyperventilation on seizure duration in patients undergoing electroconvulsive therapy. Br J Anaesth 1988 ; 60 : 70-3.*

Choi DW, Koh JY, Peters S . *Pharmacology of glutamate neurotoxicity in cortical cell culture ; Attenuation by NMDA antagonists. J Neurosci 1988 ; 8 : 185-96.*

Christensen MS . *Acid-base changes in cerebrospinal fluid and blood, and blood volume changes following prolonged hyperventilation. Br J Anaesth 1974 ; 46 : 348.*

Cook NS . *The pharmacology of potassium channels and their therapeutic potential. TIPS 1988 ; 9 : 21-8.*

Flemma RJ, Young WG . *The metabolic effects of mechanical ventilation and respiratory alkalosis in postoperative patients. Surgery 1964 ; 56 : 36-43.*

Forsythe ID, Westbrook GL, Mayer ML . *Modulation of excitatory synaptic transmission by glycine and zinc in cultures of mouse hippocampal neurons. J Neurosci 1988 ; 8 : 3733-41.*

Foster AC, Kemp JA . *Glycine maintains excitement. Nature 1989 ; 338 : 377-8.*

Fujiwara N, Higashi H, Shimoji K, Yoshimura M . *Effects of hypoxia on rat hippocampal neurons in vitro. J Physiol 1987 ; 384 : 131-51.*

Gordon E, Rossanda M . *Further studies on cerebrospinal fluid acid-base status in patients with brain lesions. Acta Anaesthesiol Scand 1970 ; 14 : 97-109.*

Hamilton TC, Weir SW, Weston AH . *Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. Br J pharmacol 1986 ; 88 : 103-11.*

Hansen AJ . *Effect of anoxia on ion distribution in the brain. Physiol Rev 1985 ; 65 : 101-48.*

Hansen AJ, Hounsgaard J, Jahnsen H . *Anoxia increases potassium conductance in hipocampal nerve cells. Acta physiol scand 1982 ; 115 : 301-10.*

Kleckner N, Dingledine R . *Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science 1988 ; 241 : 835-7.*

Kloog Y, Lamdani-Itkin H, Sokolovshy M . *The glycine site of the N-methyl-D-aspartate receptor channel ; Differences between the binding of HA-966 and of 7-chlorokynurenic acid. J Neurochem 1990 ; 54 : 1576-83.*

Larson AA, Beitz AJ . *Glycine Potentiates strychnine-induced convulsions ; Role of NMDA receptors. J Neurosci 1988 ; 8 : 3822-6.*

Ljungren B, Norberg K, Siejo BK . *Influence of tissue acidosis upon restitution of brain energy metabolism following total ischemia. Brain Res 1974 ; 77 : 173.*

Maiese K, Caronna JJ . *Coma following cardiac arrest ; A review of the clinical features, management, and prognosis. J Intensive Care Med 1988 : 3:153-63.*

Maruki Y, Koehler RC, Eleff SM, Traystman RJ . *Inracellular pH during reperfusion influences evoked potential recovery after complete cerebral ischemia. Stroke 1993 ; 24 : 697-793.*

Muizelaar JP, Marmarou A, Ward JD . *Adverse effects of prolonged hyperventilation in patients with severe head injury ; a randomized clinical trial. J Neurosurg 1991 ; 75 : 731-9.*

Muizelaar JP, Poel HG, Li ZC . *Pial arteriolar vessel diameter and CO_2 reactivity during prolonged hyperventilation in the rabbit J Neurosurg 1988 ; 69 : 923-7.*

Nicholls D . *Release of glutamate, aspartate and gamma-aminobutyric acid from isolated nerve terminals. J Neurochem 1989 ; 52 : 331-41.*

Nicholls D, Attwell D . *The release and uptake of excitatory amino acids. TIPS Special Reports 1991 ; 68-74.*

Rothman SM, Olney JW . *Glutamate and the pathophysiology of hypoxic-ischemic brain damage. Ann Neurol 1986 ; 19 : 105-11.*

Rothman SM, Thurston JH, Hauhart RE . *Delayed neurotoxicity of excitatory amino acids in vitro. Neuroscience 1987 ; 22 : 471-80.*

Sánchez-Prieto J, González P . *Occurrence of a large Ca^{2+} -independent release of glutamate during anoxia in isolated nerve terminals(synaptosomes). J Neurochem 1988 ; 50 : 1322-4.*

Siejo BK . *Mechanisms of ischemic brain damage. Critical Care Med 1988 ; 16 : 954-63.*

Siejo BK . *Pathophysiology and treatment of focal cerebral ischemia Part I ; Pathophysiology. J Neurosurg 1992 ; 77 : 169-84(a).*

Siejo BK . *Pathophysiology and treatment of focal cerebral ischemia part II ; Mechnism of damage and treatment. J Neurosurg 1992 ; 77 : 337-54(b).*

Soloway M, Nadel W, Albin MS, White RJ . *The effect of hyperventilation on subsequent cerebral infarction. Anesthesiology 1968 ; 29 : 975-80.*

Szatkowski M, Barbour B, Attwell D . *Non-vesicular release of glutamate from glial cells by reversed electrogenic glutamate uptake. Nature 1990 ; 348 : 443-6.*

Todd MM, Tommasino C, Shapiro HM . *Cerebrovascular effects of prolonged hypocarbia and hypercarbia after experimental global ischemia in cats. Critical Care Med 1985 ; 13 : 720-3.*

Vanicky I, Marsala M, Murar J, Marsala J . *Prolonged postischemic hyperventilation reduces acute neuronal damage after 15 min of cardiac arrest in the dog. Neurosci Lett 1992 ; 135 : 167-70.*

Yoshida K, Marmarou A . *Effects of tromethamine and hyperventilation on brain injury in cat. J Neurosurg 1991 ; 74 : 87-96.*

Zini S, Roisin MP, Armengaud C, Ben AY . *Effect of potassium channel modulators on the release of glutamate induced by ischaemic-like conditions in rat hippocampal slices. Neurosci Lett 1993 ; 153 : 202-5.*

Zivin JA, Choi DW . *Stroke Therapy. Sci Am 1991 ; 265 : 56-63.*