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Protective Effects of Calcium Antagonists and Vitamine E on the Ischemia-induced Neuronal Damage in Rat Brain Slices[1]

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ABSTRACT

To evaluate the protective effects of calcium antagonists, oxygen radical scavengers and excitatory amino acid (EAA) antagonist on the ischemic brain damage, we induced in vitro ischemic condition (namely, lack of oxygen and glucose) to rat hippocampal slices. And the degree of ischemic damage was determined by assaying changes in biochemical parameters such as ATP content and lactate ralease, MDA production in the presence or absence of the various drugs.

During experimental ischemia for up to 60 min, ATP content was decreased and the amount of lactate release was markedly increased time-dependently. By changing the reaction medium which contained oxygen and glucose those biochemical parameters were recovered. But the recovery was not complete in this experimental condition.

In the same ischemic conditions verapamil and vitamine E prevented the decrease of ATP content and the increase of lactate release from the slices. And verapamil and diltiazem decreased MDA release to the reaction medium.

Superoxide dismutase (SOD) and MK-801 (as EAA receptor antagonist) protected the decrease of ATP content and reduced MDA release in 20 min ischemic condition, but glutathione affected ATP content and lactate release at the same condition.

When oxygen and glucose were resupplied for 20 min after ischemic condition, verapamil showed the protective effect on the changes of ATP content and lactate release, and vitamine E decreased lactate release (at 20 min ischemia) and MDA release (at 60 min ischemia).

These results showed that calcium antagonist and vitamine E protect the ischemic biochemical changes from rat hippocampal slices and calcium antagonist is more potent than vitamine E to protect the ischemic brain damage.

Key Words: Ischemic brain damage, Oxygen radical scavengers, Antioxidants, Calcium antagonists

INTRODUCTION

In contrast to a relatively good understanding of the hemodynamic and metabolic aspects of stroke, a great deal remains to be clarified concerning the biochemical mechanisms underlying ischemia-induced cellular injury. In the past decade several mechanisms have been postulated to be involved in the production of ischemia and reperfusion injury; reduced ATP levels (Jennings and Reiner, 1981), reduced intracellar pH (Siesjo, 1984; Nemoto, 1985), increased intracellular calcium (Hass, 1981), increased free fatty acids (De-

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medio *et al.*, 1980; Rehencroma *et al.*, 1982).

Recently considerable experimental evidence indicates that excessive endogenous excitatory amino acid (EAA) release which are known to produce neurotoxic effects, contribute to the pathogenesis of ischemic hippocampal injury (Rothman and Olney, 1986; Choi, 1990). Released EAA destroy the ionic homeostasis, especially increase intracellular calcilum influx through the ion channel, through which depletion of the high energy phosphate and activation of the phospholipase and proteinase. It is followed by degradation of cell membrane, production of leukotriene, prostaglandin and oxygen free radicals. It has been recognized that damage in cerebral ischemia is at least partly due to oxidative damage caused by free radical formation and subsequent lipid peroxidation. (Flamm *et al.*, 1978; Yoshida *et al.*, 19808) Free radical damage is assumed to be initiated by increased production of the superoxide anion radical and its by-products. The abrupt increase in these active oxygen species can be very deliterious because lipids, which are abundant in the brain, are vulnerable to peroxidation. (Cooper *et al.*, 1980; Rehncrona *et al.*, 1982).

According to the results of the study about the various mechanisms of ischemic brain damage, several kinds of drugs which are expected to reduce or protect the neural damage were developed and were tried in clinical treatment. It was reported that calcium antagonists can reduce the neuronal damage because they inhibit the calcium influx through the calcium channel (Collins, 1989). Nonspecific EAA receptor antagonist, gamma-D-glutamylglycine was reported that it can protect the ischemic damage and toxic effect of glutamate/asparate (Rothman, 1983). Mannitol, phenytoin, vitamine E and dexamethasone could protect the cerebral ischemic damage in a global ischemic model of the rat.

However there were still some arguments and controversies about the protective effects of the above chemicals according to the experimental ischemic models and animal species. And also it is difficult to understand their pharmacologic mechanism and potency in in-vivo experimental conditions.

In the present study, we used the in vitro ischemic model and tested that calcium antagonist, antioxidants and free radical scavengers and EAA antagonist could protect the ischemic damage in this experimental conditions.

MATERIALS AND METHODS

Chemicals

ATP, lactate, lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH), glutamate, NAD^+, NADH, ADP, BSA, DTT, BCA, vitamine E, glutathione, superoxide dismutase (SOD), catalase, diltiazem, verapamil were purchased from Sigma chemical Co. (St. Louis, MO., U.S.A). Luciferin and luciferase were purchased from Boehringer Mannheim, MK-801 from RBI and other chemicals were reagent grade.

Preparation of hippocampal slices and induction of in vitro ischemia

Brain slices (400 μm) were prepared from the hippocampus of rat (180~200 gm) with Mcllwain tissue chopper within 15 min under ice. To recover funtion, slices were incubated in artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $MgSO_4$, 2 mM $CaCl_2$, 23 mM $NaHCO_3$ and 10 mM glucose and was equilibrated with 95% O_2/5% CO_2 for 1.5~2 hours (Miyakawa, 1985).

5~6 slices were transferred to reaction chamber and in vitro ischemic conditon was induced by changing the media to ACSF without glucose and was equilibrated with 95% N_2/5% CO_2 at 32℃ for 20 or 60 min. After the ischemic condition brain slices were transferred to determine the content of ATP and lipid peroxidation of slices, and from the reaction medium the amount of lactate releas and malondialdehyde (MDA) release were determined. To observe the effect of reoxygenation in brain, after the ischemic condition for 20 or 60 min brain slices were transferred to the ACSF which was equilibrated with 95% O_2/5% CO_2 for 20 min. And the biochemical changes were determined in slices and medium in the same way.

Drug treatment

Brain slices were incubated with the ACSF in the absence of oxygen and glucose. During ischemic condition each one of the following drugs was added to the reaction medium: 0.5 mM vitamine E

or glutathione as antioxidants, 0.05 mg/ml superoxide dismutase or catalase as oxygen radical scavengers, 0.1 mM diltiazem or verapamil as calcium antagonists, and 0.1 mM MK-801 as excitatory amino acid (EAA) antagonist respectively. To determine whether these drugs could protect or improve the ischemic damage during reoxygenation, the above same drugs were added to the reaction medium containg oxygen and glucose for 20 min.

Measurement of ATP content

After the reaction, slices were transferred to a tube containing 300 μl of 1 N perchloric acid with 0.4% Triton X-100 and then sonicated for 30 sec (Heat Systems-ultrasonics, W-385) and extracted for 15~20 min at 4℃. And then the extract was neutralized with 1 M KOH, 0.3 M KCl, 0.4 M imidazole buffer solution and centrifugated at 0℃, 10,000 rpm for 7 min and the supernatant was stored at −70℃ until use.

ATP was measured with a luminometric method (Strehler & Totter, 1952). Extract was diluted by 40 fold with ATP reagent without luciferin/luciferase (50 mM Tris acetate pH 7.75, 2 mM EDTA, 6 mM DTT, 0.075% BSA, 10 mM Mg acetate, 0.04 mM d-luciferin, 2 μg/ml luciferase). 50 μl of diluted sample were reacted with 100 μl of ATP reagent (containing 0.035 mM luciferin and 0.04 mg/ml luciferase) and the luminescence was measured by luminometer (Lumat Lb 9501, Berthold) for 10 sec and ATP content was calculated.

Measurement of lactate release from the slices

Released lactate was measured by fluorospectrometer from the reaction medium. 50 μl of reaction medium were incubated with 0.25 M glycine-0.1 M hydrazine buffer (pH 9.2), 0.4 mM NAD^+, 0.004 mg/ml LDH (from rabbit muscle) at 30℃ for 20 min and NADH produced was measured by fluorescence spectrophotometer (Kontron) at excitation 340 nm, and emission 460 nm.

Determination of lipid peroxidation

Lipid peroxidation was measured by modified thiobarbituric acid method (Bidlack and Tapple, 1973). After the reaction, slices were transferred to microfuge tube and mixed with 300 μl of 1 N PCA containing 0.4% Triton X-100 and then were sonicated. 100 μl of aliquot was mixed with 50 μl of 30% TCA and 50 μl 0.75% thiobarbituric acid containing 0.02% butylated hydroxytoluene at 100℃ for 1 hour. After cooling the mixture at room temperature, 0.5 ml of butanol was added and mixed vigorously and centrifuged. Absorbance of MDA-TBA complex which is moved to butanol fraction was measured with a spectrophotomter (Camspec) at 532 nm. Amount of MDA was calculated from the molar extinction coefficient of MDA (1.56×10^5/M/cm)(Placer *et al.*, 1966).

Measurement of protein

Protein of slices was measured with modified Lowry method (Lowry *et al.*, 1951) using bicinchoninic acid (BCA). Bovine serum albumin was used as standard protein.

RESULTS

Biochemical changes during ischemia and reperfusion

ATP content of the slices: When slices were incubated in ACSF equilibrated with 95% O_2 and 10 mM glucose for 20 or 60 min ATP content of the control group was 13.1±0.7 nmole/mg prot at 20 min incubation, 11.2±0.6 nmole/mg prot at 60 min incubation.

But in ischemic condition which was made by omitting glucose and replacing 95% N_2 instead of 95% O_2 ATP content was reduced to 2.7±0.1 nmole/mg prot for 20 min ischemia, and to 1.0± 0.1 nmole/mg prot for 60 min ischemia.

After the 20 min ischemic condition, when oxygen and glucose were supplied to the reaction medium for 20 min, ATP content of the slices was increased to 5.4±0.3 nmole/mg prot. But after the 60 min ischemic condition resupply of oxygen and glucose did not recover ATP content from the ischemic condition (Fig. 1).

These results indicated that ATP content of the slices was decreased during ischemic condition and it was slightly recovered in reoxygenation. But recovery of ATP content of the brain slices was dependent to the ischemic duration.

Lactate release from the brain slices: When brain slices were incubated with 95% O_2 and glu-

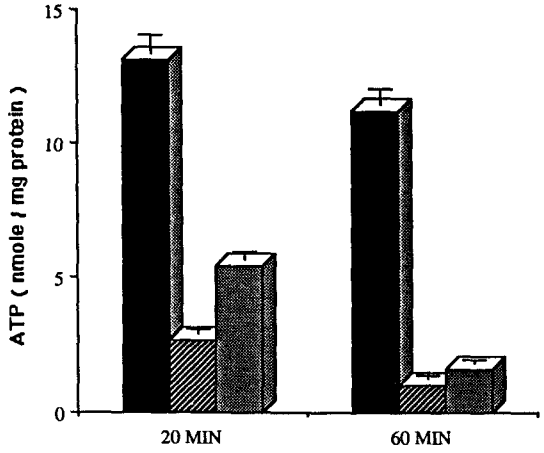

Fig. 1. Changes in ATP content of rat brain hippocampal slices after in vitro ischemic or reoxygenation condition. Brain slices were incubated with 95% O_2/5% CO_2 equilibrated artificial cerebrospinal fluid (ACSF) in the reaction chamber. And in vitro ischemic condition was produced by changing the media to ACSF without glucose and oxygen for 20 or 60 minutes. After the ischemic condition the brain slices were reoxygenated with the oxygenated ACSF for 20 minutes. At fixed time slices were prepared to determine the content of ATP by luminometric method as described in Materials & Methods. (■) control, (▨) in vitro ischemia, (▩) reoxygenation after ischemia. The Values represent mean±S.E.M. (n=5-15).

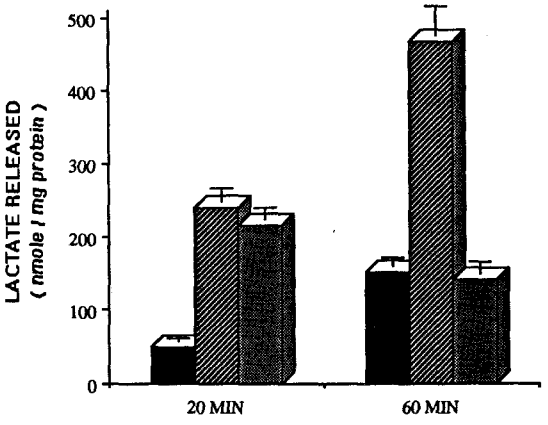

Fig. 2. Changes in lactate released to the reaction medium after in vitro ischemic or reoxy-genation condition. The condition was same as described in Fig. 1. At flxed time the reaction medium was transferred and determined the contents of lactate released fluorospectrophotometrically as described in Materials & Methods. (■) control, (▨) in vitro ischemia, (▩) reoxygenation after ischemia. The Values represent mean±S.E.M. (n=5-15).

cose for 20 min the amount of lactate release to the reaction medium was 48.5±4.9 nmole/mg prot. The amount of lactate release was increased to 151.2±12.6 nmole/mg prot for 60 min incubation with the same condition.

But when brain slices were incubated with in vitro ischemic condition the amount of lactate release was markedly increased up to 3~5 fold. In 60 min ischemia the amount of lactate release was increased more than in 20 min ischemia. However it was recovered to the control level by reoxygenation and glucose supply (Fig. 2).

MDA content and MDA release from the brain slices: To observe whether oxygen free radicals could be generated during ischemia or reoxygenation we checked the lipid peroxidation by

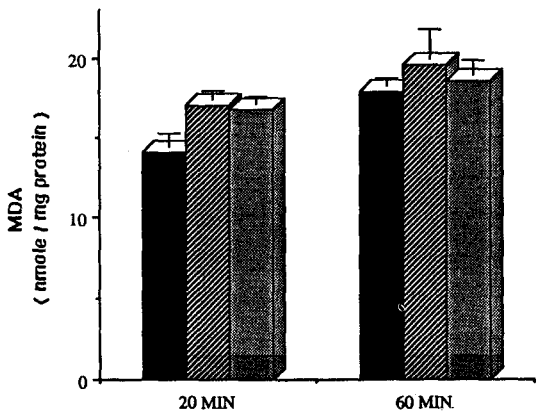

Fig. 3. Changes in MDA contents of rat brain hippocampal slices after in vitro ischemic or reoxygenation condition. The condition was same as described in Fig. 1. At fixed time the slices were prepared and determined the content of MDA spectrophotometrically as described in Materials. (■) control, (▨) in vitro ischemia, (▩) reoxygenation after ischemia. The Values are shown as mean±S.E.M. of 5-15 experiments.

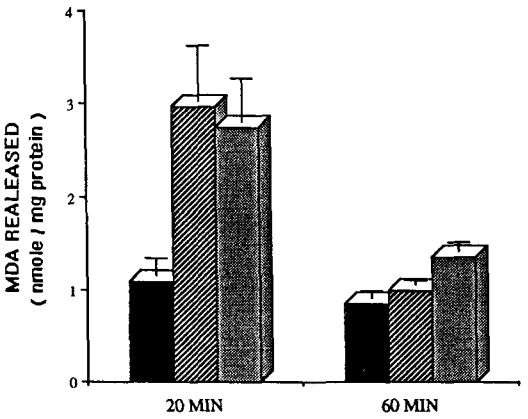

Fig. 4. Changes in MDA released to reaction medium after in vitro ischemic or reoxygenation condition. The condition was same as described in Fig. 1. At fixed time the reaction medium was transferred and determined the contents of MDA released as described in Fig. 3. (■) control, (▨) in vitro ischemia, (▩) reoxygenation after ischemia. The Values as shown as mean ± S.E.M. of 5-15 experiments.

the measurement of MDA production. MDA content in the normal control slices which were incubated for 20 min in ACSF equilibrated with 95% O_2 and 10 mM glucose was 14.1±0.8 nmole/mg prot. In ischemic condition for 20 min MDA content was slightly increased to 17.0±0.4 nmole/mg prot. After the ischemic condition, when 95% O_2 and glucose was added again to the reaction medium for 20 min, MDA content of the slices was not changed from that of ischemic condition (Fig. 3). In the case of 60 min ischemia and reoxygenation after 60 min ischemia MDA content was not changed from that of control group which was incubated with 95% O_2 and glucose for 60 min. And MDA release to the reaction medium was also increased in 20 min ischemic condition. But in 60 min ischemia and in reoxygenation after 60 min ischemia differences in MDA release were not shown (Fig. 4). These results indicated that lipid peroxidation could occur during the early phase of ischemia. But in this experimental condition the degree of lipid peroxidation was not aggravated by reoxygenation.

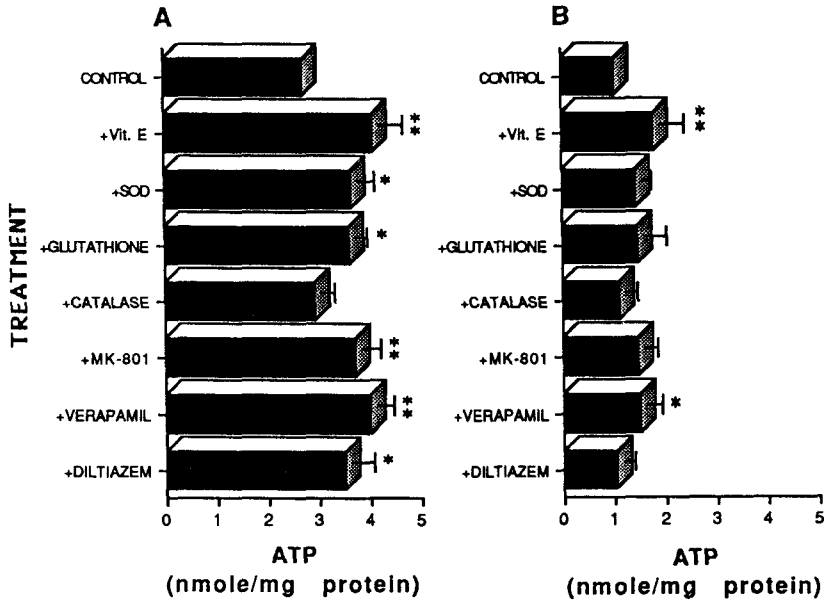

Fig. 5. Effects of various drugs on the content of ATP in ischemic brain slices. Brain slices were incubated with the ACSF without or with oxygen and glucose, with 0.5 mM vitamine E, 0.5 mM glutathione, 0.05 mg/ml superoxide dismutase, 0.05 mg/ml catalase, 0.1 mM diltiazem, 0.1 mM verapamil, and 0.1 mM MK-801 respectively for 20 or 60 min. At fixed time slices were prepared and determined the ATP content as same as described in Fig. 1. A: 20 minutes ischemia, B: 60 minutes ischemia. Values represent mean ± S.E.M. of 5-15 experiments. **: $P < 0.05$, *: $P < 0.1$ compared with control.

Protective effects of various drugs on the biochemical change in ischemic condition

ATP content and lactate release from the hippocampal slices: To evaluate which drugs could exert the protective effect on ischemia-induced neuronal damage. 0.5 mM Vit. E, 0.5 mM glutathione, 0.05 mg / ml superoxide dismutase (SOD), 0.05 mg/ml catalase, 0.1 mM diltiazem, 0.1 mM verapamil, 0.1 mM MK-801 were treated to the reaction medium from the begining of the ischemia in the same experimental condition as described above.

When the brain slices were treated with the ischemic condition for 20 min ATP content of slices was decreased to 2.7±0.1 nmole/mg prot. By adding Vit. E, SOD, glutathione, MK-801, verapamil, diltiazem to the reaction medium, ATP content were changed to 4.1±0.5, 3.6±0.3, 3.6±0.2, 3.7 ±0.3, 4.0±0.3, 3.5±0.4 nmole/mg prot respectively. And in 60 min ischemia ATP content was increased only in the presence of Vit. E and verapamil (Fig. 5).

In the case of lactate release from the slices in 20 min ischemia Vit. E, glutathione, catalase and calclum antagonist decreased the lactate release. And in 60 min ischemia these drugs showed same effects as in 20 min ischemia except catalase (Fig. 6). These results showed that those treated drugs have an action to protect the ischemia-induced biochemical changes, and also Vit. E and verapamil is more consistent and potent to protect the ischemic neuronal damage among these tested drugs.

Effects on MDA content and MDA release: In 20 min ischemia, MDA content in slices was 17.0± 0.5 nmole/mg prot. After pretreatment of 0.1 mM MK-801, it was decreased to 13.8±1.3 nmole/mg prot. But other drugs did not showed any significant effect (Fig. 7).

In the case of MDA release to the reaction medium verapamil and MK-801 decreased MDA release in 20 min and 60 min ischemia. And MDA

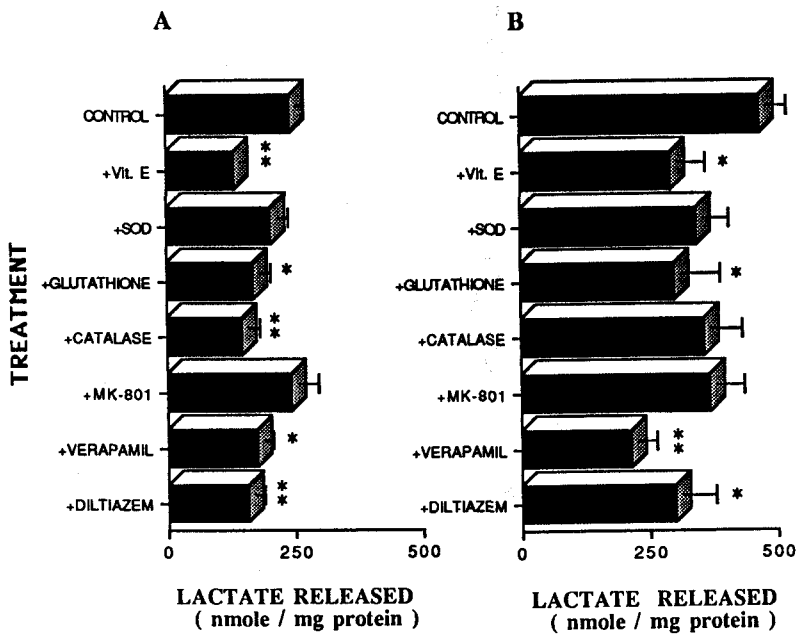

Fig. 6. Effects of various drugs on the content of lactate released to the reaction medium in ischemic condition. The reaction condition was same as described in Fig. 6. At fixed time lactate released was determined as same as described in Fig. 2. A: 20 minutes ischemia, B: 60 minutes ischemia. The Values are shown as mean±S.E.M. of 5-15 experiments. **: $P<0.05$, *: $P<0.1$, compared with control

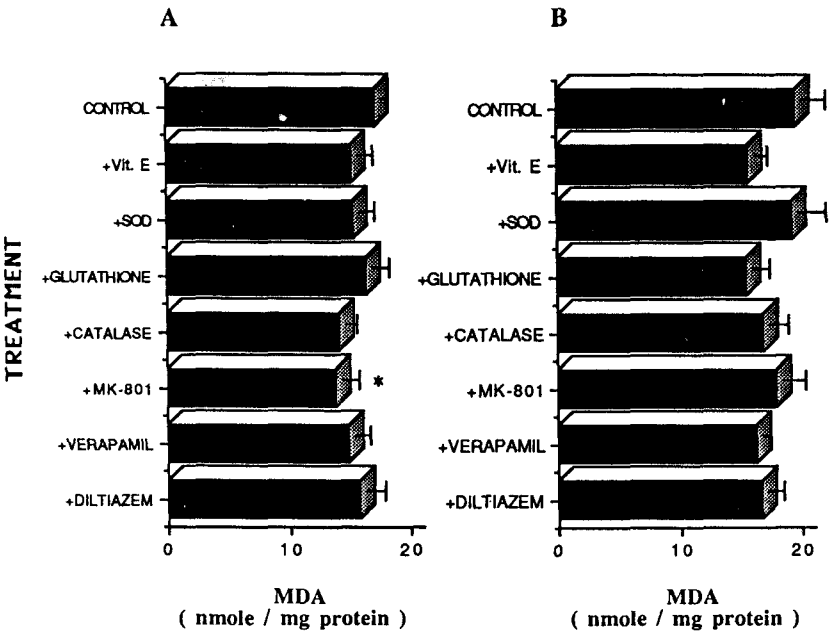

Fig. 7. Effects of various drugs on the content of MDA in ischemic brain slices. The condition was same as described in Fig. 5. At fixed time MDA was determined as same as described in Fig. 3. A: 20 minutes ischemia, B: 60 minutes ischemia. The Values are shown as mean±S.E.M. of 5-15 experiments. *: $P<0.1$ compared with control

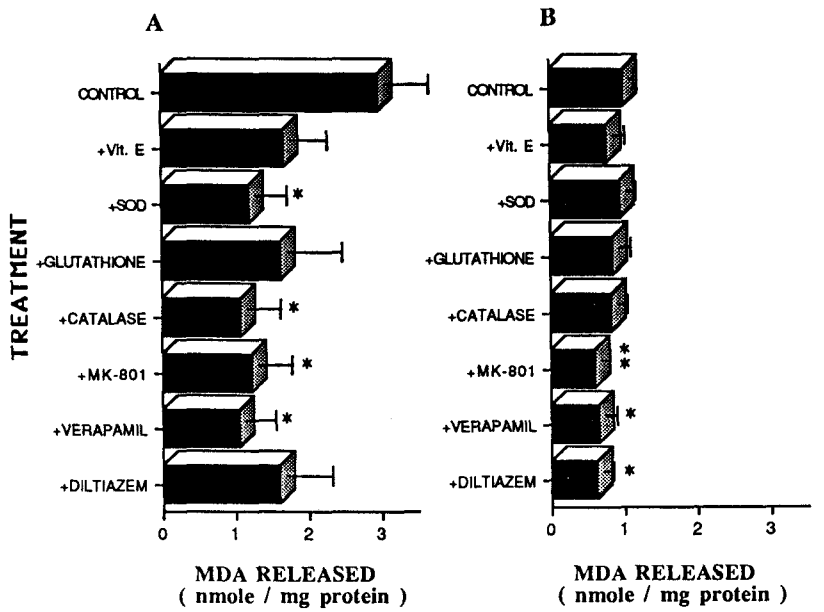

Fig. 8. Effects of various drugs on the content of MDA released to the reaction medium in ischemic condition. The condition was same as described in Fig. 5. At fixed time MDA released was determined as same as in Fig. 4. A: 20 minutes ischemia, B: 60 minutes ischemia. The Values are shown as mean±S.E.M. of 5-15 experiments. **: $P<0.05$, *: $P<0.1$ compared with control

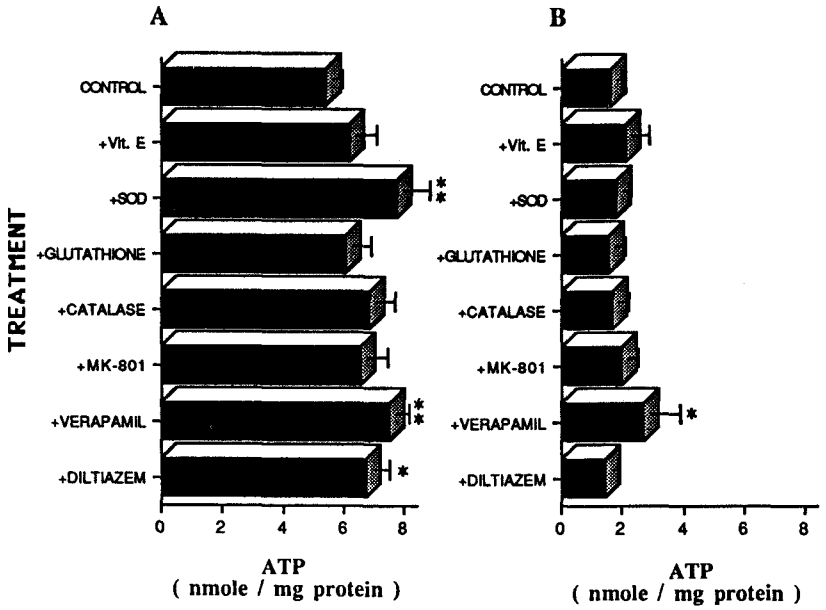

Fig. 9. Effects of various drugs on the content of ATP in reoxygenated brain slices. Brain slices were incubated with the ACSF without oxygen and glucose to 20 or 60 minutes, and then they were reoxygenated with the oxygenated ACSF for 20 minutes. The drugs which were described in Fig. 5. were added to the reaction medium respectively from the begining of the reaction. At fixed time slices were prepared and determined the ATP content as same as described in Fig. 1. A: 20 minutes ischemia, B: 60 minutes ischemia. Values represent mean ±S.E.M. of 5-15 experiments. **: $P<0.05$, *: $P<0.1$ compared with control

release was decreased by SOD and catalase in 20 min ischemia, by diltiazem in 60 min ischemia. But MDA content and MDA release were not reduced by vitamine E and glutathione as a well-known antioxidants (Fig. 8). These results showed calcium antagonist could reduce MDA content and MDA release from the slices when ischemic condtion was induced up to 60 min but Vit.E and glutathione did not show the any protective effects.

Effects of various drugs on the ischemia-induced biochemial changes in reoxygenation

ATP content and lactate release from the slices: From the beginning of ischemic condition, the same drugs were treated and at the fixed time (20 or 60 min ischemia) oxygen and glucose were supplied to the reaction medium for 20 min. After then ATP content of slices and lactate release from the slices were measured. In the pretreatment of verapamil ATP content was increased in reoxygenation after both 20 min and 60 min ischemic condition. In the case of diltiazem it was increased only in reoxygenation after 20 min ischemic condition.

Treatment of SOD also increased ATP content in reoxygenation after 20 min ischemia, but Vit. E did not show the increase of ATP content during reoxygenation.

The amount of lactate release to the reaction medium was 216.7±15.9 nmole/mg prot in reoxygenation after 20 min ischemia. In the presence of Vit. E it was decreased to 114.0±7.9 nmole/mg prot. And it was also decreased by the treatment of verapamil, diltiazem, glutathione. But, in reoxygenation after 60 min ischemia the pretective effect was shown only in the case of verapamil (Fig. 9 & 10).

These results showed that only verapamil is consistant to increase the recovery from ischemia-induced biochemical change druing reoxygen-

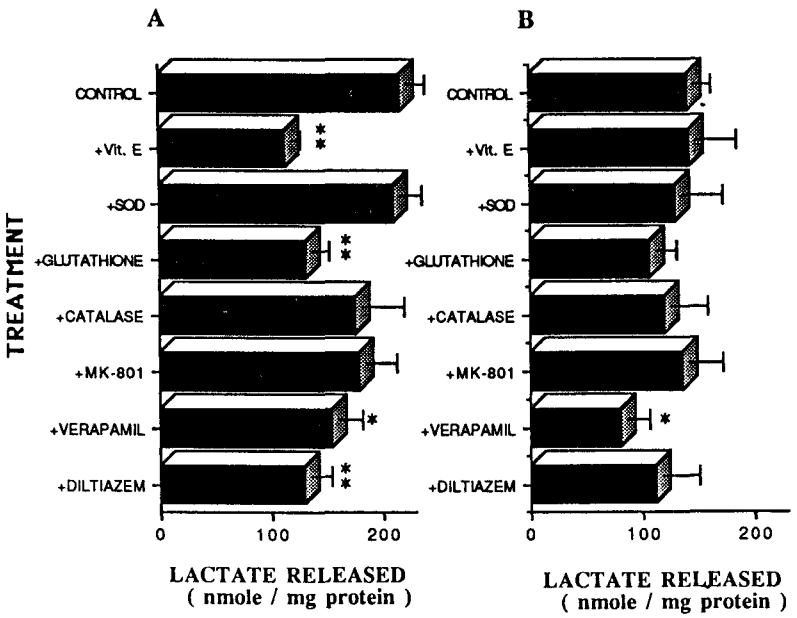

Fig. 10. Effects of various drugs on the content of lactate released to the reaction medium in reoxygenated condition. The condition was same as desribed in Fig. 9. At fixed time the reaction media was determined lactate released as same as described in Fig. 2. A: 20 minutes ischemia, B: 60 minutes ischemia. The Values are shown as mean±S.E.M. of 5~15 experiments.
**: $P<0.05$, *$P<0.1$ compared with control

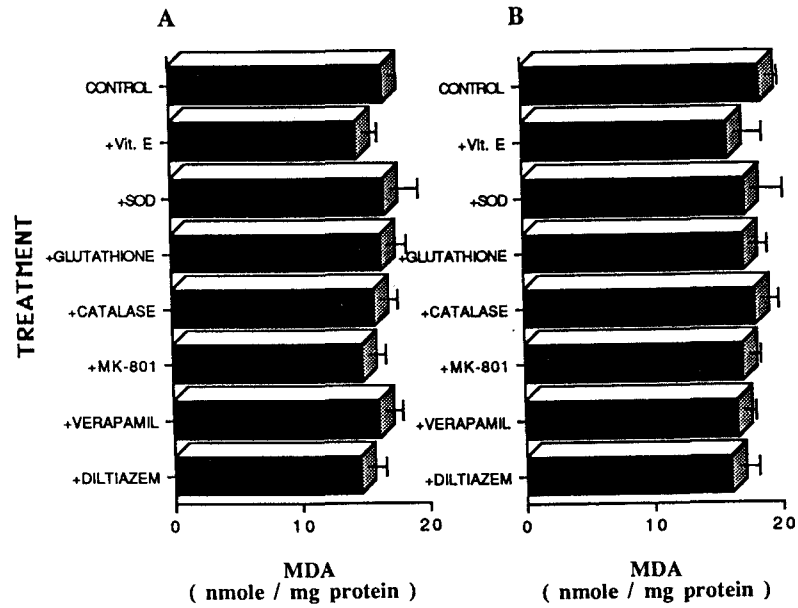

Fig. 11. Effects of various drugs on the content of MDA in reoxygenated brain slices. The condition was same as described in Fig. 9. At fixed time the slices were prepared and MDA was determined as same as described in Fig. 3. A: 20 minutes ischemia, B: 60 minutes ischemia. The Values are shown as mean±S.E.M. of 5~15 experiments.

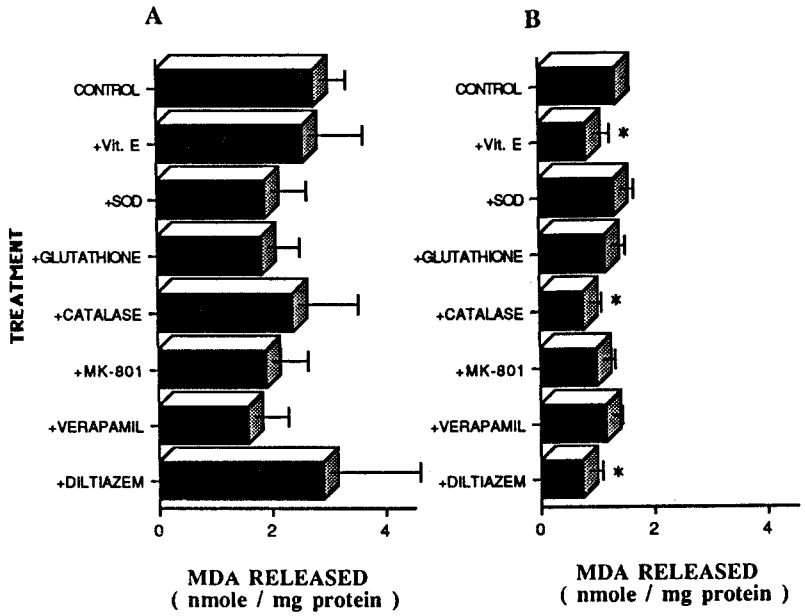

Fig. 12. Effects of various drugs on the content of MDA released to the reaction medium in reoxygenated condition. The condition was same as described in Fig. 9. At fixed time the reaction medium was determined MDA released as same as described in Fig. 4. A: 20 minutes ischemia, B: 60 minutes ischemia. The Values are shown mean±S.E.M. of 5~15 experiments.
**: $P<0.05$, *: $P<0.1$ compared with control

ation.

MDA content and MDA release: MDA content in slices was not changed in reoxygenation after 20 and 60 min ischemia by pretreatment of drugs. Also there was no effect on the amount of MDA release in reoxygenation after 20 min ischemia. But treatment of Vit. E, catalase and diltiazem slightly decreased MDA release from the slices in reoxygenation after 60 min ischemia (Fig. 11 & 12).

DISCUSSION

In this study the effects of the calcium antagonists, antioxidants and radical scavengers and EAA antagonist on the ischemic brain damage were compared using in vitro ischemic model. When verapamil was treated to the brain slices verapamil reduced the decrease of ATP content in slices and lactate release and MDA release from the slices in 20 and 60 min ischemic condition. And it increased the recovery of ATP content and inhibited lactate release during reoxygenation. Diltiazem also showed the same effects in 20 min ischemia. And vitamine E inhibited the decrease of ATP content and the increase of lactate release. But other drugs such as SOD, catalase, glutathione, MK-801 were not consistent to exert the protective effects on the ischemia-induced biochemical changes in the brain slices. These results showed that calcium antagonists like verapamil and diltiazem and vitamine E could protect the derangement of energy metabolism during ischemia.

Several mechanisms are involved in the production of ischemic and reperfusion injury: intracellular acidosis, excessive calcium influx (Demedio *et al.*, 1980; Rehencroma *et al.*, 1982), production of oxygen free radicals (Flamm *et al.*, 1978; Yoshida *et al.*, 1980), activation of excitatory amino acid receptor by excitatory neurotransmitter (Rothman & Olney, 1986; Choi, 1990) and so on. As a

result of diminished supply of oxygen and nutrients during ischemia energy-rich phosphate are rapidly depleted. This decreased energy capacity leads to reduction in efficiency of ion pumps and loss of ion homeostasis resulting in excessive calcium influx. Increased calcium ion activates proteolytic and phospholipid enzymes, leading to breakdown of membrane integrity. Additional damage may occur during oxygen resupply. When polyunsaturated fatty acids released during ischemia, are oxidized by the cyclo-oxygenase and lipoxygenase pathways, oxygen radicals are released and further induce to damage membrane lipids.

From these backgrounds application of calcium antagonists have been intensively studied in the treatment of cardicac and cerebral ischemia (Collins, 1989; Gelmers, 1987; Heiss *et al.*, 1990; Wong *et al.*, 1990).

Calcium antagonists are known to have a wide chemical variety and interact principally with the voltage-sensitive calcium channel leading to blocking the influx of calcium to the normal and ischemic cells. Most of the anti-ischemic activity of calcium antagonist can be explained by their hemodynamic effects, which induce vasodilation and reduce bascular resistance (Pirnagl *et al.*, 1990; Germano *et al.*, 1987; Jacewicz *et al.*, 1990; Marcoux *et al.*, 1987; Martinez-Vilia *et al.*, 1986; Sauter *et al.*, 1986).

We demonstrated that verapamil and diltizem protected the ischemic damage of rat hippocampal slices during in vitro ischemia. This results indicated that calcium antagonists have a direct cytoprotective action against in vitro ischemia since hemodynamic effects of calcium antagonists could be eliminated because we used in vitro ischemia model in this experiments. Although it is difficult to understand the protective mechanism of calcium antagonist against ischemic insult from these results, it can be considered that the L-type calcium channel is related to the cellular damage which occure after glucose and oxygen deprivation since these calcium antagonists are known to block the voltage-dependent L-type calcium channel.

In addition there are some reports that calcium antagonists can maintain the high energy phosphate, inhibit the abnormal movement of the electrolytes such as sodium and potassium as well as calcium, and inhibit the acidosis of focal lesion through preventing calcium overload with little or no hemodynamic activity.

In another aspect it is well known that vitamine E acts as a free radical scavenger acting particularly superoxide anion and peroxy radical and inhibit lipid peroxidation as an antioxidant. Vitamine E is also known to stabilize membranes by forming arachidonic acid-vitamine E complexes in the cellular membrane (Tominaga *et al.*, 1985; Uenohara *et al.*, 1988). Mannitol, barbiturate, and methylprednisolone as well as vitamine E could protect the cerebral damage by inhibiting the production of oxygen free radicals or lipid peroxidation (Chan and Fishman, 1978; Masahara *et al.*, 1989). In our experiments vitamine E also showed the protective effects against ischemic damage. These results were same as the reports of Acosta *et al.*, (1987) that vitamine E had the protective effects on ischemic damage in rat hippocampal slices.

However, in this study the production of malondialdehyde was slightly increased during early phase of ischemia, but there was no additional increase of MDA production during reoxygenation. This results were not usual because oxygen free radicals are particularly abundant in post-ischemic tissues (McCord, 1985). Furthermore the pretreated antioxidant like vitamine E and glutathione did not reduce MDA content and MDA release from the slices, and oxygen radical scavengers liks SOD, catalase did not show any consistent reduction on the production of MDA. These results suggested that lipid peroxidation was not significantly produced and also antioxidants including vitamine E could not show any protective effect on lipid peroxidation, even if production of MDA was slightly increased during ischemia. With regard to the protective effect of antioxidants such as Vitamine E, it is needed more study about the involvement of oxygen radical and lipid peroxidation in ischemic cerebral damage.

Recently considerable experimental evidence indicates that excessive endogenous EAA release during ischemia, and the EAA involved to the neuronal damage as a consequence of excessive stimulation of EAA receptors (Choi, 1990; Rothman, 1983; Rothman and Olney, 1986). And it is reported that glutamate antagonist, especially

NMDA receptor antagonist could reduce the cellular damage in experimental cerebral ischemia (Albers *et al.*, 1989; Choi. 1988; Clifford *et al.*, 1989; Karschin *et al.*, 1988; Siesjo and Bengtsson, 1989). Rothman and Olney (1986) have also reported that calcium dependent mechanism is involved in this EAA-mediated neuronal injury. In our experiment MK-801 showed only the protective effect on the change of ATP content in 20 min ischemic condition and this protective potency was not so potent than that of verapamil or vitamine E. These results suggested that MK-801 is not effective to protect the ischemic brain damage in this experiments. However there was a marked increase in glutamate release from slices during ischemic insult in this experimental condition (data not shown). This discrepancy can be explained that our experimental condition is not an ideal system to detect the excitotoxic effect of EAA, because we observed the neuronal damage only in a short duration of ischemia and reoxygenation. But it is well known that EAA-mediated neuronal injury is delayed phenomenon and protected by magnesium which is a physiological ionic component.

In conclusion we demonstrated that calcium antagonist and vitamine E could be potential protective agents in ischemic neuronal damage and the effectiveness of verapamil is more potent than vitamine E and diltiazem. This results support a role for calcium in the ischemic damage. However, further evaluation is needed to clarify the protective mechanism of calcium antagonists as well as vitamine E in ischemic neuronal damage.

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=국문초록=

랫트 뇌절편에서의 허혈성 신경손상에 대한 칼슘길항제와 비타민의 보호효과

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허혈성 뇌손상시 칼슘길항제, 항산화제와 산소라디칼 제거제 그리고 흥분성 아미노산 수용체 길항제의 보호효과를 검토하기 위해 본 연구에서는 랫트 뇌 해마조직 절편을 산소와 포도당을 제거한 반응액에 노출시켜 실험적 허혈상태를 유도하였다. 그리고 여러 약물을 처리한 상태에서 허혈시의 뇌세포 손상정도를 생화학적 지표들(절편내 ATP와 반응액내 lactate 및 malondialdehyde (MDA)유리량)을 측정하여 검토하였다.

60분까지 허혈상태를 유발시킨 경우 시간에 따라 절편내 ATP 함량이 감소하였고 lactate 유리량이 증가하였다. 그 후 산소와 포도당이 든 반응액으로 바꿔주니 이들 생화학적 변화들이 회복되는 양상을 보였다. 그러나 본 실험조건에서 허혈상태로부터 완전히 회복되지는 않았다.

동일한 허혈조건에서 verapamil과 비타민 E는 ATP 함량 감소와 절편으로부터의 lactate 유리량의 증가에 대해 보고효과를 보였다. 그리고 verapamil과 diltiazem은 반응액내로의 MDA 유리를 감소시켰다. Superoxide dismutase (SOD), glutathione과 MK-801 (NMDA 수용체 길항제)은 20분 허혈조건에서 ATP 함량을 증가시켰으나 그외 다른 조건에서 보호효과를 보이지 않았다. 허혈 후 20분간 산소와 포도당을 재공급한 경우 verapamil은 ATP 함량과 lactate 유리에 보호효과를 보였다. 한편 비타민 E는 20분 허혈 조건에서의 lactate 유리와 60분 허혈시의 MDA 유리 증가에 대해 감소효과를 보였다. 이상의 결과는 칼슘길항제와 비타민 E가 랫트 뇌절편에서의 허혈성 생화학적 손상을 방지함으로 나타난 결과로 해석되며, 칼슘길항제의 효과가 비타민 E보다 우수함으로 미루어 칼슘길항제는 허혈성 뇌손상에 예방 및 보고효과를 보일 것으로 믿어졌다.