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Diethylmaleate Decreased Ascorbic Acid Release Induced by Cerebral Ischemia in Cerebral Cortex of the Anesthetized Rat

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Abstract

The effect of diethylmaleate administration on ascorbic acid release following cerebral ischemia was investigated in anesthetized rat brain cortex. Cerebral ischemia, induced by ligating bilateral common carotid arteries and unilateral middle cerebral artery, significantly increased the extracellular ascorbic acid levels. Diethylmaleate (4 mmoles/kg, i.p.), which has been shown in earlier studies to decrease the ischemia-induced glutamate release, significantly reduced the ischemia-induced ascorbic acid release. The ischemia-induced ascorbic acid release was unaffected by perfusing NMDA receptor antagonist MK 801 (75 μM). Additionally, elevated extracellular glutamate levels, achieved by either externally applied glutamate solutions or by perfusing L-trans-pyrrolidine-2,4-dicarboxylate (PDC) (31.4 mM and 15.7 mM) to inhibit the glutamate uptake transporter, also significantly increased the extracellular ascorbic acid levels. These results suggested that ascorbic acid release in cerebral ischemia might be related to the elevated extracellular glutamate levels, which occurs following cerebral ischemia.

Key Words: ascorbic acid, diethylmaleate, L-trans-pyrrolidine-2,4-dicarboxylate, glutamate, glutathione, microdialysis, anesthetized rat, brain cortex

Introduction

Ascorbic acid is released and accumulated in brain extracellular space following cerebral ischemia (4, 10, 11). The elevated extracellular ascorbic acid level may play protective roles. Ascorbic acid can act as an antioxidant to scavenge oxygen-derived free radicals (or reactive oxygen species) that are produced during cerebral ischemia (12, 13), or the ascorbic acid may modulate NMDA receptors to desensitize glutamate-induced responses (7, 15). The ascorbic acid release has also been reported to be involved in the heteroexchange transport for uptaking glutamate (5, 9, 14), whose prolonged presence during cerebral ischemia (1, 17) overactivates its receptors and results in excitotoxicity (18,19).

Exogenous perfusion of glutamate can induce ascorbic acid release (3), which can also be used as an index of glutamate release (16). Both brain extracellular glutamate and ascorbic acid levels were increased following cerebral ischemia, however, whether the accumulated extracellular glutamate is responsible for ascorbic acid release has not been specifically investigated.

Our earlier results showed that cerebral ischemia-induced glutamate release can be lowered by diethylmaleate administration (21), probably because diethylmaleate can lower intracellular glutathione (g-glutamyl cysteinylglycine). Therefore, in present study we investigated the effect of diethylmaleate administration, which can lower ischemia-induced glutamate release, on extracellular ascorbic acid levels in anesthetized rat brain cortex following cerebral ischemia. Furthermore, the effect of L-trans-pyrrolidine-2,4-dicarboxylate (PDC), an inhibitor of sodium-dependent glutamate transporter (8), on extracellular ascorbic acid levels was also studied.

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Materials and Methods

Animal Preparations and Microdialysis Procedure

Male Sprague-Dawley rats (260-350 gm) were used. The animals were anesthetized with urethane (1.2 g/kg, i.p.), and body temperature was maintained at 37 °C with a heating pad. Polyethylene catheters were inserted into the femoral artery for monitoring the systemic arterial blood pressure (SAP) with a Gould pressure processor. The rat's head was mounted on a stereotaxic apparatus (Davis Kopf Instruments, Tujunga, CA, U.S.A.) with the nose bar positioned 3.3 mm below the horizontal. Following a midline incision the skull was exposed and one burr hole was drilled on the skull for inserting a dialysis probe. The microdialysis probe was implanted into cortex (-0.5mm anterior and 5.5 mm lateral to the bregma and 4.0 mm from the brain surface). The microdialysates were collected for every 10-min perfusion with a CMA-140 fraction collector. When diethylmaleate was used, it was administered (4 mmole/kg, i.p.) one hour prior to the onset of cerebral ischemia. Cerebral ischemia was induced by the ligation of bilateral common carotid arteries and unilateral middle cerebral artery.

The microdialysis system was perfused with Ringer solution at a flow rate of 2 µl/min. Microdialysis probes were purchased from CMA (CMA/20 , membrane length 4 mm). The probe was perfused in corresponding outer medium for 30 - 60 min before starting the measurement to avoid the changes of relative recovery with time.

HPLC Analysis

The LC system consisted of a BAS PM-80 isocratic pump (Bioanalytical System, Lafayete, IN, U.S.A.) a CMA on-line degasser (CMA 260) and a BAS LC-4C electrochemical detector with dual glassy carbon electrodes. Separation was achieved by using a Merck Associates (Darmstadt, Germany) Lichrospher 100, and LichroCART (5 mm, Econosphere) 250 mm × 4 mm RP-18 cartridge column. The mobile phase consisted of 40 mM sodium acetate, 0.54 mM Na2EDTA, 1.5 mM tetrabutylammonium hydroxide, 7.5% methanol (final pH 4.75). Elution was isocratic with a flow-rate of 0.7 ml/min. The working potential for electrochemical detector was +0.6 V vs. Ag/AgCl) were described previously. Data collection and analysis were performed with a Chem Station Chromatographic Management System (Hewlett Packard, Taiwan Branch, Taipei, Taiwan).

Amino acid analyses were performed according to the following procedure. O-phthdialdehyde (OPA) stock solution (0.02 M) was prepared in methanol (10%) containing 0.005% b-mercaptoethanol (v/v). The stock solution was stored at -20 °C, if not used immediately. Prior to derivatization, the OPA stock solution was mixed with 0.25 M sodium borate buffer (1:2). To this mixture, microdialysates or standard solution (2:1) were added. Then, the solution was mixed at 4 °C for 60 seconds followed by immediate injection onto the HPLC system equipped with a fluorescence detector (excitation wavelength 340 nm, emission wavelength 450 nm). Separation was carried out on an Alltech reversed C-18 column (150 × 4.6 mm, 3 mm). Ternary gradient elution was used. Mobile phase A consisted of 93 mM sodium acetate buffer (pH=6.0) in 7% acetonitrile. Mobile phase B consisted of 10 mM sodium acetate buffer in 90% acetonitrile. Mobile phase C was distilled H_2O. Flow rate was 0.8 ml/min. The elution profile was: 0 min 92% A, 8% B; 3 min 60%A, 40% B; 8.5 min, 60% A, 40% B; 9 min 30% B, 70% C; 13 min, 30% B, 70% C; 14 min 10% A, 90% B; 19 min 92% A, 8% B.

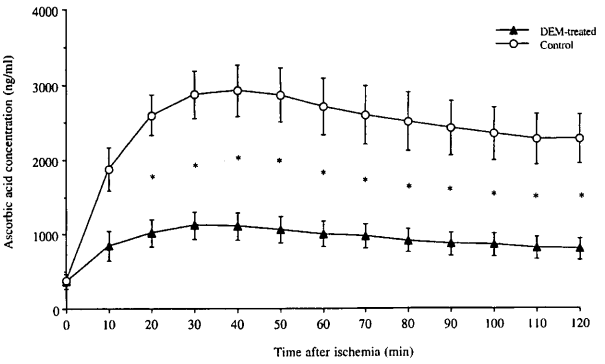

Fig. 1. Effect of diethylmaleate (4 mmole/kg) on extracellular ascorbic acid levels in anesthetized rat brain cortex after cerebral ischemia. Diethylmaleate was administered 60 minutes prior to the onset of cerebral ischemia. Data shown (mean±SEM) are from the average of 8 rats in each group. *Statistically significant ($p<0.05$) by Student's t-test.

Results

Effect of Cerebral Ischemia on Extracelluar Ascorbic Acid Levels

Cerebral ischemia significantly increased extracellular ascorbic acid levels (Fig. 1). This increase can be observed immediately after the onset of cerebral ischemia, and the ascorbic acid levels remained elevated for the entire 60-min ischemic period. Administration of diethylmaleate (4 mmole/kg, i.p.), which can lower the ischemia-induced glutamate release in anesthetized rat brain cortex (Fig. 2), can also significantly lower the ischemia-induced ascorbic acid release (Fig. 1).

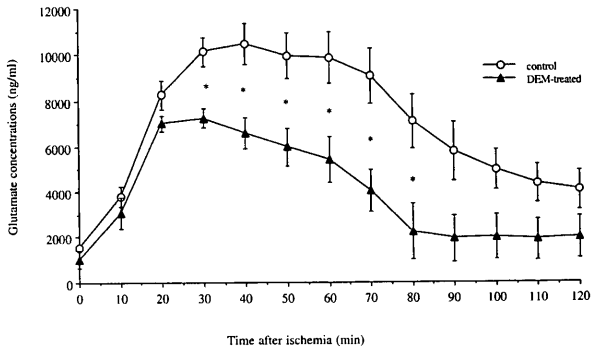

Fig. 2. Effect of diethylmaleate (4 mmole/kg) on extracellular glutamate levels in anesthetized rat brain cortex after cerebral ischemia. Diethylmaleate was administered 60 minutes prior to the onset of cerebral ischemia. Data shown (mean±SEM) are from the average of 8 rats in each group. *Statistically significant ($p<0.05$) by Student's t-test.

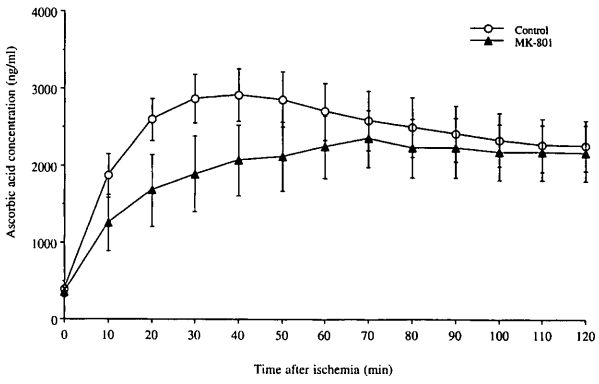

Fig. 3. Effect of MK801 (75 μM) on cerebral ischemia-induced ascorbic acid release. Data shown (mean±SEM) were the average from 12 rats.

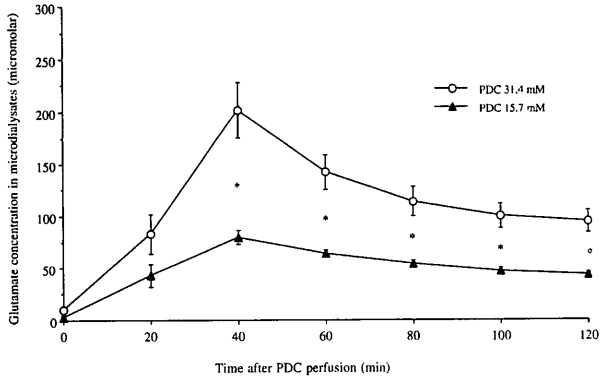

Fig. 4. Effect of PDC (31.4 mM and 15.7 mM) on extracellular glutamate levels in anesthetized rat brain cortex. Data shown (mean±SEM) were the average from 8 rats in each group. *Statistically significant ($p<0.05$) by Student's t-test.

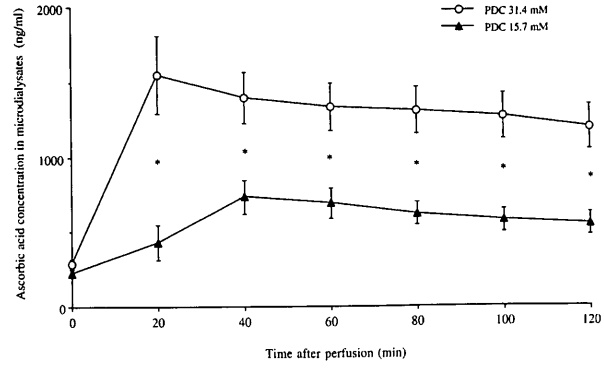

Fig. 5. Effect of PDC (31.4 mM and 15.7 mM) on extracellular ascorbic acid levels in anesthetized rat brain cortex. Data shown (mean±SEM) were the average from 8 rats. *Statistically significant ($p<0.05$) by Student's t-test.

Effect of MK-801 on Ischemia-Induced Accumulation of Extracellular Ascorbic Acid Level

To examine whether glutamate-induced ascorbic acid release during cerebral ischemia is related to the ionotropic glutamate receptor, MK-801 (a NMDA receptor antagonist) was perfused through the microdialysis probe 60 min prior to the onset of cerebral ischemia and was continuously perfused throughout the entire ischemic period. MK-801 perfusion (75 μM) seemed to lower the ischemia-induced ascorbic acid level, the decreases were of no significant difference (Fig. 3).

Effect of Glutamate Uptake Transporter Inhibition on Extracellular Ascorbic Acid Levels

To further clarify the relationship between glutamate and ascorbic acid, we used PDC (31.4 mM and 15.7 mM), a competitive and transportable inhibitor of sodium-dependent glutamate transporter, to achieve the elevated extracellular glutamate levels. PDC perfusion (31.4 mM and 15.7 mM) through the microdialysis probe significantly increased extracellular glutamate levels (Fig. 4), as expected. Interestingly, the PDC perfusions could also significantly increase extracellular ascorbic acid levels (Fig. 5).

Discussion

The results that cerebral ischemia significantly increased extracellular ascorbic acid levels are in accordance with other published results (4, 10, 11). The accumulated ascorbic acid may play protective functions against ischemia-induced toxicity either by acting as an antioxidant to scavenge reactive oxygen species that are known to be produced during the cerebral ischemia (12, 13), or by modulating the redox status of NMDA receptor so that the NMDA

receptor-mediated glutamate currents are diminished (7, 14).

Although the released ascorbic acid may play protective roles, the pathways leading to extracellular accumulation of ascorbic acid is not very clear. One possibility is that ascorbic acid might be released to uptake the ischemia-induced glutamate accumulation through a heteroexchange transporter (5, 9, 15). There are several pieces of evidence indicating that elevated extracellular glutamate levels, induced either by cerebral ischemia or by exogenous perfusion of glutamate solutions, can induce ascorbic acid release (3, 20). Although the increased extracellular glutamate levels have been known to be positively correlated with increased extracellular ascorbic acid levels, however, the decrease of glutamate levels on ascorbic acid concentration has been little investigated. In the present study, the administration of diethylmaleate administration (4 mmole/kg, i.p.), which can lower the ischemia-induced glutamate release in anesthetized rat brain cortex (Fig. 2), can also significantly lower the ischemia-induced ascorbic acid release (Fig. 1). These results suggest that in brain cortex, the extracellular ascorbic acid levels are directly related to the extracellular glutamate levels.

The results that MK-801 perfusion did not significantly alter ischemia-induced ascorbic acid level suggest that the ascorbic acid elevation during cerebral ischemia was not directly related to the NMDA receptor. However, whether other ionotropic receptors or metabotropic receptors are involved in the regulation of extracellular ascorbic acid remain to be further clarified. These observations suggest that during cerebral ischemia, extracellular glutamate levels had a significant effect on ascorbic acid release in that lowered glutamate levels can result in subsequent lowered ascorbic acid levels. These results indirectly demonstrated that ascorbic acid release is involved in the glutamate uptake system.

PDC is a commonly used inhibitor for sodium dependent glutamate transporter. Our results showed that PDC perfusions can significantly increase extracellular ascorbic acid levels (Fig. 5). These results, when combined with published results that glutamate solution can increase ascorbic acid release according to other published results (3), again suggest that ascorbic acid release should be closely related to the extracellular glutamate levels since ascorbic acid release can be observed by either exogenous increase in glutamate due to uptake inhibition or by exogenous perfusion of glutamate solution.

Our results, however, do not rule out other possibilities for the DEM-induced decrease in extracellular ascorbic acid levels following cerebral ischemia. For example, a major source of ascorbic acid is the reduction of dehydroascorbic acid, and this reaction requires glutathione as a co-factor. DEM can decrease brain glutathione level (6, 21), and may consequently decrease cellular ascorbic acid level, whose release following cerebral ischemia is therefore diminished. More investigations are needed to clarify the detailed mechanism underlying the interrelation between glutamate acid and ascorbic acid levels have been known to be decreased following cerebral ischemia.

In conclusion, we observed that after diethylmaleate administration, cerebral ischemia-induced ascorbic acid release was diminished. This effect may be the consequence of lowered extracellular glutamate levels following cerebral ischemia after diethylmaleate administration. Furthermore, elevated extracellular glutamate levels, resulting from either exogenous perfusion of glutamate or from inhibition of the glutamate uptake transport, can also induce an increase in ascorbic acid release.

Acknowledgments

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