Korean J Physiol Pharmacol
Vol 3: 137−146, April, 1999

Effects of Dexamethasone and DHEA on the Changes of Glutamate and Polyamine Uptake in Rat Astrocytes by Lipopolysaccharide and Antimycin A

Sang-Hyun Choi, Bum Lee, Kyung-Ho Shin, Bon-Hong Min, Yeon-Sook Chun, and Boe-Gwun Chun

Department of Pharmacology, Korea University College of Medicine, Seoul 136−705, Korea

Interactions among dexamethasone, dehydroepiandrosterone (DHEA), lipopolysaccharide (LPS), and antimycin A on the glutamate uptake and the polyamine uptake were investigated in primary cultures of rat cerebral cortical astrocytes to examine the effects of dexamethasone and DHEA on the regulatory role of astrocytes in conditions of increased extracellular concentrations of glutamate or polyamines. 1. [^{3}H]Glutamate uptake: LPS and antimycin A decreased V_{max}, but both drugs had little effect on K_m. Dexamethasone also decreased basal V_{max} without any significant effect on K_m. And dexamethasone further decreased the antimycin A-induced decrease of V_{max}. DHEA did not affect the kinetics of basal glutamate uptake and the change by LPS or antimycin A. 2. [^{14}C]Putrescine uptake: LPS increased V_{max}, and antimycin A decreased V_{max}. They showed little effect on K_m. Dexamethasone decreased V_{max} of basal uptake and further decreased the antimycin A-induced decrease of V_{max}, and also decreased V_{max} to less than control in LPS-treated astrocytes. DHEA did not affect K_m and the change of V_{max} by LPS or antimycin A. 3. [^{14}C]Spermine uptake: Antimycin A decreased V_{max}, and LPS might increase V_{max}. K_m was little affected by the drugs. Dexamethasone decreased basal V_{max} and might further decrease the antimycin A-induced decrease of V_{max}. And dexamethasone also decreased V_{max} to less than control in LPS-treated astrocytes. DHEA might increase basal V_{max} and V_{max} of LPS-treated astrocytes. 4. V_{max} of glutamate uptake by astrocytes was increased by putrescine (1000 μM & 2000 μM) and spermidine (200 μM, 500 μM & 2000 μM). Spermine, 200 μM (and 100 μM), also increased V_{max}, but a higher dose of 2000 μM decreased V_{max}. K_m of glutamate uptake was not significantly changed by these polyamines, except that higher doses of spermine showed tendency to decrease K_m of glutamate uptake. In astrocytes, dexamethasone inhibited the glutamate uptake and the polyamine uptake in normal or hypoxic conditions, and the polyamine uptake might be stimulated by LPS and DHEA. Polyamines could aid astrocytes to uptake glutamate.

Key Words: Astrocytes, Dexamethasone, Dehydroepiandrosterone, Lipopolysaccharide, Antimycin A, Glutamate uptake, Putrescine uptake, Spermine uptake

INTRODUCTION

Glutamate is one of the major excitatory neurotransmitters in the brain and affect neuronal activity through ionotropic and metabotropic receptors (Rothman & Olney, 1986). Glutamate is ubiquitous in brain and compartmentalized intracellularly. At high extracellular concentration, glutamate is neurotoxic because of the overactivation of glutamate receptors on neurons. The excessive release of glutamate implicated in neurodegenerative processes associated with ischemia, epilepsy, and other neuropathologic states is an early and critical event in the Ca^{2+}-mediated cell death of

Corresponding to: Boe-Gwun Chun, Department of Pharmacology, Korea University College of Medicine, Seoul 136-705, Korea. (Tel) 920-6287, (Fax) 927-0824, E-mail: bgchun@kuccnx.korea.ac.kr

neurons.

Polyamines such as putrescine, spermidine, and spermine are essential for cell growth and proliferation (Tabor & Tabor, 1984; Pegg, 1986; Heby & Persson, 1990), and the cellular levels of polyamines are regulated by the activities of polyamine transporters (Pegg, 1988; Lessard et al, 1995) as well as by the activities of synthetic enzymes, ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase, spermidine/spermine N^1-acetyltransferase, and oxidases (Seiler, 1987; Pegg, 1988; Casero & Pegg, 1993). ODC is activated in various pathologic conditions of the brain such as seizure (Martinez et al, 1991), excitotoxic conditions (Reed & de Belleroche, 1990; Gardiner et al, 1992; Porcella et al, 1992), and ischemia (Dempsey et al, 1988; Paschen, 1992). Putrescine is a putative neurotoxic substance (Paschen et al, 1992), because of the activation of Ca^{2+} influx (Komulainen & Bondy, 1987) and the release of excitotoxic amino acids from nerve endings (Reed & de Belleroche, 1990). And extracellular level of putrescine in hippocampus showed a rapid and sustained increase by 5 min occlusion of bilateral carotid arteries (Shin et al, 1994).

Astrocytes form contacts with blood vessels and other astrocytes and surround neurons and their processes (Kuffler et al, 1984). Their intimate relationship with neurons put them in an ideal position to respond to and modify events at synapses. The transport and metabolic functions of astrocytes have been known to support the signaling functions of neurons. Brain contains multiple transport systems for excitatory amino acids, and astrocytes possess multiple uptake systems for glutamate (Ferkany & Coyle, 1986; Erecinska & Silver, 1990; Balcar & Li, 1992). Mitigating effect of astroglial uptake of glutamate and other excitatory amino acids on neuronal injury has been well established both *in vivo* and *in vitro* (Choi, 1988; Rosenberg et al, 1992).

Lipoplysaccharide (LPS), an inflammatory signal in brain, leads to the transformation of resting astrocytes to reactive astrocytes (Lieberman et al, 1989; Chung & Benveniste, 1990).

Metabolic inhibition by hypoxia, hypoglycemia, and ischemia leads to neuronal hyperpolarization accompanying increased extracellular $[K^+]$. This hyperpolarization may contribute to EEG silence, synaptic transmission failure, and astrocytic swelling (Hansen et al, 1982; Fujiwara et al, 1987; Kawasaki et al, 1988; Walz et al, 1993). Also, inhibition of astrocyte metabolism can lead to alterations of the astrocyte reactivities (Walz, 1989; Yu et al, 1989; Nicholls & Attwell, 1990). Antimycin A, a blocker of mitochondrial respiration, can induce failure of aerobic metabolism and act to inhibit oxidative ATP production in cultured astrocytes (Erecinska et al, 1981; Olson et al, 1986). It can cause incomplete ischemia where oxygen but not glucose is depleted (Swanson, 1992). Steroids can be synthesized *de novo* in the brain from cholesterol, mainly by glial cells (Baulieu & Robel, 1990; Robel et al, 1991). The concentrations of dehydroepiandrosterone (DHEA) and its sulfate form (DHEA-S) remain virtually unchanged in the rat brain after the removal of steroidogenic endocrine glands, and thus DHEA can be referred to as a neurosteroid (Corpéchot et al, 1981; Baulieu, 1991; Korneyev et al, 1993; Schumacher et al, 1996). The neurosteroids affect neuronal excitability (Majewska, 1992; ffrench-Mullen et al, 1994) and glial cells as well (Chvatal & Kettenmann, 1991; Del Cerro et al, 1995; Garcia-Segura et al, 1996).

Therefore, this study was carried out to investigate the effect of DHEA on the changes of glutamate uptake and of polyamine uptake by primary clutured astrocytes in conditions of LPS-activation and of antimycin A-induced hypoxia and to compare it with the effect of dexamethasone.

METHODS

Primary culture of astrocytes and drug treatment

Astrocytes were prepared and cultured by the method modified from McCarthy & de Vellis' (1980). Cerebral cortices were obtained from neonatal rats (Sprague-Dawley, 2 days old or younger). They were free from meninges and vessels aseptically in cold phosphate-buffered saline without Ca^{2+} (PBS). The cortices were dissociated through mesh, and then suspended in DMEM containing 10% fetal bovine serum (FBS) and penicillin-streptomycin (100 IU/ml-100 μg/ml). The suspended cells were washed and resuspended in DMEM with FBS and penicillin-streptomycin, and cell count and viability were examined. The cells were cultured in humidified 5% CO_2 atmosphere at 37°C. The medium was changed after 24 hours and then twice a week, so that the culture cells could reach to confluence. And then the confluent cultures were shaken (250 rpm) at 37°C for

12 hours. The adherent cells were washed and subcultured after trypsinized with 0.125% trypsin containing 1.3 mM EDTA. Immunoreactivity of the cultured cells to glial fibrillary acidic protein (GFAP) was more than 95%.

Astrocytes were plated and incubated in 24-well plates. When the cultures were grown to be confluenced, the culture medium was changed to a fresh serum free DMEM. The astrocytes were treated with dexamethasone (10^{-6} M; control, ethanol 0.1%) or DHEA (5×10^{-5} M; control, ethanol 0.1%) 4 hours before the treatment with LPS (from *E. coli* 055: B5; 1 μg/ml; control, PBS) or antimycin A (50 μg/ml; control, PBS). After 20 hours of final treatments, uptake assays were performed.

[^{3}H]Glutamate uptake measurement

Glutamate uptake by astrocytes was measured as described by Drejer et al (1982), Bender et al (1989), and Yu et al (1986). Culture medium was changed to DMEM containing L-glutamate and L-[^{3}H]glutamate (Amersham). The kinetics of glutamate uptake was measured in terms of the function of glutamate concentration (200 μM glutamate with 0.4 μCi/ml L-[^{3}H]glutamate and 2-fold decrements to 6.25 μM). The uptake was performed for 7 min at 37°C in 5% CO_2 atmosphere, and another batch was incubated at 4°C to determine the nonspecific binding of glutamate to cell membrane. The uptake reaction was terminated by two washes with 1 ml of ice-cold PBS, followed by cell lysis in 0.5 ml of 1 N NaOH. The radioactivity of one aliquot was measured by β-scintillation counter, and another was used for protein assay (Bradford, 1976).

To study the influences of polyamines on the glutamate uptake, astrocytes were incubated with L-glutamate and L-[^{3}H]glutamate in the presence of putrescine, spermidine, or spermine.

[^{14}C]Putrescine and [^{14}C]spermine uptake measurement

Polyamine uptake by astrocytes was measured as described by Porter et al (1985) and Lessard et al (1995).

Astrocytes cultured and treated in 24-well plates were washed with PBS (37°C) and incubated in 500 μl/well of PBS containing [^{14}C]putrescine (40 μM to 2.5 μM; Amersham) or [^{14}C]spermine (2.5 μM to 0.04 μM; Amersham) for 60 min at 37°C in 5% CO_2 atmosphere. Another batch was incubated at 4°C to determine the nonspecific binding. At the end of the incubation, 1 ml of ice-cold PBS containing 1 mM putrescine or 1 mM spermine was added to the well, and astrocytes were washed twice with ice-cold PBS. And then astrocytes in each well were dissolved in 200 μl of 1 N NaOH at 60°C for 60 min. Aliquots of the solution neutralized with 200 μl of 1 N HCl were used for the radioactivity measurement using β-scintillation counter and for protein assay (Bradford, 1976).

The kinetic parameters of uptake, K_m and V_{max}, were determined by Edie-Scatchard plot and linear regression analysis.

RESULTS

Effects of dexamethasone and DHEA on the change of [^{3}H]glutamate uptake by LPS or antimycin A

LPS and antimycin A decreased V_{max} of glutamate uptake by astrocytes, but both drugs had little effect on K_m of glutamate uptake. Dexamethasone also decreased basal V_{max} without a significant effect on K_m. And dexamethasone further decreased the antimycin A-induced decrease of V_{max}. DHEA did not affect the kinetics of basal glutamate uptake and the change by LPS or antimycin A (Fig. 1 & Table 1).

Effects of dexamethasone and DHEA on the change of [^{14}C]putrescine uptake by LPS or antimycin A

LPS increased V_{max} of putrescine uptake, and antimycin A decreased the V_{max}. They showed little effect on K_m. Dexamethasone decreased V_{max} of basal uptake and further decreased the antimycin A-induced decrease of V_{max}, and also decreased V_{max} to less than control in LPS-treated astrocytes. DHEA did not affect K_m and the change of V_{max} by LPS or antimycin A (Fig. 2 & Table 2).

Effects of dexamethasone and DHEA on the change of [^{14}C]spermine uptake by LPS or antimycin A

Antimycin A decreased V_{max}, and LPS might increase V_{max} of spermine uptake. K_m was only marginally affected by the drugs. Dexamethasone decreased basal V_{max} and might further decrease the

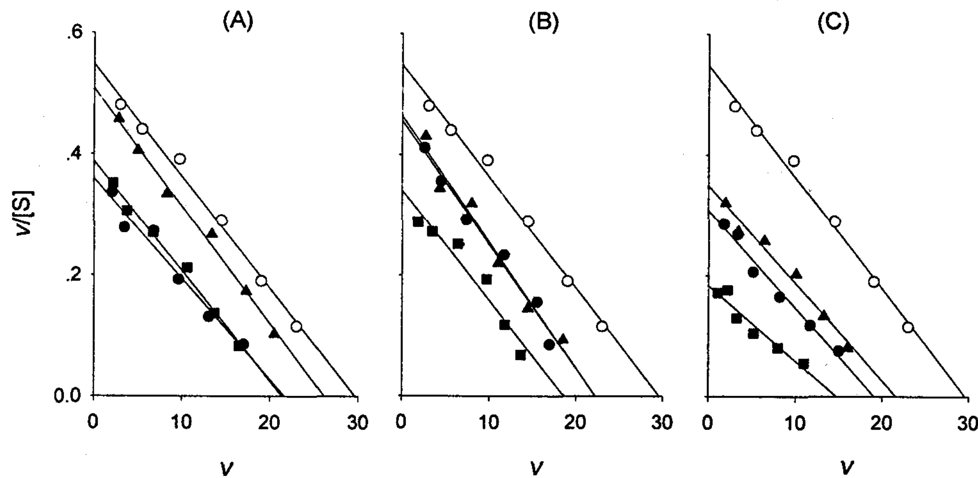

Fig. 1. Effects of dexamethasone and DHEA on the change of [^{3}H]glutamate uptake by LPS and antimycin A in astrocytes. Data were analysed by Edie-Scatchard plot; (A) ○-○: control (r^2 = 0.99), ■-■: dexamethasone (r^2 = 0.99), ▲-▲: DHEA (r^2 = 0.99), ●-●: dexamethasone & DHEA (r^2 = 0.96); (B) ○-○: control (r^2 = 0.99), ●-●: LPS (r^2 = 0.98), ■-■: LPS & dexamethasone (r^2 = 0.94), ▲-▲: LPS & DHEA (r^2 = 0.97); (C) ○-○: control (r^2 = 0.99), ●-●: antimycin A (r^2 = 0.98), ■-■: antimycin A & dexamethasone (r^2 = 0.93), ▲-▲: antimycin A & DHEA (r^2 = 0.98). Each point represents mean±S.E. of 4 independent experiments.

Table 1. Effects of dexamethasone (Dex) and DHEA on the changes of [^{3}H]glutamate uptake by LPS or antimycin A (AA) in astrocytes

Treatment	K_m	V_{max}
Control	54.03±5.01	29.62±2.52
Dex	55.01±5.67	21.39±2.63*
DHEA	51.49±4.92	26.16±2.85
Dex&DHEA	60.27±5.84	21.69±2.42*
LPS	48.82±5.32	22.24±2.02*
LPS&Dex	54.78±5.49	18.67±2.12**
LPS&DHEA	47.93±5.23	22.22±2.46*
AA	61.90±6.30	19.07±2.05**
AA&Dex	80.24±7.65**	14.71±1.02**,†
AA&DHEA	61.79±5.62	21.59±2.23**

K_m: uM, V_{max}: nM/mg protein/min
Each value represents mean±S.E. of 4 independent experiments.
*, **: $p<0.1$ & $p<0.05$, respectively, in comparison to the control value
†, ††: $p<0.1$ & $p<0.05$, respectively, in comparison to LPS- or AA-treated value

antimycin A-induced decrease of V_{max}. And dexamethasone also decreased V_{max} to less than control in LPS-treated astrocytes. DHEA might increase basal V_{max} and V_{max} of LPS-treated astrocytes (Fig. 3 & Table 3).

Effects of polyamines on the [^{3}H]glutamate uptake by astrocytes

V_{max} of glutamate uptake by astrocytes was increased by putrescine (1000 μM & 2000 μM) and spermidine (200 μM, 500 μM & 2000 μM). Spermine, 200 μM (and 100 μM), also increased V_{max}, but higher dose of 2000 μM decreased V_{max}.

K_m of glutamate uptake by astrocytes was not significantly changed by these polyamines, except that higher doses of spermine showed tendency to decrease K_m of glutamate uptake (Fig. 4 & Table 4).

DISCUSSION

In our recent study, DHEA is supposed to inhibit the changes of astrocyte responses by dexamethasone. Dexamethasone are involved in the process of neuronal injury by inhibiting the release of arachidonic

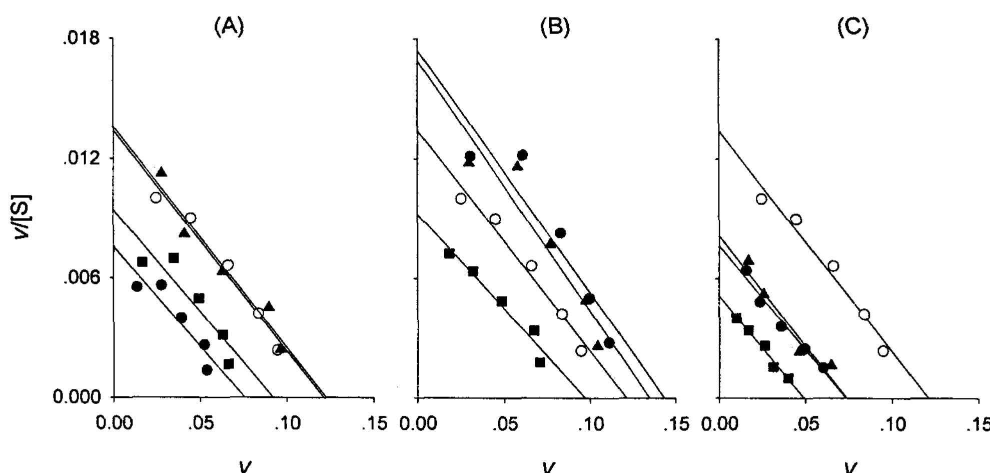

Fig. 2. Effects of dexamethasone and DHEA on the change of [^{14}C]putrescine uptake by LPS and antimycin A in astrocytes. Data were analysed by Edie-Scatchard plot; (A) ○-○: control (r^2 = 0.97), ■-■: dexamethasone (r^2 = 0.84), ▲-▲: DHEA (r^2 = 0.95), ●-●: dexamethasone & DHEA (r^2 = 0.85); (B) ○-○: control (r^2 = 0.97), ●-●: LPS (r^2 = 0.87), ■-■: LPS & dexamethasone (r^2 = 0.95), ▲-▲: LPS & DHEA (r^2 = 0.88); (C) ○-○: control (r^2 = 0.97), ●-●: antimycin A (r^2 = 0.97), ■-■: antimycin A & dexamethasone (r^2 = 0.97), ▲-▲: antimycin A & DHEA (r^2 = 0.92). Each point represents mean±S.E. of 4 independent experiments.

Table 2. Effects of dexamethasone and DHEA on the changes of [^{14}C]putrescine uptake by LPS or antimycin A in astrocytes

Treatment	K_m	V_{max}
Control	9.04±0.91	121.07±10.42
Dex	9.77±1.02	92.05±11.06*
DHEA	9.01±0.95	122.66±10.32
Dex&DHEA	9.98±0.97	75.62±9.50**
LPS	8.23±0.87	143.35±9.86
LPS&Dex	10.53±0.93	97.11±10.26††
LPS&DHEA	7.97±0.81	134.75±11.96
AA	9.57±0.92	73.18±9.18**
AA&Dex	9.61±1.02	49.20±5.86**,†
AA&DHEA	9.04±0.85	73.70±8.54**

K_m: uM, V_{max}: pM/mg protein/min

acid, IL-6, and NO from astrocytes exposed to LPS (Choi et al, 1999). DHEA is synthesized and presented in brain as well as in circulation, though the precursors and enzymes responsible for its biosynthesis in brain are unknown (Corpéchot et al, 1981; Schumacher et al, 1996). The actions of DHEA have been said to be state-dependency and to buffer the actions of dexamethasone in periphery (Regelson et al, 1990). And interactions of dexamethasone and DHEA in the regulation of proliferation and differentiation of astroglial cells (Jung-Testas et al, 1992; Del Cerro et al, 1995; Crossin et al, 1997) proposed the significance of the interactions of dexamethasone and DHEA concerning the responses of astrocytes to brain injury.

Therefore, the effects of DHEA and dexamethasone on glutamate uptake and polyamine uptake by astrocytes in conditions of LPS treatment or of antimycin A-induced hypoxia were studied to investigate the different roles of these steroids in the neuropathologic conditions in which excitatory amino acids or polyamines, putative neurotoxic substances, may overwhelm the neuronal viability.

Several activities of astrocytes directed toward the maintenance of the extracellular milieu are energy dependent (Nicholls & Attwell, 1990; Walz, 1989; Yu et al, 1989); glutamate uptake into astrocytes is an energy demanding process since glutamate is co-transported with two or three Na^+ which have to be transported out of the cells by the Na^+/K^+ ATPase (Dennis et al, 1976; Sonnewald et al, 1997). So antimycin A markedly inhibited glutamate uptake

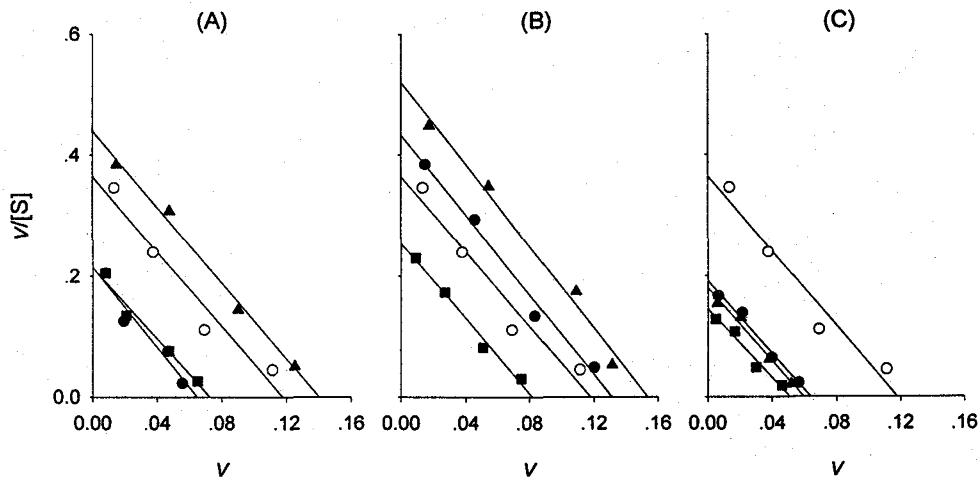

Fig. 3. Effects of dexamethasone and DHEA on the change of [^{14}C]spermine uptake by LPS and antimycin A in astrocytes. Data were analysed by Edie-Scatchard plot; (A) ○-○: control (r^2 = 0.95), ■-■: dexamethasone (r^2 = 0.97), ▲-▲: DHEA (r^2 = 0.99), ●-●: dexamethasone & DHEA (r^2 = 0.93); (B) ○-○: control (r^2 = 0.95), ●-●: LPS (r^2 = 0.99), ■-■: LPS & dexamethasone (r^2 = 0.99), ▲-▲: LPS & DHEA (r^2 = 0.99); (C) ○-○: control (r^2 = 0.95), ●-●: antimycin A (r^2 = 0.98), ■-■: antimycin A & dexamethasone (r^2 = 0.96), ▲-▲: antimycin A & DHEA (r^2 = 0.98). Each point represents mean±S.E. of 4 independent experiments.

Table 3. Effects of dexamethasone and DHEA on the changes of [^{14}C]spermine uptake by LPS or antimycin A in astrocytes

Treatment	K_m	V_{max}
Control	0.323±0.029	117.78±9.84
Dex	0.338±0.033	72.59±7.92**
DHEA	0.318±0.030	139.95±13.94
Dex&DHEA	0.300±0.028	64.61±8.30**
LPS	0.303±0.027	131.12±12.86
LPS&Dex	0.321±0.028	81.82±8.74**,††
LPS&DHEA	0.295±0.032	153.62±13.84*
AA	0.333±0.027	63.77±6.48**
AA&Dex	0.354±0.027	51.21±5.96**
AA&DHEA	0.332±0.031	59.63±6.72**

K_m: uM, V_{max}: pM/mg protein/min

with little effect on the affinity of the transporters. In case of glutamate uptake, the decrease of V_{max} by antimycin A was about 35.6% of the control, and it did not differ from other reports that the uptake was maintained at 54% to 63% of control despite maximal inhibition of oxidative ATP production (Swanson, 1992). The remarkable viability of astrocytes in oxygen-depleted conditions (Goldberg et al, 1987; Tombaugh & Sapolsky, 1990) and the maintenance of ability to uptake may show the persistent significance of astrocytes to uptake excitotoxic glutamate in hypoxic conditions.

However, the steroids used in this study could not reverse the antimycin A-induced inhibition of glutamate uptake. Moreover, dexamethasone further decreased the uptake velocity and the affinity of transporter to glutamate. This inhibitory effect of dexamethasone on the glutamate uptake opposes the ability to induce glutamine synthetase activity; the activity of glutamine synthetase which determines the proportion of glutamate converted to glutamine is regulated by glucocorticoids in brain and cultured astrocytes (Patel et al, 1983; Patel & Hunt, 1985), and transcription of the glutamine synthetase gene is inducible by corticosteroids (O'Banion et al, 1994). These results pose a possibility that glucocorticoids may impair the ability of astrocytes to help neurons during the pathologic states involving hypoxia.

In spite of the significance of polyamine metabolism in the pathophysiology of brain such as the putative neurotoxicity of polyamines (Paschen et al, 1992) and the linkage of polyamine metabolism to

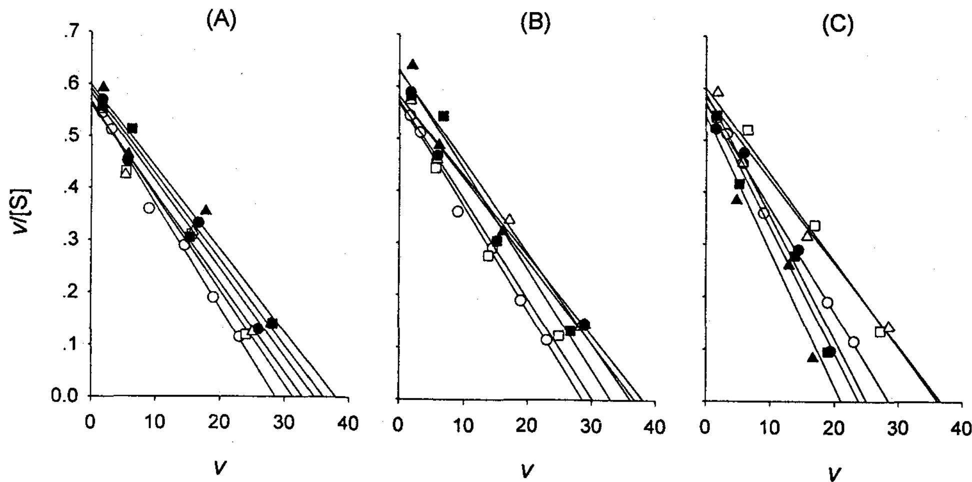

Fig. 4. Effect of putrescine, spermidine, and spermine on the [^{3}H]glutamate uptake by astrocytes. Data were analysed by Edie-Scatchard plot; (A) ○-○: control (r^2 = 0.99), □-□: pureescine 100 μM (r^2 = 0.97), △-△: 200 μM (r^2 = 0.96), ●-●: 500 μM (r^2 = 0.97), ■-■: 1000 μM (r^2 = 0.98), ▲-▲: 2000 μM (r^2 = 0.96); (B) ○-○: control (r^2 = 0.99), □-□: spermidine 100 μM (r^2 = 0.97), △-△: 200 μM (r^2 = 0.98), ●-●: 500 μM (r^2 = 0.97), ■-■: 1000 μM (r^2 = 0.97), ▲-▲: 2000 μM (r^2 = 0.97); (C) ○-○: control (r^2 = 0.99), □-□: spermine 100 μM (r^2 = 0.98), △-△: 200 μM (r^2 = 0.97), ●-●: 500 μM (r^2 = 0.96), ■-■: 1000 μM (r^2 = 0.97), ▲-▲: 2000 μM (r^2 = 0.95). Each point represents mean ± S.E. of 4 independent experiments.

Table 4. Effects of putrescine, spermidine, and spermine on the [^{3}H]glutamate uptake by astrocytes

Treatment	K_m	V_{max}
Control	50.21±5.52	28.57±2.71
Putrescine 100 uM	55.33±5.54	31.29±2.77
200 uM	58.47±5.68	32.79±2.65
500 uM	59.37±5.91	34.58±2.79
1000 uM	60.90±6.01	36.02±2.84*
2000 uM	63.28±6.08	37.91±2.83**
Spermidine 100 uM	52.58±5.53	30.15±2.89
200 uM	65.54±5.96	38.12±2.91**
500 uM	63.38±5.97	36.83±2.69*
1000 uM	52.21±5.62	33.04±2.65
2000 uM	57.33±5.49	36.16±2.71*
Spermine 100 uM	60.22±6.23	36.20±3.01
200 uM	62.79±6.45	36.61±2.96*
500 uM	42.39±5.01	25.04±2.77
1000 uM	41.49±4.79	23.81±2.56
2000 uM	38.60±4.01	21.09±2.50*

K_m: uM, V_{max}: nM/mg protein/min

epiletogenic GABA metabolism (De Sarro et al, 1986), only a small number of studies have been conducted on the polyamine uptake by astrocytes. Specific polyamine transport systems have been reported to have Michaelis-Menten type kinetics in enterocytes (Kumagai & Johnson, 1988), pneumocytes (Wyatt et al, 1988), neuroblastoma cells (Chen & Rinehart, 1981), and in astrocytes of epileptic mice (Laschet et al, 1992). The putrescine uptake and the spermine uptake showed similar kinetic changes by dexamethasone and DHEA in antimycin A-treated astrocytes. Comparatively, polyamine transport systems of astrocytes seemed to be activated by LPS. And DHEA clearly increased velocity of spermine uptake by non-hypoxic astrocytes. Considering these results, astrocyte activities with LPS- or DHEA-treatment seem to be partly ascribed to the polyamine uptake, though astrocytes may be less capable of uptaking polyamines than of uptaking glutamate.

Many studies say that polyamines play an important role in biological responses to brain ischemia (Paschen, 1992; Paschen et al, 1992). Also, the activation of NMDA receptor has been known to be positively modulated by polyamines (Nussenzveig et al, 1991; Williams et al, 1991; Lazarewicz et al,

1992; Araneda et al, 1993). In relation to these reports, the effects of various extracellular concentrations of polyamines on the kinetics of glutamate uptake by astrocytes were studied. Higher levels of spermidine or putrescine could increase the velocity of glutamate uptake. However, higher level of spermine could inhibit the velocity of uptake and had tendency to increase the transporter's affinity to glutamate. These findings suggested that polyamines might facilitate glutamate uptake by astrocytes as well as participate in the activation of NMDA receptors in brain.

Conclusively, dexamethasone inhibited the glutamate uptake and the polyamine uptake by astrocytes in normal or hypoxic conditions, and polyamine uptake was stimulated by LPS and DHEA. Polyamines might influence to aid astrocytes to uptake glutamate.

ACKNOWLEDGEMENTS

This work was supported by the Basic Medical Research Fund from the Ministry of Education in 1996.

REFERENCES

Araneda RC, Zukin RS, Bennett MV. Effects of polyamines on NMDA-induced currents in rat hippocampal neurons: a whole-cell and single-channel study. *Neurosci Lett* 152: 107−112, 1993

Balcar VJ, Li Y. Heterogeneity of high affinity uptake of L-glutamate and L-aspartate in the mammalian central nervous system. *Life Sci* 51: 1467−1478, 1992

Baulieu EE, Robel P. Neurosteroids: A new brain function? *J Steroid Biochem Mol Biol* 37: 395−403, 1990

Baulieu EE. Neurosteroids: A new fucnction in the brain. *Biol Cell* 71: 3−10, 1991

Bender AS, Woodbury DM, White HS. β-DL-methylene-aspartate, an inhibitor of aspartate aminotransferase, potently inhibits L-glutamate uptake into astrocytes. *Neurochem Res* 14: 641−646, 1989

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal Biochem* 72: 248−254, 1976

Casero RA Jr, Pegg AE. Spermidine/spermine N^1-acetyltransferase - the turning point in polyamine metabolism. *FASEB J* 7: 653−661, 1993

Chen KC, Rinehart CA Jr. Difference in putrescine transport in undifferentiated versus differentiated mouse NB-15 neuroblastoma cells. *Biochem Biophys Res Commun* 101: 234−249, 1981

Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1: 623−634, 1988

Choi SH, Kim HG, Kim CG, Park NH, Choi DH, Chun YS, Chun BG. Effects of dexamethasone and DHEA on the responses of rat cerebral cortical astrocytes to lipopolysaccharide and antimycin A. *Korean J Physiol Pharmacol* 3: in press, 1999

Chung IY, Benveniste EW. Tumor necrosis factor-α production by astrocytes. Induction by lipopolysaccharides, IFN-γ and IL-1β. *J Immunol* 144: 2999−3007, 1990

Chvatal A, Kettenmann H. Effects of steroids on γ-aminobutyrate-induced currents in cultured rat astrocytes. *Pflugers Arch* 419: 263−266, 1991

Corpéchot C, Robel P, Axelson M, Sjövall J, Baulieu EE. Characterization and measurement of dehydroepiandrosterone sulfate in the rat brain. *Proc Natl Acad Sci USA* 78: 4704−4707, 1981

Crossin KL, Tai MH, Krushel LA, Mauro VP, Edelman GM. Glucocorticoid receptor pathways are involved in the inhibition of astrocyte proliferation. *Proc Natl Acad Sci USA* 94: 2687−2692, 1997

De Sarro GB, Ascioti C, Bagetta G, Libri V, Nistocò G. Behavioural and electrocortical changes of the polyamines after microinfusion in several areas of the brain. In: Nistocò G, Morselli PL, Lloyd KG, Fariello RG, Engel J Jr ed, *Neurotransmitters, Seizures and Epilepsy III.* Raven Press, New York, p 423−438, 1986

Del Cerro S, Garcia-Estrada J, Garcia-Segura LM. Neuroactive steroids regulate astroglia morphology in hippocampal cultures from adult rats. *Glia* 14: 65−71, 1995

Dempsey RJ, Maley BE, Cowen D, Olson JW. Ornithine decarboxylase and immunohistochemical location in postischemic brain. *J Cereb Blood Flow Metab* 11: 979−985, 1988

Dennis SC, Land JM, Clark JB. Glutamate metabolism and transport in rat brain mitochondria. *J Biochem* 156: 323−331, 1976

Drejer J, Larsson OM, Schousboe A. Characterization of L-glutamate uptake into and release from astrocytes and neurons cultured from different brain regions. *Exp Brain Res* 47: 259−269, 1982

Erecinska M, Silver IA. Metabolism and role of glutamate in mammalian brain. *Prog Neurobiol* 35: 245−296, 1990

Erecinska M, Wilson D, Heytler PG, Rieske JS. Inhibitors of mitochondrial functions. In: Erecinska M, Wilson DF ed, *International Encyclopedia of Pharmacology*

and Therapeutics. Section 107. Pergamon, New York, p 109−205, 1981

Ferkany J, Coyle JT. Heterogeneity of sodium-dependent excitatory amino acid uptake mechanisms in rat brain. *J Neurosci Res* 16: 491−503, 1986

ffrench-Mullen J, Danks P, Spence KT. Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G-protein-coupled mechanism. *J Neurosci* 14: 1963−1977, 1994

Fujiwara N, Higashi H, Shimoji K, Joshimura M. Effects of hypoxia on rat hippocampal neurons *in vitro*. *J Physiol* 29: 315−322, 1987

Garcia-Segura LM, Chowen JA, Naftolin F. Endocrine glia: Roles of glial cells in the brain actions of steroid and thyroid hormones, and in the regulation of hormonal secretion. *Front Neuroendocrinol* 17: 180−211, 1996

Gardiner IM, Li A, Patel N, Ball S, de Belleroche J. Excitotoxin induction of ornithine decarboxylase in cerebral cortex is reduced by phospholipase A_2 inhibition. *Life Sci* 51: PL77−PL81, 1992

Goldberg MP, Weiss JH, Pham PC, Choi DW. N-methyl-D-aspartate receptors mediate hypoxic neuronal injury in cortical culture. *J Pharmacol Exp Ther* 243: 784−791, 1987

Hansen J, Hounsgaard J, Jahnsen H. Anoxia increases potassium conductance in hippocampal nerve cells. *Acta Physiol Scand* 115: 301−310, 1982

Heby O, Persson L. Molecular genetics of polyamine synthesis in eukaryotic cells. *Trends Biochem Sci* 15: 153−158, 1990

Jung-Testas I, Renoir M, Bugnard H, Greene GL, Baulieu EE. Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. *J Steroid Biochem Mol Biol* 41: 621−631, 1992

Kawasaki K, Czeh G, Somjen GG. Prolonged exposure to high potassium concentration results in irreversible loss of synaptic transmission in hippocampal tissue slices. *Brain Res* 457: 322−329, 1988

Komulainen H, Bondy SC. Transient elevation of intrasynaptosomal free calcium by putrescine. *Brain Res* 401: 50−54, 1987

Korneyev A, Pan BS, Polo A, Romer E, Guidotti A, Costa E. Stimulation of brain pregnenolone synthesis by mitochondrial diazepam binding inhibitor receptor ligands *in vivo*. *J Neurochem* 61: 1515−1524, 1993

Kuffler SW, Nicholls JG, Martin AR. Physiology of neuroglial cells. In: Kuffler SW ed, *From Neuron to Brain*. Sinauer, p 323−360, 1984

Kumagai J, Johnson LR. Characteristics of putrescine uptake in isolated rat enterocytes. *Am J Physiol* 254: G81−G86, 1988

Laschet J, Grisar T, Bureau M, Guillaume D. Characteristics of putrescine uptake and subsequent GABA formation in primary cultured astrocytes from normal C57BL/6J and epileptic DBA/2J mouse brain cortices. *Neuroscience* 48: 151−157, 1992

Lazarewicz JW, Salinska E, Puka M. Glycine enhances extracellular $^{45}Ca^{2+}$ response to NMDA application investigated with microdialysis of rabbit hippocampus *in vivo*. *Acta Neurobiol Exp (Warsz)* 52: 83−91, 1992

Lessard M, Zhao C, Singh SM, Poulin R. Hormonal and feedback regulation of putrescine and spermidine transport in human breast cancer cells. *J Biol Chem* 270: 1685−1694, 1995

Lieberman AP, Pitha PM, Shin HS, Shin ML. Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. *Proc Natl Acad Sci USA* 86: 6348−6352, 1989

Majewska MD. Neurosteroids: Endogenous bimodal modulators of the GABA receptor. Mechanisms of action and physiological significance. *Prog Neurobiol* 38: 379−395, 1992

Martinez E, de Vera N, Artigas F. Differential response of rat brain polyamines to convulsant agents. *Life Sci* 48: 77−84, 1991

McCarthy KD, de Vellis J: Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. *J Cell Biol* 85: 890−902, 1980

Nicholls D, Attwell D. The release and uptake of excitatory amino acids. *Trends Pharmacol Sci* 11: 462−468, 1990

Nussenzveig IZ, Sircar R, Wong ML, Frusciante MJ, Javitt DC, Zukin SR. Polyamine effects upon N-methyl-D-aspartate receptor functioning: differential alteration by glutamate and glycine site antagonists. *Brain Res* 561: 285−291, 1991

O'Banion MK, Young DA, Bohn MC. Corticosterone-responsive mRNAs in primary rat astrocytes. *Brain Res Mol Brain Res* 22: 57−68, 1994

Olson JE, Sankar R, Holtzman D, James A, Fleischhacker D. Energy-dependent volume regulation in primary cultured cerebral astrocytes. *J Cell Physiol* 128: 209−215, 1986

Paschen W, Widmann R, Weber C. Changes in regional polyamine profiles in rat brains after transient cerebral ischemia (single versus repetitive ischemia): evidence for release of polyamines from injured neurons. *Neurosci Lett* 135: 121−124, 1992

Paschen W. Polyamine metabolism in reversible cerebral ischemia. *Cerebrovasc Brain Metab Rev* 4: 59−88, 1992

Patel AJ, Hunt A, Tahourdin CSM. Regulation of *in vivo* glutamine synthetase activity by glucocorticoids in the developing rat brain. *Dev Brain Res* 10: 83−91, 1983

Patel AJ, Hunt A. Observations on cell growth and regulation of glutamine synthetase by dexamethasone

in primary cultures of forebrain and cerebellar astrocytes. *Dev Brain Res* 18: 175−184, 1985

Pegg AE. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res* 48: 759−774, 1988

Pegg AE. Recent advances in biology of polyamines in eukaryotes. *Biochem J* 234: 249−262, 1986

Porcella A, Fage D, Voltz C, Bourdiol F, Benavides J, Scatton B, Carter C. Implication of the polyamines in the neurotoxic effects of N-methyl-D-aspartate. *Neurol Res* 14: 181−183, 1992

Porter CW, Cavanaugh PF Jr, Stolowich N, Ganis B, Kelly E, Bergeron RJ. Biological properties of N^4- and N^1,N^8-spermidine derivatives in cultured L1210 leukemia cells. *Cancer Res* 45: 2050−2057, 1985

Reed LJ, de Belleroche J. Induction of ornithine decarboxylase in cerebral cortex by excitotoxin lesion of nucleus basalis: association with postsynaptic responsiveness and N-methyl-D-aspartate receptor activation. *J Neurochem* 55: 780−787, 1990

Regelson W, Kalimi M, Loria R. Dehydroepiandrosterone (DHEA): the precursor steroid: inductory remarks. In: Kalimi M, Regelson W ed, *The Biologic Role of Dehydroepiandrosterone (DHEA)*. Walter de Gruyter, Berlin, New York, p 1−6, 1990

Robel P, Akwa Y, Corpéchot C, Hu ZY, Jung-Testas I, Kabbadj K, Le Goascogne C, Morfin R, Vourc'h C, Young J, Baulieu EE. Neurosteroids: biosynthesis and function of pregnenolone and dehydroepiandrosterone in the brain. In: Motta M ed, *Brain Endocrinology*. Raven Press, New York, p 105−132, 1991

Rosenberg PA, Amin S, Leitner M. Glutamate uptake disguises neurotoxic potency of glutamate agonists in cerebral cortex in dissociated cell culture. *J Neurosci* 12: 56−61, 1992

Rothman SM, Olney JW. Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann Neurol* 19: 105−111, 1986

Schumacher M, Robel P, Baulieu EE. Development and regeneration of the nervous system: A role for neurosteroids. *Dev Neurosci* 18: 6−21, 1996

Seiler N. Inhibition of enzymes oxidizing polyamines. In: McCann PP, Pegg AE, Sjoerdsma A ed, *Inhibition of Polyamine Metabolism - Biological Significance and Basis for New Therapies*. Academic Press, p 49−77, 1987

Shin KH, Kim HG, Choi SH, Cho SH, Chun YS, Chun BG. Changes of glutamate and polyamine levels of hippocampal microdialysates in response to occlusion of both carotid arteries in Mongolian gerbils. *Kor J Pharmacol* 30: 273−289, 1994

Sonnewald U, Westergaard N, Schousboe A. Glutamate transport and metabolism in astrocytes. *Glia* 21: 56−63, 1997

Swanson RA. Astrocyte glutamate uptake during chemical hypoxia in vitro. *Neurosci Lett* 147: 143−146, 1992

Tabor CW, Tabor H. Polyamines. *Annu Rev Biochem* 53: 749−790, 1984

Tombaugh GC, Sapolsky RM. Mechanistic distinctions between excitotoxic and acidotic hippocampal damage in an *in vitro* model of ischemia. *J Cereb Blood Flow Metab* 10: 527−535, 1990

Walz W, Klimaszewski A, Paterson IA. Glial swelling in ischemia: A hypothesis. *Dev Neurosci* 15: 216−225, 1993

Walz W. Role of glial cells in the regulation of the brain microenvironment. *Prog Neurobiol* 33: 309−333, 1989

Williams K, Romano C, Dichter MA, Molinoff PB. Modulation of the NMDA receptor by polyamines. *Life Sci* 48: 469−498, 1991

Wyatt I, Soames AR, Clay MF, Smith LL. The accumulation and localization of putrescine, spermidine, spermine and paraquat in the rat lung. *In vitro* and *in vivo* studies. *Biochem Pharmacol* 37: 1909−1918, 1988

Yu ACH, Chan PH, Fishman RA. Effects of arachidonic acid on glutamate and γ-aminobutyric acid uptake in primary cultures of rat cerebral cortical astrocytes and neurons. *J Neurochem* 47: 1181−1189, 1986

Yu ACH, Gregory GA, Chan PH. Hypoxia-induced dysfunction and injury of astrocytes in primary cell cultures. *J Cereb Blood Flow Metab* 9: 20−28, 1989