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Excitatory Amino Acid Receptors, Oxido-reductive Processes and Brain Oedema Following Transient Ischaemia in Gerbils

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Summary

A key mechanism of brain injury after cerebral ischaemia is supposed to be the iron-dependent formation of highly reactive oxygen free radicals initiated by the intracellular accumulation of calcium and promoted by the excess release of glutamate. Oxido-reductive processes (formation of superoxide radicals and lipid peroxidation) are mediated through NMDA-receptors, while non-NMDA receptors, associated with (or being a part of) Na,K-ATPase, are responsible for postischaemic brain swelling. The hypothesis was put forward for consideration that release of glutamate (and other related endogenous excitatory amino acids) due to depolarization in the early minutes of ischaemia and (non)-NMDA antagonists may have roles in the development and prevention of metabolic brain impairment and cytotoxic oedema, respectively, in the ischaemic state.

Introduction

Experimental evidence indicates that excitatory neurotransmission plays a significant role in the development of ischaemic/hypoxic neuronal cell death. However, exact mechanism(s) of the excitatory amino acid (EAA)-induced neurotoxicity during ischaemia is yet to be defined. Massive calcium influx due to activation of the NMDA-receptors is supposed to mediate a prolonged cell degeneration by a number of reactions including free radical initiation and membrane lipid peroxidation[3].

It was intriguing to investigate whether or not excitatory amino acid receptors are involved in the development of ischaemic brain swelling and free radical formation. The obtained data suggest that activation of NMDA receptors leads to an oxidative stress, while blockade of non-NMDA receptors (and/or preservation of Na,K-ATPase activity) is essential for the brain to recover from swelling induced by ischaemia.

Materials and Methods

Mongolian gerbils of both sexes were subjected to 15 min bilateral ischaemia and up to 96 hours of reflow. 2-amino-5-phosphonovaleric acid (APV, 4 mg/kg b.w., i.p.) and propentofylline (HWA 285, 25 mg/kg b.w., i.p.) were administrated to the gerbils at the end of ischaemia, alone or in combination, and animals were sacrificed by decapitation 1 hour thereafter; heads were immediately frozen in the liquid nitrogen. Separate groups of gerbils were taken to estimate brain swelling[5]. Effects of postischaemia and drug application were also tested on the production of superoxide anion ($\cdot O_2^-$), superoxide dismutase (SOD) activity and lipid peroxidation (LP) in the crude mitochondrial fraction[7] of the striatum. Detection of the superoxide radicals was based upon the reduction of nitro-blue tetrazolium (NBT)[2], superoxide dismutase activity was measured as an inhibition of spontaneous epinephrine autoxidation[12] and lipid peroxidation as the content of thiobarbituric acid (TBA)-reactive material formed during the *in vitro* stimulation by Fe^{2+}-salts and ascorbic acid[13]. Protein content was determined according to Lowry *et al.*[8]. Statistical evaluation was performed using the analysis of variance (ANOVA) followed by the least squares difference testing between the means; $p < 0.05$ was considered as significant.

Results and Discussion

During ischaemia alone, only slight increase in the superoxide anion production in the gerbil striatum was observed (Table 1). However, pronounced changes were seen in the first minutes of reperfusion: marked increase in the $\cdot O_2^-$ production followed by the increase in the amount of TBA-reactive material which is indicative for enhanced lipid peroxidation (Table 1). One hour after the circulation had been reestablished, SOD activity was lowered at 73% of control values (Table 1). During the remaining period of reperfusion (until the fourth day), $\cdot O_2^-$ production was permanently enhanced, SOD activity restored and lipid per-

Table 1. *Changes of the Superoxide Anion Production ($\cdot O_2^-$), Superoxide Dismutase (SOD) Activity and Lipid Peroxidation (LP) in the Gerbil During Ischaemia, 60 min of Postischaemia, and the Effects of Drugs*

Time	$\cdot O_2^-$ [a]	SOD [b]	LP [c]
A. Control	88.7 ± 4.9 (5)	538.1 ± 40.6 (6)	92.2 ± 7.0 (6)
A'. Isch. (15')	105.7 ± 7.5* (6)	489.1 ± 43.3 (6)	96.2 ± 7.7 (6)
B. Reperfusion			
1 min	282.2 ± 22.8* (6)	673.3 ± 52.2* (6)	211.8 ± 13.1* (6)
3 min	223.5 ± 15.5* (6)	845.2 ± 68.1* (6)	142.3 ± 4.6* (6)
15 min	199.2 ± 16.9* (5)	693.3 ± 57.7* (6)	171.1 ± 6.8* (6)
1 h	145.1 ± 5.9* (6)	392.9 ± 28.3* (6)	179.7 ± 7.9* (6)
C. Drugs after ischamia; reperfusion 1 h			
HWA 285	120.6 ± 10.7* (6)	402.8 ± 28.3* (6)	144.2 ± 6.4* (6)
APV	115.3 ± 6.3* (6)	493.6 ± 49.4 (6)	157.7 ± 7.6* (6)
HWA + APV	105.6 ± 11.2* (6)	494.3 ± 27.9 (6)	108.6 ± 8.5 (6)

Values are Means ± SEM (n); [a] nmol NBF/min/mg prot; [b] units; [c] nmol MDA/min/mg prot. *$p < 0.05$ (ANOVA) in comparison to A.

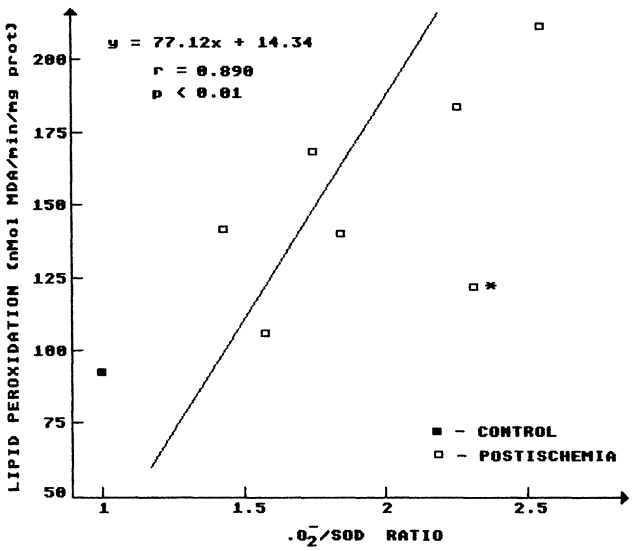

Fig. 1. Linear correlation between the $\cdot O_2^-$/SOD ratio and lipid peroxidation in the crude mitochondrial fraction of the gerbil striatum during the four days of reperfusion. * The value which is beyond the confidence level of 0.95

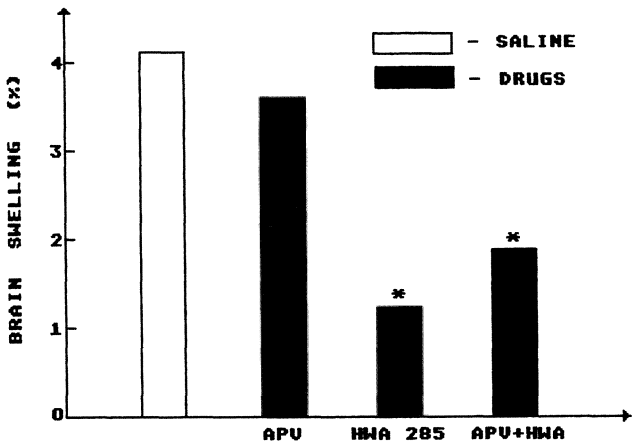

Fig. 2. Effects of APV, HWA 285 and APV + HWA 285 on the brain swelling one hour after 15-min of bilateral ischaemia in the gerbil. Brain water content in the control group was 78.29 ± 0.04; after the application of: saline − 79.14 ± 0.10; APV − 79.05 ± 0.14; HWA 285 − 78.57 ± 0.12; APV + HWA 285 − 78.67 ± 0.09. * Statistically significant difference as compared to saline-treated group (Student's t-test; $p < 0.05$)

oxidation decreased to control value (data not shown). Hence, at least in the first hour of reperfusion a disbalance exists between the enhanced superoxide anion production and the ability of SOD to quench it adequately. Since the intensity of oxidative stress to the tissue depends on its relative capacity for the protective response, it can be adequately expressed by the $\cdot O_2^-$/SOD ratio. Indeed, considering the $\cdot O_2^-$/SOD ratio, a relative incapacity for the $\cdot O_2^-$ removal was observed not only at the time of decreased SOD activity, but during the entire period of reperfusion (data not shown). $\cdot O_2^-$/SOD ratio, also, correlates well with the amount of TBA-reactive material formed during the reperfusion ($r = 0.89$) (Fig. 1).

Production of oxygen free radicals starts with the reperfusion in the electron transport chain. Oxygen and lipid free radicals further originate from a different metabolic reaction promoted by ischaemia-increase in cytosolic calcium concentration, activation of arachidonic acid cascade, increase in the monoamine turnover rate; all these reactions are stimulated during the *in vivo* activation of the NMDA-receptors[3]. Selective NMDA antagonist APV given immediately after 15-min bilateral ischaemia reduced $\cdot O_2^-$ production and

$\cdot O_2^-$/SOD ratio in the gerbil striatum one hour after ischaemia (Table 1). Nevertheless, it was without (significant) effect on the brain swelling (Fig. 2); obviously, receptors other than NMDA are responsible for the main sodium and water influx and cell swelling. When the HWA 285 was applied, the effects on the $\cdot O_2^-$/SOD ratio was less expressed, lipid peroxidation was diminished (Table 1) and brain swelling almost completely attenuated (Fig. 2). Finally, when the both drugs were applied together, $\cdot O_2^-$/SOD ratio and lipid peroxidation were close to control values (Table 1, Fig. 2).

HWA 285 is known to prevent postischaemic inhibition of Na,K-ATPase as well as brain swelling[9]. On the other hand non-NMDA receptors are linked to channels that allow sodium and potassium to pass through the membrane, causing acute cellular swelling; this would suggest that HWA 285 is a non-NMDA receptor antagonist or that Na,K-ATPase is linked with (or is a part of) non-NMDA receptor. Intracellular accumulation of sodium also causes depolarization and thus voltage-gated calcium channels may be activated secondarily leading to further intracellular calcium increase; new conditions develop for free radical formation.

Activation of the EAA-receptors (NMDA and non-NMDA) can be reduced either by specific alosteric inhibition using certain drugs, or indirectly, *e.g.* by the inhibition of EAA release, depolarization, Ca^{2+} entry. All these properties are intensive and may respond to endogenous adenosine or exogenous adenosine analogues[1]. HWA 285 acts as adenosine uptake inhibitor[6], decreases monoamine- and EAA-neurotransmitter release[11] and inhibits intracellular accumulation of Ca^{2+} during ischaemia[4]. Inhibition of the EAA release in addition to "indirect NMDA-blockade" also prevents the activation of non-NMDA receptors, and consequently, development of brain oedema. Since the HWA 285 is shown to prevent postischaemic reduction of Na,K-ATPase activity and increase in sodium concentration[9], direct action of HWA 285 on the non-NMDA receptors is possible. Thus, direct blockade of the NMDA-receptors by the APV and simultaneous inhibition of the non-NMDA receptors by means of HWA 285 effectively protect against lipid peroxidation and, at the same time, prevent development of postischaemic brain oedema.

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