Effects of Glutamate and γ-aminobutyric Acid of Cerebral Ischemia on Rhesus Monkey

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Article

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Abstract

Objective To explore the effect of cerebral ischemia in rhesus monkeys on the metabolism of glutamate and γ-aminobutyric acid.

Method Bilateral common carotid artery was blocked in 9 rhesus monkeys. The extracellular fluid was collected by microdialysis. Perform liquid chromatography analysis .

Result After cerebral ischemia, the content of glutamate and gamma-aminobutyric acid was significantly increased compared with that of ischemia. P <0.05, the difference was statistically significant.

Conclusions Cerebral ischemia hypoxia leads to the large release of glutamate, which leads to increased synthesis of GABA and antagonism to it’s excitatory toxicity.

Introduction

Ischemic strokes is one of the main diseases that harming human's health and lead to serious and long term disability [1].Acute cerebral ischemic injury appears in a few minutes to hours after ischemia at most times.Beacsue of ischemia and hypoxia,the neuron releasing a large number of glutamic acids with the calcium ion accumulating in cell, so that the effect called “Excitotoxicity” arises. That makes cell membrane depolarize ,aggravate the phenomenon we described above and lead to a vicious cycle. At the same time,a large number of water molecules get into the cell along with calcium ion and produce cytotoxic edema what destroyed the cell membrane, wire and DNA[2–3]. This stimulation will lead to neuronal damage include neurons apoptosis, necrosis and inflammation [3]. In this experiment, we blocked rhesus monkeys' bilateral common carotid arteries for 10 mins for simulating animal model of ischemic stroke at room temperature. Extracellular fluid in brain tissue will be collect continuously by using micro dialysis technology in the different periods before and after ischemia .Using HPLC - uv method to separate and analysis the content amino acid neurotransmitters in extracellular fluid. The quantity of amino acid neurotransmitter metabolism was observed in different time periods before and after cerebral ischemia and statistical analysis was performed try to understand the damage mechanism of ischemia and hypoxia on neurons further from the perspective of neurotransmitter metabolism.

Materials And Methods

All experimental protocols have been reviewed and approved by the local ethics committee and in accordance with relevant guidelines (including the ARRIVE guidelines) and regulations.

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

1.1 Experimental Animals

We choiced the number of 9 healthy male responsive and adult rhesus monkeys. Its' neural function were normal, aged at 4 to 10 years old with an average age of 7.90 ± 1.82 and weight 4.2 ~ 14 Kg with an average of 8.22 ± 2.15 Kg.

1.2 Model

Routine blood test, coagulation function test ,Liver and kidney function test etc. was completed preoperate. The monkey did not drink and eat at the night before the operate. The monkeys were fixed on the operating table in the supine position with cervical hyperextension after ketamine hydrochloride (10 mg / kg) and diazepam (0.5 mg/kg) go into effect.Maintaining general anesthesia by using propofol(0.1-0.2mg/kg/min)via venous pump. Endotracheal intubation after eyelash reflex disappearing for keeping spontaneous breathing(100% oxygen concentration). Skin preparation of head and neck was completed and multi lead monitor were connected for lasting ECG monitor. And then cut bilateral carotid sheath to expose bilateral common carotid artery and clamping for 10 min.

1.3 Micro Dialysis Sample Collectting And Processing

Using micro electric drill to drill a hole about 0.5 cm in diameter in the right parietal lobe (1 cm after coronal suture and 0.5 cm beside the center line). Cuted the dura mater, then implanted probes guide tube fixating by dental cement,and inserted the micro dialysis(dialysis membrane effective length is 4mm, the molecular weight is 20 ku). Microinjection pump was used to dialyse(2.5ml/min) the area we mentioned above at the time of 90mins befor block and gather specimens from extracellular fluid of brain at the time of 10mins before ischemia.We collected the extracellular fluid of 10min before ischemia and 10min after ischemia and quickly stored at -80℃.The retention time of the known amino acid peak of each standard was compared with that of the extracellular solution to be tested, and the chromatographic peak area of the amino acid standard was converted and quantified according to the following formula by the external standard method: C = R1/R2·D·N. R1 = Peak area of the sample to be measured ; R2 = Peak area of amino acid standard sample ; D = Concentration of amino acid standard sample(μmol/L);D = Dilution multiple; C = Sample concentration to be tested(μmol/ L)

Picture 1. the picture of Glu in HPLCPicture 2.the picture of Gluin HPLC

Picture 3. the picture of GABA in HPLC Picture 4. the picture of GABA in HPLC

1.4 Statistical Processing

SPSS17.0 software was used for statistical analysis, and the concentration values of Glu and GABA among different groups were analyzed. All quantitative data were expressed by mean ± standard deviation (x ± s). The data were tested by normal test. Comparison of multiple groups of means satisfying normal distribution and homogeneity of variance one-way analysis of variance

((OneWayANOVA),) was used to compare the differences with LSD method. There was significant difference between the two groups ($P < 0.05$).

Results

Analysis of the changes of Glu and GABA in extracellular fluid of brain.

Tab 1 Changes in Glu GABA content μmol L in extracellular fluid $\bar{x} \pm s$ n

Time	Glu	GABA
before ischemia	150.81±12.02*	45.16±3.38*▲
10mins after ischemia	398.99±34.03	91.66±4.01
F	279.66	351.74
P	0.00	0.00

* Compared with before ischemia *P<0.05

The concentrations of Glu and GABA in extracellular fluid of brain before and after ischemia were analyzed, and all quantitative data were expressed by mean ± standard deviation (x ± s) (Table 1). One-way ANOVA showed that there were significant differences in Glu concentrations before and after ischemia (Fang 279.66), GABA concentrations were significantly different between Fang 351.74); LSD groups. Glu concentration in extracellular fluid reached 398.99 ± 34.03 umol / L), in ischemic 10min group was significantly higher than that in pre-ischemic group. The concentration of GABA in extracellular fluid in ischemic 10min (91.66 ± 4.01 umol / L),) was significantly higher than that in pre-ischemic group.

Discussion

The blood supply of the brain tissue is very rich, and the blood flow accounts for about 20% of the blood supply of the whole body, so the brain tissue is highly dependent on the blood supply and has a poor tolerance to ischemia and hypoxia. When the blood flow of every 100g brain tissue decreases to 40ml/min, the brain tissue will be damaged by ischemia and hypoxia. At room temperature, complete interruption of blood supply to the brain tissue for a few seconds will lead to disturbance of consciousness, lasting more than 5-6min, and irreversible damage will occur to the brain tissue [4–6].

The neurotransmitter (neurotransmitter) refers to the information transmission substance synthesized by neurons and released at the end of the presynaptic membrane, which specifically acts on the postsynaptic membrane and produces postsynaptic potential. Glutamate (glutamicacid,Glu) is the main excitatory neurotransmitter in mammalian brain. After electrical stimulation, nerve cells release glutamate and bind to another cell surface receptor, which leads to the influx of sodium and calcium ions and the formation of postsynaptic potential. It participates in many important physiological functions, such as

cognition, memory, learning, movement, sensation and so on. Olney et al. [7] put forward the theory of "excitotoxicity (excitotoxicity)" of glutamate in the study of brain injury, which proved that systemic application of glutamate can lead to degenerative changes of neurons in the central nervous system. Studies have shown that the content of excitatory amino acids in extracellular fluid increases during cerebral ischemia, and the content of glutamate increases more significantly [8–10], which is consistent with our results. The massive accumulation of glutamate can lead to continuous depolarization of neurons, which in turn leads to the massive release of glutamate in neurons. Cerebral ischemia and hypoxia can also cause a series of chain reactions, such as energy metabolism disturbance, inflammatory reaction, calcium overload, free radical reaction, cell necrosis and so on, which together with excitotoxicity constitute the central link of ischemic brain damage [11–13].

Gamma-aminobutyric acid (γ-aminobutyricacid,GABA) is the main inhibitory amino acid neurotransmitter in the central nervous system of mammals, which mainly exists in the cerebrum and cerebellar cortex, and partly in the projection fibers and striatum. Under physiological conditions, GABA has the effects of hypnosis, sedation, lowering blood pressure, anticonvulsant and so on. Our study confirmed that the level of GABA in extracellular fluid increased sharply in the early stage of cerebral ischemia. Form postsynaptic and presynaptic inhibition, jointly antagonize the excitotoxicity of glutamate, reduce cell injury, enhance the tolerance of neurons to ischemia and hypoxia, and have significant neuroprotective effects [15]

This experimental study found that the concentration of Glu increased rapidly after ischemia (398.99 ± 34.03 umol / L), and the concentration of ischemic 10minGABA (91.66 ± 4.01 umol / L) was also significantly higher than that before ischemia (45.16 ± 3.38 umol / L). During cerebral ischemia, with the release of a large amount of glutamate, feedback caused an increase in GABA synthesis and antagonized its "excitotoxicity". The experimental results are consistent with the results of Li and Melani et al. [16–17].

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Figures

Figure 1

Caption not included with this version.

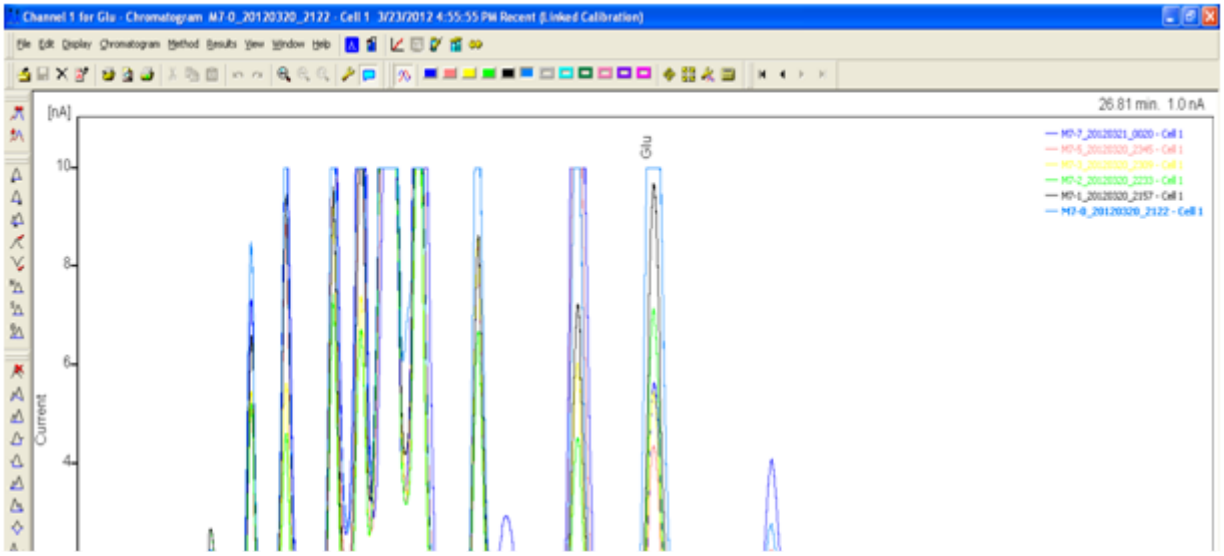

Figure 2

the picture of Glu in HPLC

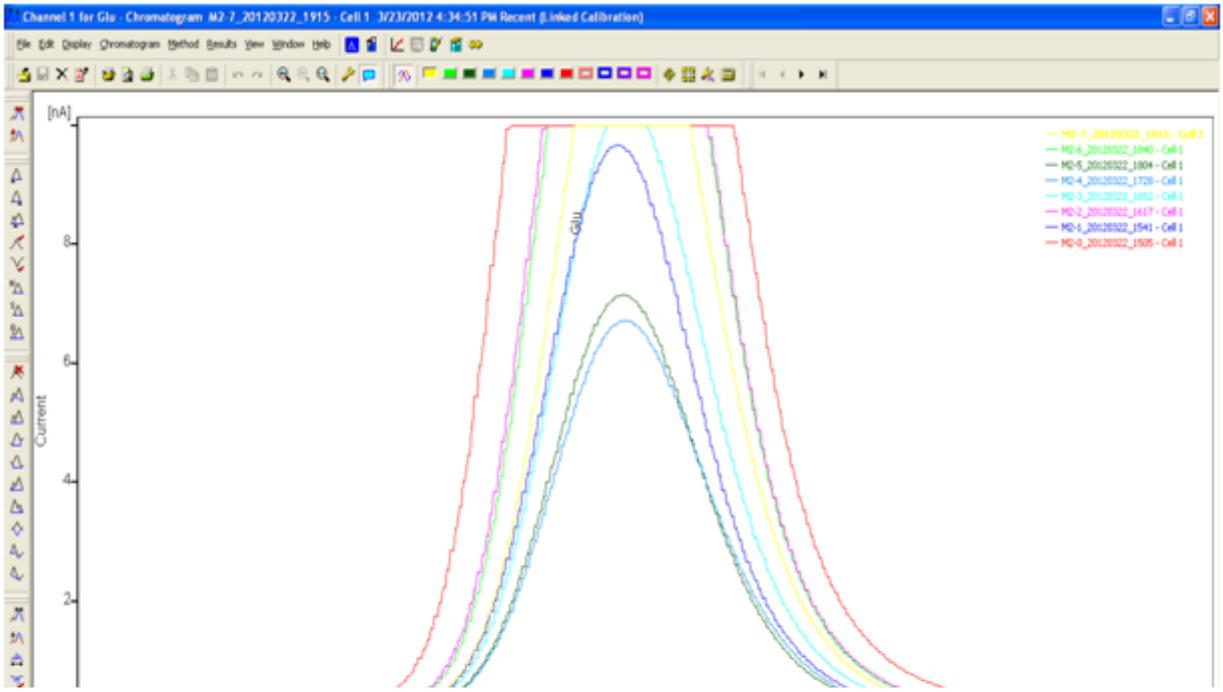

Figure 3

the picture of Glu in HPLC

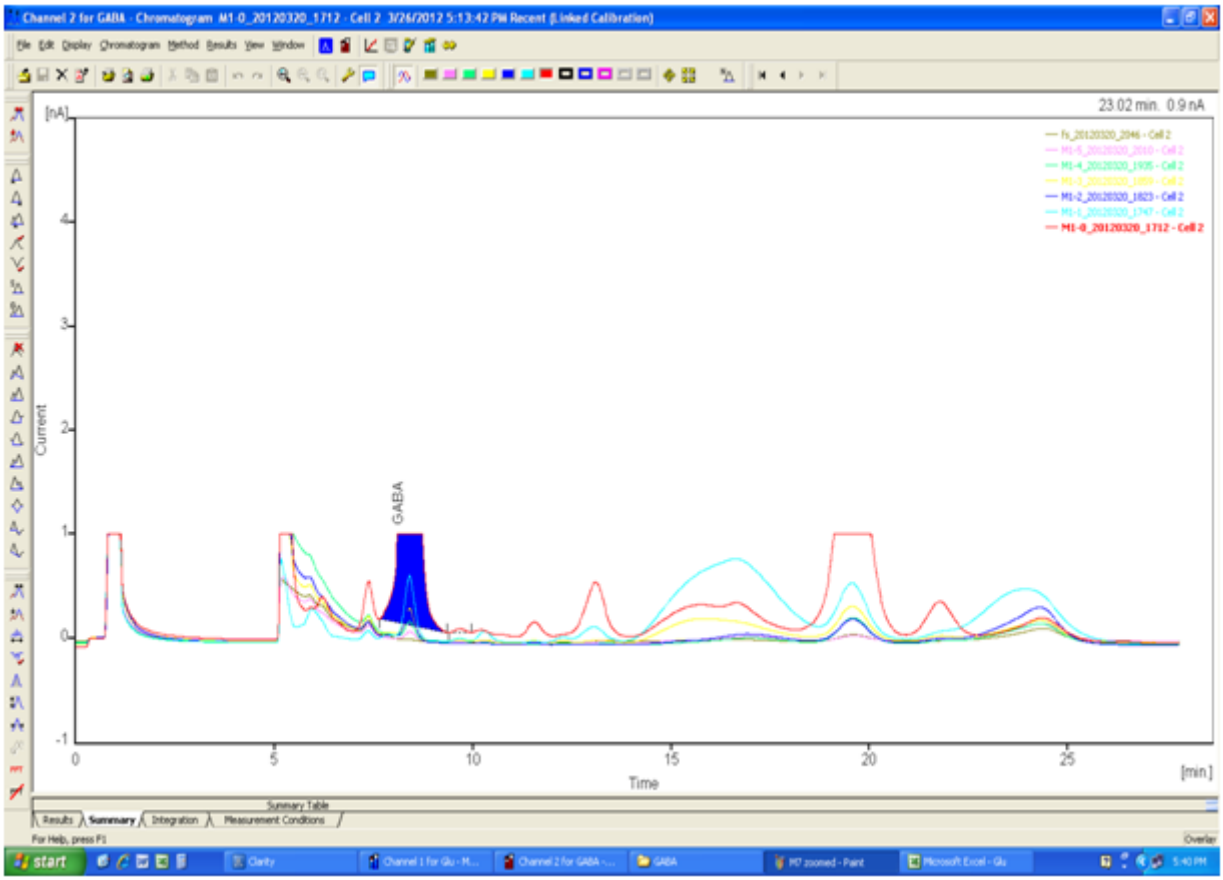

Figure 4

the picture of GABA in HPLC

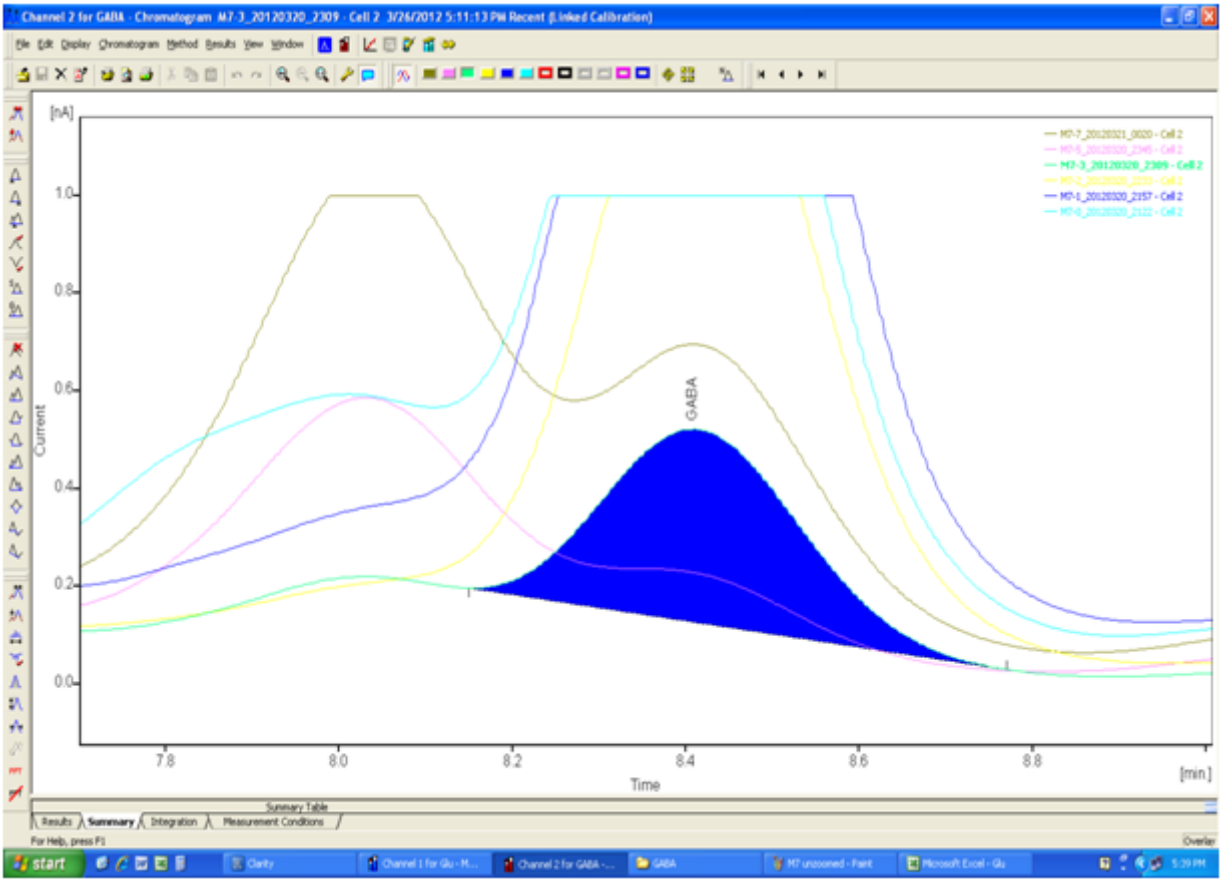

Figure 5

the picture of GABA in HPLC

Supplementary Files

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- GABA.xls