Altered Expression of Metabolic Glutamate Receptor 5 in Methamphetamine Intoxication in the Rats*

ZHANG Jing[1], ZHANG Hao[1,2], ZENG Xiao-feng[1], WANG Shang-wen[1], ZHAO Yong-he[1], LI Zhen[1△]

(1 Kunming Medical College, Kunming 650031, China;

2 Public Security Bureau Of Yunnan Province, Kunming 650228, China)

ABSTRACT Objective: To investigate the effect of mG1uR5 receptors on methamphetamine hydrochloride in the rats. Methods Forty male Wistar rats were randomly divided into control group (n=20) and experimental group (n=20). Rats in EG were peritoneal injection with methamphetamine hydrochloride (20 mg/kg-HDG, 10 mg/kg-MDG, bid). CG was administered with the same normal saline. Within 24 h of the last methamphetamine injection, rats were anesthetized by intraperitoneal injection of pentobarbital sodium and then were perfused by 4 % paraformaldehyde. After the removement of brain ,mGluR5 by immunohistochemical staining were observed and counted in different brain areas. Results: The expression of mG1uR5 in the hippocampus and the striatum of the brain for experimental group was enhanced markedly compared with control group (P<0.05) and it had dose dependent. Conclusion: MG1uR5 participated in the damage mechanism of methamphetamine poisoning.

Key words: Methamphetamine; Intoxication; MG1uR5

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Introduction

Methylamphetamine is an amphetamine-type stimulant. Long-term abuse can induce ethologic change, neurochemical and neuropathological damage of central nervous system. Its neurotoxical roles are as follows: disfunctions of monoaminergic system's (DA and 5-HT), study shows that methylamphetamine's neurotoxicity is correlated with generation of active compounds (ROS、$ONOO^-$)[1]. Current evidences have been accumulated to indicate that nerve injury mechanism primarily through basic action pathways,generating active compounds which was known to have impaired effect can induce nerve injury. These are as follows: ①the oxidation of DA[2] ②disfunction of mitochondrium[3] ③neuron excitability toxicity. Previous researches are mainly about the oxidation of DA, however study on neurons' excitability toxicity is being focus on this field. Some scholar had study the NMDA receptors' contribution in nerve injury of MA poisoning , this experiment were try to investigate the excitability toxicity's mechanism by morphology of rats MA subacute poisoning and mGluR5, to make further study of MA poisoning mechanism and to provide new evidence, to provide MA poisoning's treatment and pharmaceutical research with pathologic data.

1 Materials and Methods

1.1 Sample

40 adult Sprague Dawley male rats weighing 240± 20 g were purchased from Experimental Animal Center of Kunming Medical College.

1.2 Methods

Experimental group was divided into: high dose group (10 rats) Ip (intraperitoneal injection) MA 20 mg/kg bid * 4 d, moderate dose group (10rats)Ip MA 10 mg/kg bid * 4 d,(8am,8pm).The control group was treated with saline. MA was offered by The Ministry of Public Security of PRC. By model building, meanwhile, immunohistochemical method was used to investigate the expression of mGluR5 in the hippocamp and the striatum.

1.3 Experimental Operations

1.3.1 Collection of brain specimens All rats were anaesthetized by 1 % pentobarbital sodium through peritoneal cavity injection,then they were fixed on icefilled bag to lower the heart rates and given cardiac perfusion. According to the rat brain stereotaxic atlas, the position of the hippocampus and the striatum was located, and the tissue respectively from the same section was collected, then imbedded with paraffin.

1.3.2 Immunohistochemical staining of anti-mGluR5 (S-P) Act according to operation steps on the kit.

1.4 Statistical analysis

SPSS 12.0 software package was used for statistical analysis. Data were presented as mean± SD, the groups were analyzed by one-way ANOVA. Covariance was analyzed by the SNK. When the variance was irregular K Independent Sample rank sum test and 2 Related Sample rank sum test was used.

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Author:ZHANG Jing(1984-),female,Graduate Students in Reading, Forensic Pathology,E-mail:sunflower_zj@sina.com

△Correspondence author:LI Zhen (1959-),female,Professor. E-mail:lizhenlaura@126.com

ZHANG Jing, ZHAO Hao,both are the first author.

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2 Results

2.1 The expresssion of mG1uR5 positive cells in the hippocampus and the striatum

mG1uR5 positive cells significantly increased in the hippocampus and the striatum after the intraperitoneal injection of methamphetamine.The growth in HG was more than in MDG,and the growth in HG and MDG were more than in CG. Hippocampal area (Fig.1~3), striatum (Fig. 4~6).

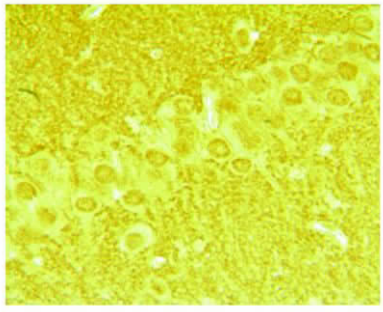

Fig.1 CG mG1uR5 positive cell in the hippocampus

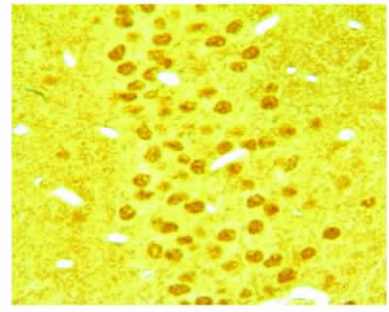

Fig.2 HDG mG1uR5 positive cell in the hippocampus

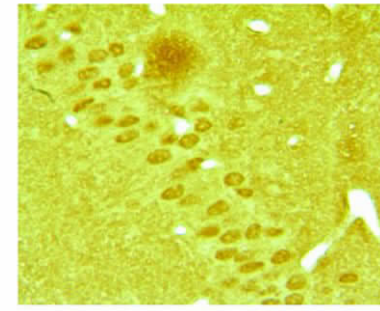

Fig.3 MDG mG1uR5 positive cell in the the striatum

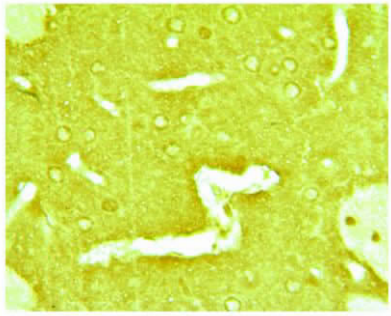

Fig.4 CG mG1uR5 positive cells in hippocampus

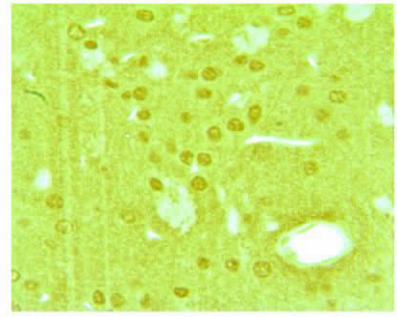

Fig.5 HDG mG1uR5 positive cells in the striatum

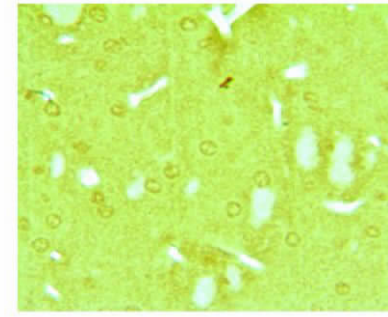

Fig.6 MDG mG1uR5 positive cells in the striatum

2.2 Cell counting results

The microscopic photography system at 400 times magnification was used to observe the conduct random site plans and to count mG1uR5 positive cells of the hippocampus and the striatum under the same vision. The hippocampus and the striatum had significant differences ($p < 0.05$). (Table. 1)

Table 1 The mG1uR5 positive cells counting in the hippocampus and striatum($\bar{x} \pm s$)

Group	N	The mG1uR5 positive cells counting in the hippocampus	The mG1uR5 positive cells counting in the striatum
Control group	10	28.4± 2.6	10.3± 2.3
20mg/kg	10	58.5± 3.5	21.4± 2.5
10mg/kg	10	39.3± 3.2	16.5± 2.2

P<0.05, compared with the control

3 Discussion

From the results of this experiment, it can find that there were the expression differences of mG1uR5 positive cells between the experimental group and control group,which indicated that the mG1uR5 methamphetamine poisoning play a role in the neuron injury. Excitotoxicity referring to an excessive amount of glutamate aspartate or excitatory amino acids lead to a high degree of excitement in the corresponding receptors by the receptors of neurons, then led to the toxic effects and cell death process. Glutamate, as the chemical messenger of the central nervous system excitatory synapses regulates excitatory synaptic transmission through the excitement of G-protein-coupled glutamate metabolic receptors (mG1uRs)[4]. Glutamate is the most important excitatory neurotransmitter of the central nervous system, it plays an important role in the induction of synaptic plasticity, neurogenesis, nerve

degeneration. Under pathological condictions, glutamate excited by glutamate receptor-mediated nerve produced excitotoxicity, which was the main reason for the nervous system ischemia, trauma other nerve disorders caused by other reasons, and nerve degeneration. Metabotropic glutamaterceptor(mGluR) belongs to the third members of G protein-coupled receptor superfamily. Eight different mG1uRs genes have been cloned [5]. They were named mG1uR1~8. It was showed that human mG1uR5 was related to many physiological and pathological processes[6]. Under pathological condictions, mG1uR5 was related to neurons' excitotoxicity and incoordination. mG1uR5 was widely distributed and high expressed in the striatum and the hippocampus in rats [7]. Related investigation showed that human mGluR5 had high expression in the striatum and the hippocampus [8]. MGluR5 can be activated by several pathways after MA intraperitoneal injection, heightened intra-cellular Ca2+ to induced a series of injury response, then caused excitotoxicity to the cells and cellular ischemia, hypoxic damage even cell death. It was confirm that human nervous system mG1uR5 and rat mG1uR5 had the same effects[8].

mGluR5 mainly activates phospholipase C (PLC)and promotes phosphoric acid lipid inositol(PIP2) hydrolysis and lipid inositol triphosphate (IP3) generation, which lead to the release of Ca^{2+} [9]. It affects cell metabolism by multiple channels, produces different biological effects, including the composition of oxygen (ROS) [10] and O_2^-. Na^+- K^+-ATPase is the target of oxygen free radicals [4], and it is important in energy metabolism. The damagement of Na^+- K^+-ATPase will certainly cause hypoxic damage, including the injury of neurons. In addition, glutamate transporter is Na^+ and K^+-dependent,and the driving force comes from the inward Na^+ iron gradient by Na^+- K^+-ATPase producing. Therefore, energy metabolism, ATP formation and the resulting reduction of Na^+- K^+ -ATP activity decreased, the change of the ion gradient can cause the barrier of glutamate remove. Glutamate transporter also has an oxidation and reduction mechanism based on sulfhydryl. The reversible redox regulation not only represents a physiological regulatory mechanism, but also may have pathological significance, particularly when it is not reversible protein regulator. Oxygen free radicals' of ongoing oxidization of sulfhydryl in protein may influence glutamate uptake through Na^+- K^+-ATPase indirectly. Two conditions will further cause concentration increase of glutamate, which can activate mGluR5 and intensify the excitotoxicity.

Although MA does not directly cause the increase of glutamate concentration, it causes the release of glutamate indirectly through the DA. It can be considered that the DA is the media of glutamate's concentration increase caused by MA. After MA intraperitoneal injection, DA and 5-HT nerve terminal loss and related pathological changes were found,which may cause by a direct chemical injury leading to cell death [11]. At the same time, DA caused the release of glutamate leading to the increase of extracellular glutamate concentration, which activates mG1uR5 to generate excitotoxicity.

Previous studies had shown that the cyclopentane dicarboxylic acid (ACPD) injection of hippocampus could cause epilepsy and neuron injury in the dentate gyrus of hippocampus[12]. Past studies have proved that ionic glutamate N-methyl-D-aspartate(NMDA) receptors can cause neuron excitotoxicity damage in the striatum.Research shows that mG1uRs strengthen the excitotoxicity damage of striatum neuron NMDA receptors.

Based on results of this experiment and combined with other results of past researchers, we can speculate mG1uR5 has played a role in body toxicity caused by subacute methamphetamine poisoning, mG1uR5 was involved in the methamphetamine poisoning injury.

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甲基苯丙胺中毒 mG1uR5 的表达 *

张 晶[1] 张 浩[1,2] 曾晓锋[1] 王尚文[1] 赵永和[1] 李 桢[1△]

(1 昆明医学院法医学院 云南 昆明 650031;2 中国云南省公安厅 云南 昆明 650228)

摘要 目的:研究代谢性谷氨酸受体 5 (mG1uR5) 在甲基苯丙胺中毒的损伤机制中的作用。方法:设立实验组,对照组。实验组分别给予 20 mg/kg,10 mg/kg 腹腔注射 MA;对照组分别给予同剂量的生理盐水。末次注射后 24 h 内腹腔注射戊巴比妥钠麻醉大鼠后用 4%多聚甲醛灌注、取脑后行代谢性谷氨酸受体 5 的免疫组织化学染色,观察并计数 mG1uR5 在不同脑区的表达。结果:实验组 mG1uR5 在大脑纹状体、海马的表达较对照组显著增强,差异有显著性 (P<0.05),并呈剂量依赖性。结论:mG1uR5 参与了甲基苯丙胺中毒的损伤机制。

关键词:甲基苯丙胺;中毒;mG1uR5

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作者简介:张晶(1984-),女,硕士研究生,法医病理学专业。E-mail:sunflower_zj@sina.com

△通讯作者:李桢 (1959-),女,教授。E-mail:lizhenlaura@126.com

张晶,张浩,同为第一作者

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