关键词 脑缺血；再灌注损伤；多聚 ADP-核糖多聚酶；3-氨基苯甲酰胺；尼克酰胺；单 ADP-核糖酰转移酶；维生素 K_1

目的：研究多聚 ADP-核糖多聚酶（PARP）在局灶性脑缺血再灌注损伤中的作用. **方法**：雄性 Wistar 大鼠用插丝法阻塞大脑中动脉 3.5 h 后再灌注，梗塞灶用 TTC 染色显示，图象分析测量；神经功能缺损采用 0－5 级评分. **结果**：低剂量 PARP 抑制剂 3-氨基苯甲酰胺（10 mg·kg^{-1}）或尼克酰胺（20 mg·kg^{-1}）具有明显的神经保护作用，治疗窗近 6 h；高剂量反而加重脑损伤，特别是尼克酰胺在再灌注起始给药. 选择性单 ADP-核糖酰转移酶抑制剂对脑梗塞无明显作用. **结论**：暂时非完全性抑制 PARP 对脑缺血再灌注损伤产生神经保护作用，然而完全抑制该酶（尤其是在再灌注期）则产生损害作用.

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Enhancing effects of β-endorphin on glutamate neurotoxicity[1]

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KEY WORDS beta-endorphin; endorphins; calcium; sodium glutamate; arcuate nucleus

AIM: To study the effects of β-endorphin (β-End) on monosodium glutamate (MSG)-induced neurotoxicity (GNT). **METHODS**: Image analysis of neuronal areas and determination of mitochondrial membrane protein-bound Ca^{2+} and intracellular free Ca^{2+} ($[Ca^{2+}]_i$) were used. **RESULTS**: β-End aggravated MSG-induced neuronal injury in arcuate nucleus of hypothalamus in a dose-dependent manner in the range from 0.5 to 5.0 mg·kg^{-1}. MSG-induced increase in mitochondrial membrane protein-bound Ca^{2+} was enhanced when treated with β-End 2 g·L^{-1}. MSG-induced elevation in $[Ca^{2+}]_i$ in single neuron was also augmented from 320 ± 84 to 589 ± 78 nmol·L^{-1} by the treatment with β-End 2 g·L^{-1}. **CONCLUSION**: β-End enhanced GNT via aggravating the disruption of intracellular Ca^{2+} homeostasis induced by MSG.

Excessive activation of glutamate receptors results in neuronal injury and death, associated with acute brain injury and chronic neurodegenerative disorders, eg, hypoxic-ischemic injury, eclamptic seizures, Alzheimer's disease, and Huntington's diseases[1,2]. Our previous work showed that morphine enhanced the glutamate neurotoxicity (GNT)[3,4], suggested that morphine-like substances might produce neuronal damage in CNS via enhancing GNT.

β-Endorphin (β-End) exerts a wide spectrum of actions, such as analgesic effect and stress-induced reproductive dysfunction[5]. The purpose of this study was to investigate whether β-End might has the same effect as morphine on GNT.

MATERIALS AND METHODS

Morphological observation and image analysis of neuronal areas Neonatal mice (n = 64) of either sex of 7－9 d, weighing 5.8 ± 0.5 g were divided into 8 groups at random. The mice in each group were daily injected sc with saline, MSG (1.0 g·kg^{-1}) or MSG + β-End (0.5, 1.5, 2.0, 3.0, 5.0 mg·kg^{-1}) for 7 d. β-End was injected 15 min after MSG. Mice were killed 1 wk after the final treatment and 6 μm thick serial coronal sections in hypothalamus were stained with cresyl violet[3]. Morphological changes of neurons were photographed, and neuronal areas of arcuate nucleus (AN) were measured by 540-Biological Medicine Color Image Analyser.

Detection of mitochondrial membrane protein-bound Ca^{2+} The 0.2 mm thick brain slices of mice[6] were

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divided into 3 groups at random and treated with saline, MSG (100 μmol·L^{-1}, 10 μL), and MSG + β-End (0.2 g·L^{-1} or 2 g·L^{-1}, 10 μL) under the condition of extracellular Ca^{2+} concentration (1 or 2 mmol·L^{-1}). β-End was added 1 min after the treatment of MSG, and the brain slices were incubated at 37 ℃ for 15 min in artificial cerebrospinal fluid (pH 6.9) with 95 % O_2 + 5 % CO_2. The center areas of hypothalamus, hippocampus, and cortex were dissected from the above slices according to atlas and homogenated. The preparation of mitochondria was obtained and Tb^{3+} relative fluorescent intensity was detected by Hitachi RF-540 fluorescent spectrophotometer[7].

Determination of $[Ca^{2+}]_i$ in single neuron The procedure for determination of the fluorescence of calcium was modified from the previous methods[8]. Brain cells were freshly dissociated from 1 – 2-day-old mice. The cell suspension was loaded with Fura 2-AM 2 μmol·L^{-1} (Sigma, final concentration), incubated at 37 ℃ for 45 min and collected by centrifugation at 300 × g for 5 min. The 100 μL suspensions of dissociated cells were kept at 37 ℃ and administrated with MSG (100 μmol·L^{-1}, 10 μL), MSG + β-End (0.2 – 2 g·L^{-1}, 10 μL). $[Ca^{2+}]_i$ was determined from the ratio of fluorescence (λ_{ex} = 340 and 380 nm; λ_{em} = 505 nm) by Spex AR-CM-MIC Cation Measurement System, and the morphological changes were also observed through the inversed microscope simultaneously.

Statistical analysis Results were expressed as $\bar{x} \pm s$ and compared with ANOVA.

RESULTS

Effects of β-End on GNT *in vivo* MSG (0.5 g·kg^{-1}, sc) given to immature mice induced pyknotic nuclei and loss of neurons in the arcuate nucleus of hypothalamus compared with the saline group, which were aggravated by the treatment with MSG 15 min before sc β-End (1.0 mg·kg^{-1}) (Fig 1).

The above damage was quantitatively analyzed using image analysis in AN. The MSG-induced decreases of neuronal areas and NA/TA were further reduced after the addition of β-End, which indicated that the enhancing effects of β-End on MSG-induced loss of neurons were dose-dependent (Tab 1).

Tab 1. Effects of β-End on MSG-induced decrease of neuronal areas (NA) in arcuate nucleus (AN) of hypothalamus. n = 8 sections from 3 – 4 mice in one group and 30 mice were used in the whole experiment. TA: total areas = 199 ± 9 μm². $\bar{x} \pm s$. $^cP < 0.01$ vs control. $^eP < 0.05$, $^fP < 0.01$ vs MSG.

MSG, g·kg^{-1}	β-End, mg·kg^{-1}	NA, μm²	NA/TA, %
0	–	48.3 ± 4.0	24.4
1	–	35.0 ± 2.3^c	17.6
1	0.5	32.2 ± 1.5^{ce}	16.2
1	1.0	30.4 ± 1.9^{cf}	15.3
1	1.5	24.4 ± 2.1^{cf}	12.3
1	2.0	20.0 ± 2.1^{cf}	10.1
1	3.0	15.1 ± 1.3^{cf}	7.6
1	5.0	12.6 ± 1.6^{cf}	6.4

Effects of β-End on mitochondrial membrane protein-bound Ca^{2+} levels induced by MSG Low dose of β-End (0.2 g·L^{-1}, 10 μL) did not affect the MSG-induced increase in mitochondrial membrane protein-bound Ca^{2+} under the condition of extracellular Ca^{2+} (1.0 or 2.0 mmol·L^{-1}), while high dose of β-End (2 g·L^{-1}, 10 μL) enhanced the increase in mitochondrial membrane

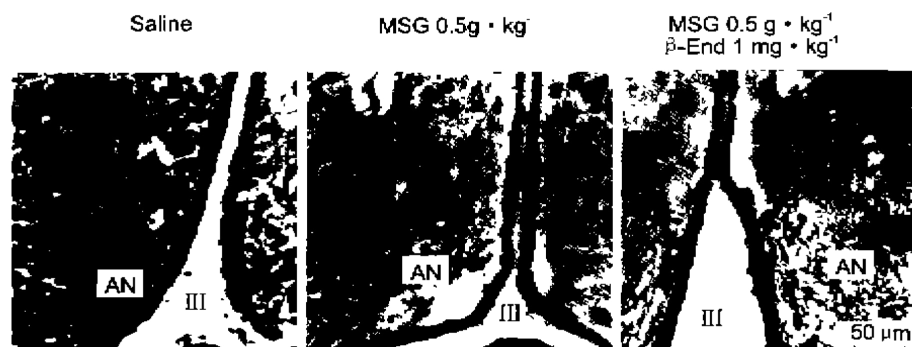

Fig 1. Sections through arcuate nucleus (AN) of hypothalamus from mice sc saline, MSG, and MSG + β-End. III: the third ventricle.

protein-bound Ca^{2+} in hypothalamus, hippocampus, and cortex (Tab 2). These data suggested that high dose of β-End could aggravate the dysfunction of mitochondria to sequester or deplete Ca^{2+} induced by MSG.

Tab 2. Effects of β-End on mitochondrial membrane protein-bound Ca^{2+} induced by MSG (100 μmol·L^{-1}, 10 μL) in 1.0 and 2.0 mmol·L^{-1} extracellular Ca^{2+}. n = 8 – 10 samples, each was prepared from 3 pieces of brain slices which were cut from 1 mouse, and 10 mice in one group altogether. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control. $^dP > 0.05$ *vs* MSG, in Ca^{2+}-free Hanks', L = β-End (0.2 g·L^{-1}, 10 μL), H = β-End (2 g·L^{-1}, 10 μL).

Added Ca^{2+} /mmol ·L^{-1}	Group	Tb^{3+} relative fluorescent intensity		
		Hypothalamus	Hippocampus	Cortex
1	Control	54 ± 7	63 ± 8	57 ± 8
1	MSG	38 ± 3^c	49 ± 5^c	41 ± 7^c
1	MSG + β-End (L)	36 ± 5^{cd}	43 ± 6^{cd}	39 ± 3^{cd}
2	Control	51 ± 5	59 ± 6	54 ± 7
2	MSG	36 ± 4^c	41 ± 5^c	31 ± 4^c
2	MSG + β-End (L)	38 ± 4^{cd}	39 ± 3^{cd}	35 ± 5^{cd}
1	Control	56 ± 3	65 ± 8	59 ± 7
1	MSG	42 ± 4^c	48 ± 6^c	43 ± 6^c
1	MSG + β-End (H)	33 ± 4^{cf}	34 ± 5^{cf}	32 ± 6^{cf}
2	Control	58 ± 4	66 ± 6	61 ± 8
2	MSG	42 ± 6^c	49 ± 7^c	48 ± 4^c
2	MSG + β-End (H)	30 ± 4^{cf}	32 ± 6^{cf}	29 ± 4^{cf}

Effects of β-End on MSG-induced elevation of $[Ca^{2+}]_i$ Only the higher dose of β-End (≥0.8 g·L^{-1}, 10 μL) had the enhancing effect on the elevation of $[Ca^{2+}]_i$ induced by MSG. $[Ca^{2+}]_i$ increased from 188 ± 21 to 228 ± 26 nmol·L^{-1} when treated with high dose of β-End (2 g·L^{-1}, 10 μL) alone, the elevation level was smaller than that of MSG 100 μmol·L^{-1} (320 ± 84 nmol·L^{-1}, $P < 0.05$ *vs* control, $P < 0.01$ *vs* MSG) (Fig 2).

DISCUSSION

In the present study, the dose-dependent enhancing effects of β-End on GNT were observed by the morphological evidence and quantitative image analysis, as well as the determination of mitochondrial membrane protein-bound Ca^{2+} level which reflect the extent of the mitochondrial dysfunction following the

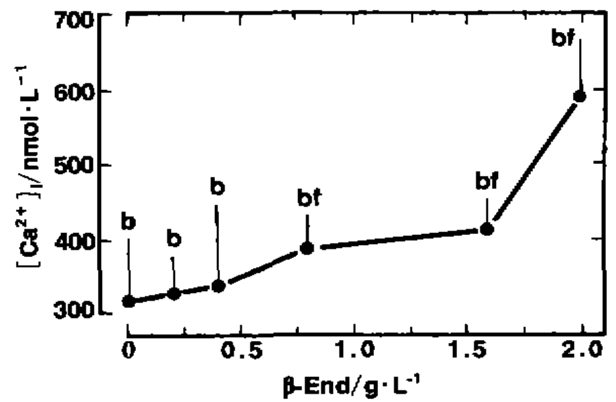

Fig 2. $[Ca^{2+}]_i$ after MSG (100 μmol·L^{-1}, 10 μL) + β-End (0 – 2 g·L^{-1}, 10 μL) in suspension of freshly dissociated neurons. n = 20 – 25 neurons from 6 neonatal mice. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control, $[Ca^{2+}]_i$ = 188 ± 21 nmol·L^{-1}. $^fP < 0.01$ *vs* MSG.

treatment with MSG or β-End + MSG. The above phenomenon was further confirmed by the determination of $[Ca^{2+}]_i$ in single neuron following the treatment with various drugs. It is interesting to note that β-End itself increased $[Ca^{2+}]_i$, though the elevation extent was smaller than that of MSG. Meanwhile, the MSG-induced increase in $[Ca^{2+}]_i$ was augmented by the treatment with β-End.

It is generally accepted that opioids generate their physiological effect by reducing free cytosolic calcium and inhibiting neurotransmitter release[9,10]. In recent years, however, evidence has been accumulated indicating excitatory activity of opioids as well: elevated $[Ca^{2+}]_i$ in NG 108 – 15 cells and other cell lines[11,12], increased Ca^{2+} uptake by synaptosomes and potentiated the release of various neurotransmitters[13,14]. Our previous research showed that morphine could enhance MSG-induced neuronal injury in both immature and adult mice, which might be due to Glu receptor overactivation or up-regulation and the severer dysfunction of neuronal abilities to sequester or deplete intracellular Ca^{2+} [4,6]. β-End, as an endogenous opioid, may enhance GNT via the similar mechanisms and ultimately result in the overloading of intracellular Ca^{2+} as well as irreversible neuronal injury and death.

In conclusion, β-End enhanced GNT in a dose-dependent manner, and the ionic mechanism was related to the aggravation of the disruption of intracelluar Ca^{2+} homeostasis induced by MSG.

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β-内啡肽增强谷氨酸神经毒性[1]

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关键词 β-内啡肽；内啡肽类；钙；谷氨酸钠；弓状核

目的：探讨β-内啡肽对谷氨酸单钠诱导的神经毒性的影响．**方法**：形态学观察、神经元面积图象分析、线粒体膜蛋白结合钙和单细胞内游离钙浓度测定．**结果**：β-内啡肽在 0.5 – 5.0 $mg \cdot kg^{-1}$范围内以剂量依赖方式加剧谷氨酸单钠诱导的下丘脑弓状核神经元损伤，谷氨酸单钠诱导的线粒体膜蛋白结合钙增多可以被β-内啡肽 2 $g \cdot L^{-1}$加强，谷氨酸单钠诱导的单细胞内钙浓度升高也被β-内啡肽 2 $g \cdot L^{-1}$从 320 ± 84 提高至 589 ± 78 $nmol \cdot L^{-1}$．**结论**：β-内啡肽以剂量依赖方式通过加剧胞内钙自稳态失衡来增强谷氨酸单钠诱导的神经毒性．

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