301-304

Pharmacol Biochem Behav 1994; 48: 689－92.

11 Domino EF, Yao L, Sheng JG, Grekin RJ, Malvin RL. Is nitric oxide involved in mediating rat open field behavior? Pharmacol Commun 1993; 3: 175－81.

12 Morley JE, Flood JF. Evidence that nitric oxide modulates food intake in mice. Life Sci 1991; 49: 707－11.

13 Morley JE, Farr SA, Suarez MD, Flood JF. Nitric oxide synthase inhibition and food intake: effects on motivation to eat and in female mice.
Pharmacol Biochem Behav 1995; 50: 369－73.

14 Brennan PA, Kishimoto J. Local inhibition of nitric oxide synthase activity in the accessory olfactory bulb does prevent the formation of an olfactory memory in mice. Brain Res 1993; 619: 306－12.

Nω-硝基-L-精氨酸对大鼠八臂迷宫工作记忆的作用

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目的：研究一氧化氮合成酶抑制剂 Nω-硝基-L-精氨酸(NNA)对大鼠空间工作记忆的作用. **方法**：采用八臂迷宫延迟插板的程序. **结果**：腹腔注射 NNA 100 mg kg^{-1}对大鼠八臂迷宫选择的准确性没有显著影响，只能增加反应的潜伏期. 东莨菪碱0.25 mg kg^{-1}使大鼠延迟后的错误选择显著增加. 脑室内注射 NNA (10, 50, 100 nmol/1 μL)没有影响准确性. **结论**：急性 NNA 给予对大鼠空间工作记忆的形成和使用没有显著影响.

关键词 记忆；迷宫学习；Nω-硝基-L-精氨酸

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Protection of methylflavonolamine against acute cerebral ischemia reperfusion injury in rats

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AIM: To examine the possible beneficial action of methylflavonolamine (MFA) on cerebral ischemia/reperfusion injury. **METHODS**: Acute cerebral ischemia-reperfusion injury was produced by 4-vessel occlusion and subsequent 1-h release. MFA, 20 mg kg^{-1}, was injected intravenously 5 min before occlusion and again before release. **RESULTS**: The brain water content in the reperfusion group (Rep) was elevated (82.7 %±1.1 % *vs* control 79.7 %±0.5 %, $P<0.01$), while MFA alleviated the brain edema (80.9 %±0.9 % *vs* Rep, $P<0.01$). The CK level of brain tissue in Rep decreased (4.7±1.4 *vs* control 8.4±1.2 U/mg protein, $P<0.01$), but MFA restored it (7.2±1.1 U/mg protein *vs* Rep, $P<0.01$). Reperfusion caused the rise of lipid peroxides (2.3±0.5 *vs* control 1.5±0.4 nmol/mg protein, $P<0.01$) and weakened the superoxide dismutase (SOD) (3.1±1.6 *vs* control 10.5±3.9 U/mg protein $P<0.01$), MFA reduced the rise of lipid peroxides (1.6±0.4 nmol/mg protein *vs* Rep, $P<0.05$) and protected the activity of SOD (7.9±1.6 U/mg protein *vs* Rep, $P<0.01$) in brain. **CONCLUSION**: MFA has the protective effects on cerebral ischemia/reperfusion,

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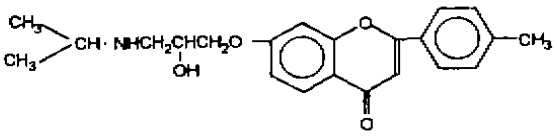
and these effects are relative to scavenging free radicals and anti-lipid peroxidation.

KEY WORDS methylflavonolamine; cerebral ischemia; reperfusion injury; brain edema; creatine kinase; free radicals; superoxide dismutase; lipid peroxides

Methylflavonolamine [4'-methyl-7-(2-hydroxy-3-isopropylaminopropoxy)-flavone hydrochloride, MFA] protects ischemia-reperfusion injury in heart[1], prevents arrhythmia[2], has the effect of calcium antagonist[3], and the effect of anti-lipid peroxidation[2]. Similarly, postischemic brain damage is closely associated with lipid peroxidation[4-7]. The drugs with anti-lipid peroxidation effect such as 21-aminosteroids and ginsenosides, have also protective effect on cerebral ischemia-reperfusion injury[4,6]. The present investigation is to examine the possible beneficial action of MFA on postischemic brain damage.

MATERIALS AND METHODS

MFA was a white powder which was odorless, bitterish, and soluble in water, alcohol, ethyl ether, and Me_2SO. MFA was provided by Shanghai Institute of Pharmaceutical Industry, and its solution (0.8 %) was prepared in distilled water immediately before use. CK-NAC kits were from Zhongsheng Corporation, Biophysical Institute, Chinese Academy of Sciences.



Methylflavonolamide, M_r 367

The 4-vessel occlusion was made on Wistar rats ($n = 21$) of either sex, weighing 230 ± 30 g. Rats were anesthetized with urethane (1 g kg^{-1}, ip) and both common carotid arteries (CCA) were separated. In order to find the alar foramina of the first cervical vertebra, the head was positioned on stereotactic ear bars and tilted at approximately 30° to the horizontal line, while its tail was anchored to stretch the spine gently[8]. The initial electroencephalogram (EEG) was recorded by a physiological recorder (SJ-42, China) with a silver electrode on the skull 1 cm left to the midline in front of coronary suture and another one on the midline over the frontal sinus. The first alar foramina were cauterized until the perimeter of the foramen and the little bone bridge underneath it were seen. After 24 h, both CCA were clamped with atraumatic arterial clamps. The EEG was recorded again when the rat calmed down. The amplitude of EEG should be decreased to 25 %[9] and the righting reflex should be lost, but the spontaneous breath should be maintained. After 45 min of ischemia, the clamps were removed to reperfuse for 1 h.

Rats ($n = 21$) were randomly divided into 3 groups: 1) Sham operation; 2) Ischemia for 45 min and reperfusion for 1 h; 3) Reperfusion+MFA 20 mg kg^{-1}, iv 5 min before occlusion and again before reperfusion. After reperfusion, rats were killed by cervical dislocation and the brain was stored at −30 ℃. About 0.1 g forebrain tissue was put into distilled water 1.9 mL to make a 5 % homogenate, which was centrifuged at $3500 \times g$ for 15 min. CK was determined by CK-NAC kits, SOD by pyrogallol autoxidation[10], lipid peroxides by thiobarbituric acid reaction[11], and protein by colorimetric method[12]. The water content of the brain was estimated according to dry-wet weight method[13].

The statistical significance of the results was determined by ANOVA and the water contents were analyzed after transformation of square root and inverse sine.

RESULTS

The brain water content in the reperfusion group was elevated *vs* control, while MFA suppressed its increase, though it was still higher than that in control group (Tab 1).

The CK level of brain tissue in reperfusion group decreased (Tab 1), but MFA restored it.

During ischemia/reperfusion the SOD activity was weakened while MDA content increased greatly. MFA enhanced the SOD activity while reduced the rise of MDA and made them close to that in the control group.

Tab 1. Effects of methylflavonolamine (MFA, 40 mg kg^{-1}, iv) on brain injury. $n=7$, $\bar{x}\pm s$. $^{c}P<0.01$ *vs* **sham-operation**; $^{a}P<0.05$, $^{f}P<0.01$ *vs* **reperfusion.**

Group	Water content %	CK U/mg protein	SOD U/mg protein	MDA nmol/mg protein
Sham-operation	79.7±0.5	8.4±1.2	10.5±3.9	1.5±0.4
Reperfusion	82.7±1.1^{c}	4.7±1.4^{c}	3.1±1.6^{c}	2.3±0.5^{c}
MFA	80.9±0.9^{f}	7.2±1.1^{f}	7.9±1.6^{f}	1.6±0.4^{a}

DISCUSSION

Effects of MFA on brain were studied for the first time in this experiment though there are many studies on heart[1-3].

4-Vessel occlusion in rat had proven to be a highly reproducible method to achieve reversible but neartotal forebrain ischemia[8,9]. In fact, the key was to find the first alar foramina and cauterize the artery through it thoroughly. Besides that the details such as tilting the head must be noticed, we found the criteria of ischemia was usually met when the little bridge below the first foramen was seen.

The high mortality was the shortcoming of this method[8], which was higher than 50 % in our experiment. In order to decrease mortality, we adopted this model by 2 d to avoid the effect of anesthetic. And when the rats were awake, the righting reflex was also easy to be observed.

In our study, the changed of brain edema and CK release were significant, which reflected the brain damage in reperfusion group, and this was similar to other's reports[6,13]. While MFA suppressed these changes, indicating that MFA had protected the brain tissue during ischemia/reperfusion.

Meanwhile, the decrease of SOD activity and increase of MDA during reperfusion in our study confirmed the view that the free radicals played an important role in reperfusion injury[4,5]. MFA protected SOD and prevented the lipid peroxidation, suggested the above protection was relative to suppressing free radicals.

Under our present circumstances, CK type was not measured in our study. However the CK that we measured was directly taken from forebrain tissue and mainly reflected the CK level of forebrain tissue. Certainly, it would be more convincible to measure the CKBB.

MFA is also a typical calcium antagonist[3]. Calcium overloading is another important role besides free radicals in reperfusion injury, and these are mutually promoting[14]. Then, the above protection of MFA may also associate with calcium antagonism, but it is not clarified which is the main factor.

Taken altogether, our results suggest that MFA has the protective effects on cerebral ischemia/reperfusion, and these effects are relative to scavenging free radicals and anti-lipid peroxidation.

REFERENCES

1 Yang LZ. Dun W, Zhou EF. Protective effects of methylflavonolamine on ischemia-reperfused injury in isolated working rabbit hearts. Chin J Pharmacol Toxicol 1991; **5**: 21—3.

2 Dun W. Yang LZ, Zhou EF, Liang YQ. Anti-arrhythmia and anti-lipid peroxidation effects of methylflavonolamine. Acta Pharmacol Sin 1991; **12**: 177—80.

3 Zhang MS, Zhou EF. Methylflavonolamine hydro-

chloride inhibits contractions induced by noradrenaline, calcium and potassium in rabbit isolated aortic strips. Br J Pharmacol 1988; **94**: 1184－8.

4 Schmidley JW. Free radicals in central nervous system ischemia. Stroke 1990; **21**: 1086－90.

5 Demopoulos HB, Flamm ES, Pietronigro DD, Seligman ML. The free radical pathology and the microcirculation in the major central nervous system disorders. Acta Physiol Scand 1980; Suppl **492**: 91－199.

6 Chu GX, Chen X. Protective effect of ginsenosides on acute cerebral ischemia/reperfusion injury of rats. Chin J Pharmacol Toxicol 1989; **3**: 18－22.

7 Bromont C, Marie C, Bralet J. Increased lipid peroxidation in vulnerable brain regions after transient forebrain ischemia in rats. Stroke 1989; **20**: 918－24.

8 Pulsinelli WA, Buchan AM. The four-vessel occlusion rat model: Method for complete occlusion of vertebral arteries and control of collateral circulation. Stroke 1988; **19**: 913－4.

9 Zhao WD, Wang ZC, Li LX, Zhou FM. Investigation on the model of rat cerebral reperfusion syndrome. Chin J Nerv Ment Dis 1987; **13**: 193－5.

10 Yuan QS, Wang ZY, Weng QQ, Hu Y, Wu HF, Chen BX. Assay of superoxide dismutase by pyrogallol autoxidation method. Pharm Ind 1983; (1): 16－9.

11 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; **95**: 351－8.

12 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; **193**: 265－8.

13 Young W, Rappaport ZH, Chalif DJ, Flamm ES. Regional brain sodium, potassium, and water changes in the rat middle cerebral artery occlusion model of ischemia. Stroke 1987; **18**: 751－9.

14 Koller PT, Bergmann SR. Reduction of lipid peroxidation in reperfused isolated rabbit hearts by diltiazem. Circ Res 1989; **65**: 838－46.

甲基黄酮醇胺对大鼠急性脑缺血再灌注损伤的保护

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目的：研究 MFA 对大鼠脑缺血再灌注损伤的作用。**方法**：用四动脉结扎法造成大鼠急性全脑缺血再灌损伤，MFA 在缺血和再灌前 5 min 分别 iv 20 mg kg^{-1}。**结果**：再灌组的脑水份含量显著增高（82.7 %±1.1 % *vs* 对照组 79.7 %±0.5 %；$P<0.01$），MFA 能抑制这一水肿（80.9 %±0.9 %，*vs* 再灌组 $P<0.01$）。再灌组的 CK 含量下降明显（4.7±1.4 *vs* 对照组 8.4±1.2 U/mg protein，$P<0.01$），MFA 能减少这下降（7.2±1.1 U/mg protein *vs* 再灌组 $P<0.01$）。再灌组能引起脂质过氧化物 MDA 含量的增加（2.3±0.5 *vs* 对照组 1.5±0.4 nmol/mg protein，$P<0.01$ 和 SOD 的减少（3.1±1.6 *vs* 对照组 10.5±3.9 U/mg protein $P<0.01$），而 MFA 则抑制 MDA 的升高（1.6±0.4 nmol/mg protein *vs* 再灌组 $P<0.05$），同时保护了 SOD 的活性（7.9±1.6 U/mg protein *vs* 再灌组 $P<0.01$）。**结论**：MFA 能保护急性脑缺血再灌损伤，而此作用可能与保护内源性自由基清除系统、抑制脂质过氧化有关。

关键词 甲基黄酮醇胺；脑缺血；再灌注损伤；脑水肿；肌酸激酶；自由基；超氧化物歧化酶；过氧化脂质类