BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Mar; 17 (2): 161 - 163

Effect of anisodine on acute forebrain ischemia-reperfusion damage in rats

XU Wei (*Guangdong Medical College, Zhanjiang 524023, China*)
DENG Yi-Feng (*Food Quality Monitor and Testing Center under Ministry of Agriculture, Zhanjiang 524001, China*)

KEY WORDS anisodine; scopolamine; transient cerebral ischemia; calcium; Evans blue; scopolamine derivatives; cerebral arteries

AIM: To study the protective effect of anisodine (Ani) on acute forebrain ischemia-reperfusion injury in rats. **METHODS**: Both vertebral arteries were occluded by electrocautery. Severe, but transient bilateral cerebral ischemia was produced by clamping both common carotid arteries in rats. Atomic absorption spectrophotometric and spectrophotometric methods were used to determine the contents of calcium and extravasated Evans blue (EB), respectively, remained in forebrain at 60-min recirculation after 30-min ischemia. **RESULTS**: At 60-min recirculation, the brain calcium contents were increased from 112 ± 6 μg/g brain dry weight in control (sham operation) group to 165 ± 7 μg/g brain dry weight with marked increase of EB extravasation. Ani (2.5 mg·kg^{-1}, ip), and scopolamine (Sco, 0.25 mg·kg^{-1}, ip) decreased the elevated calcium and extravasated EB contents. **CONCLUSION**: Ani prevented the brain from ischemia insults through reducing intracellular calcium accumulation resulted from ischemia and reperfusion.

Interest has been centered on the role of calcium in irreversible ischemic brain damage[1-2]. Henbane drugs had action to unspecifically block calcium channel and improve microcirculation in infectious shock[3-5]. Anisodine (Ani), an M-receptor blocking agent, was first isolated from *Scopolia tangutica* in China. Its structure is similar to scopolamine (Sco). Ani was used for ischemic cerebralvascular disease[6]. In the present study, our purpose was to test whether Ani could prevent the brain from injury consequent upon recirculation after temporary cerebral ischemia compared with Sco.

Received 1995-05-25 Accepted 1995-08-30

MATERIALS AND METHODS

Rats Sprague-Dawley rats (n = 40) weighing 327 ± 15 g were obtained from the breedmg colony of Guandong Medical College. Rats were randomized into 4 groups. Group A: sham operation. Groups B - D: ischemia for 30 min and recirculation for 60 mm. Group B was treated with saline. Group C: treated with ip Ani (Chengdu № 1 Pharmaceutical Factory) 0.5 mg · kg^{-1}. Group D: ip Sco (Chengdu № 1 Pharmaceutical Factory) 0.05 mg · kg^{-1}. Saline, Ani, and Sco were injected 3 times before the bilateral carotid arteries occlusions, at the onset and at 30-min recirculation.

The 4-vessel occlusion method[7] Rats were anesthetized with ketamine 150 mg·kg^{-1}, ip. Bilateral vertebral arteries were occluded by electrocautery. Severe, transient bilateral cerebral ischemia was produced by clamping both common carotid arteries for 30 min. Then recirculation was started by removal of the carotid clamps.

EEG recording[8] The EEG monitoring was continuously recorded from stainless steel screws inserted bilaterally in the pariatal bones, with the tips in an extradural position, against a reference inserted in the prefrontal bone. EEG was recorded with LMS-2B double channel physiologic recording system (Chengdu Instrument Factory).

Determination of brain calcium and water contents[9] After experiments, the rats were decapitated and the brain hemisphere were weighed, dried at 100 ℃ for 12 h, and reweighed to determine the water content. Dry samples were digested in nitric acid. The calcium concentrations were analyzed with a Hitachi Z-8100 polarized Zeeman atomic absorption spectophotometer.

Assessment of blood brain barrier (BBB) permeability[10] Evans blue (EB, Fluka) 2 % saline solution 0.2 mL was injected iv 20 min prior to sacrifice. EB binds to albumin which is normally excluded from the brain parachyma by BBB. The extravasated EB remained in the brain was determined with a 756 MC UV-VIS spetrophotometer (Shanghai № 3 Analytical Instrument Factory).

Surgery took about 45 min, after which the rats were left to stabilize for 1 h. Then both carotid arteries were clamped. Clamps were removed after 30 mm and restoration of carotid blood flow was verified by naked eyes. A heating bulb was placed to maintain the body temperature at 37 ℃.

Data were analyzed by t test and Chi-square (χ^2) test.

RESULTS

EEG changes after clamping of bilateral carotid arteries occurred in Group B – D rats. Initially, the EEG became suppressed to isoelectric. After 10 – 20 min of recirculation, a spontaneous EEG gradually reappeared. At 60-min reperfusion, 1/10 rats in Group B, 6/10 rats in Group C, and 6/10 rats in Group D regained their EEG amplitude to 50 % of the pre-ischemia level ($P<0.05$ *vs* Group B).

At 60-min recirculation, the brain calcium and water contents were higher in Groups B, C, and D *vs* Group A (Tab 1), but markedly lower in Groups C and D *vs* Group B.

Tab 1. Effect of anisodine (2.5 mg·kg^{-1}, ip) and scopolamine (0.25 mg·kg^{-1}, ip) on brain calcium, Evans blue and water contents in rats. $n=10$, $\bar{x}\pm s$. $^{c}P<0.01$ *vs* A, $^{f}P<0.01$ *vs* B.

Group		Calcium, μg/g dry brain	Water, %	Evans blue, μg/g wet brain
A	Sham	112 ± 6	69.9 ± 1.2	3.3 ± 0.3
B	SN	165 ± 7^{c}	74.8 ± 1.4^{c}	6.7 ± 0.5^{c}
C	Anisodine	138 ± 8^{f}	70.7 ± 1.2^{f}	3.8 ± 0.3^{f}
D	Scopolamine	141 ± 8^{f}	71.1 ± 1.3^{f}	4.1 ± 0.4^{f}

The brain EB content was higher in Groups B, C, and D than that in Group A (Tab 1), indicating an increased permeability of BBB. The brain EB content was markedly lower in Groups C and D than that in Group B, indicating that Ani and Sco could partially prevent BBB from damage induced by ischemia-reperfusion.

DISCUSSION

This study has shown that during reperfusion period, in comparision between Group B, C, and D, the lower brain calcium contents, the better EEG recovery; the lower brain calcium contents, the slighter the BBB damage. These results supported the viewpoint[11] that cerebral ischemia-reperfusion injuries were closely related to a precipitous influx of Ca^{2+} from the extracellular to the intracellular compartment, as a consequence, intracellular calcium increase, and demonstrated that Ani and Sco have calcium-antagonist effect to protect forebrain from ischemia-reperfusion damage, this results was agreed with previous report[12].

According to clinic report[6], that the low dosage of Ani was administered orally at several times, which could exert the same pharmacological action as the high dosage of Ani could treated at one time, and decreased side effects. Therefore, in this experiment, Ani (0.5 mg · kg^{-1}) and Sco (0.05 mg · kg^{-1}) was injected ip at three times. The dosage of treated Ani was 10-fold as high as that of treated Sco, this was due to the lipophilic difference of the two compounds.

There have been many research reports about henbane drugs, most of them were concerned over anisodamine and Sco pharmacological mechanisms[3,4,13], which, in generally, were to promoted erythrocyte membrane Na^+/K^+ ATPase activity, antilipid peroxidation, unspecifically block calcium channel, favorably altering the regional cerebral blood flow, inhibits thromboxane synthesis, granulocyte aggregation, and platelet aggregation. Ani is one of henbane drugs, therefore, all previously combined pharmacological machanisms of anisodamine could be used to explain the protective effect of Ani on acute forebrain ischemia-reperfusion damage.

REFERENCES

1 Djuricic B, Rohn G, Paschen W, Hossmann KA Calcium activity and post-ischemic suppression of protein synthesis. Experientia 1995; **51**: 245 – 8.

2 Sakaki T, Tsujimoto S, Sasarka Y, Tsunoda S. The effect of a new calcium antagonist, TA3090 (clentazem) on experimental transient focal cerebral ischemia in cats. Stroke 1993; **24**: 872 – 8.

3 Ren DY, KE J, Weng SA Effects of henbane drugs on electrophysiological and mechanical properties of guinea pig papillary muscles. Acta Pharmacol Sin 1987; **8**: 131 – 5.

4 Liu YB, Ke J, Weng SA, Zhang GQ Effects of henbane drugs on isolated rabbit cerebral vascular mesentric vascular smooth muscles and papillary muscles. Chin J Pharmacol Toxicol 1988; **2**: 148 – 50.

5 Zhu SH, Fang HS, Gao HY. Anisodamine in compreheosive treatment of fulminant epidemic meningitisits — its effect and mechanism of action. Natl Med J China 1983; **63**: 257 – 60.

6 Chen BJ, Lu QX, Yan GH. The inhibitive effect of anisodine on platelet aggregation and adhesion Chin J Neurol Psychiatry 1987; **20**: 161 – 3.

7 Pulsinelli WA, Brierley JB. A new model of bilateral hemi-

161-163

sphere ischemia in the unanesthetized rat.
Stroke 1979; 10: 267 - 72.

8 Mabe H, Nagai H, Takagi T, Umemura S, Ohno M.
Effect of nimodipine on cerebral functional and metabolic recovery following ischemia in the rat brain
Stroke 1986; 17: 501 5.

9 Bradbury MWB, Kleeman CR, Bagdoyan H, Berberian A.
The calcium and magnesium content of skeleral muscle, brain, and cerebrospinal fluid as determined by atomic absorption flame photometry J Lab Clin Med 1968; 71: 884 - 92.

10 Doczi T, Joo F, Sonkodi S, Adam G.
Increased vulnerability of the blood-brain barrier to experimental subarachnoid hemorrhage in spontaneously hypertensive rat.
Stroke 1986; 17: 498 - 501.

11 Degraba TJ, Ostrow PT, Grotta JC.
Threshold of calcium disturbances after focal cerebral ischemia in rats: implications of the window of therapeutic opportunity.
Stroke 1993; 24: 1212 - 7

12 Peng XQ, Ke J Effects of 3 henbane drugs on acute forebrain ischemia and reperfusion injury in rats.
Acta Pharmacol Sin 1992; 13: 357 - 8.

13 Xiu RJ, Hammerschidt DE, Coppo PA, Jacob HS.
Anisodamine inhibits thromboxane sythesis, granulocyte aggregation, and platelet aggregation. A possible mechanism for its efficacy in bacteremic shock.
J Am Med Assoc 1982; 274: 1458 - 60.

樟柳碱对大鼠脑缺血再灌注损伤的影响

徐 伟 (广东医学院药理教研室, 湛江 524023, 中国)
邓亦峰
(农业部食品监督检验测试中心, 湛江 524001, 中国)

关键词 樟柳碱; 东莨菪碱; 暂时性脑缺血; 钙; 伊文思蓝; 东莨菪碱衍生物; 脑动脉 再灌注

目的: 研究樟柳碱对大鼠急性脑缺血及再灌注损伤的影响. **方法**: 电灼闭塞锥动脉并夹闭颈动脉, 使大鼠前脑缺血 30 min, 放开双侧颈总动脉重灌 60 min, 并在重灌 40 min 时 iv 2 % 伊文思蓝 0.2 mL. 分别用原子吸收分光光度法, 分光光度法测定前脑钙含量和伊文思蓝含量. **结果**: 缺血重灌后, 大鼠脑钙含量由对照的 112 ± 6 μg/g 干重脑增加至 165 ± 7 μg/g 干重脑, 伊文思蓝含量由对照的 3.3 ± 0.3 μg/g 湿重脑增加至 6.7 ± 0.5 μg/g 湿重脑, 樟柳碱, 东莨菪碱可使异常增高的脑钙含量以及伊文思蓝含量明显降低. **结论**: 樟柳碱和东莨菪碱通过降低缺血及重灌引起的脑钙积累, 减轻脑损伤改善脑功能.

R743.3
R971.92