

mRNA. 结果: POMC mRNA 主要表达于弓状核, 而 SHR 表达量大于 WKY. PPD mRNA 表达于海马、下丘脑、中脑中央灰质、孤束核、胸髓. 在齿状回、孤束核、内侧视前区, SHR PPD mRNA

表达量少于 WKY; 在弓状核、胸髓、海马 CA1、CA2、CA3 两者之间无差别. 结论: SHR 弓状核 POMC mRNA 增加和齿状核等 PPD mRNA 减少可能与高血压的发病有关.

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## Melatonin decreases production of hydroxyl radical during cerebral ischemia-reperfusion<sup>1</sup>

LI Xue-Jun, ZHANG Ling-Mei, GU Jing, ZHANG An-Zhong, SUN Feng-Yan<sup>2</sup>

(State Key Laboratory of Medical Neurobiology, Department of Neurobiology, Shanghai Medical University, Shanghai 200032, China)

**KEY WORDS** melatonin; hydroxyl radical; cerebral ischemia; microdialysis

**AIM:** To study the effect of melatonin on hydroxyl radical ( $\cdot\text{OH}$ ) contents during cerebral ischemia-reperfusion in rats. **METHODS:** Ischemia was induced by occluding left lateral middle cerebral artery for 30 min following reperfusion. The salicylate trapping method coupled with ipsilateral striatal microdialysis for measurement of hydroxyl radicals generated during ischemia and reperfusion. **RESULTS:** The contents of dihydroxybenzoic acid (DHBA) were increased at 15 min after ischemia and remained high for 30 min after reperfusion. Melatonin ( $4\text{ mg}\cdot\text{kg}^{-1}$ , sc, 30 min before ischemia) decreased the production of DHBA during ischemia for 16-30 min and reperfusion for 1-30 min. **CONCLUSION:** Melatonin inhibits the production of hydroxyl radicals in rat brain during ischemia and reperfusion.

Tissue injury following cerebral ischemia and reperfusion is mediated by various mechanisms, among which hydroxyl radical ( $\cdot\text{OH}$ )-mediated processes play an important role<sup>[1,2]</sup>. Due to its high reactivity,  $\cdot\text{OH}$  has a very short life and is therefore difficult to be

measured. In the present study, the salicylate trapping method coupled with brain microdialysis<sup>[3]</sup> was used to examine the  $\cdot\text{OH}$  production in a rat model of middle cerebral artery (MCA) ischemia-reperfusion.

Melatonin, *N*-acetyl-5-methoxytryptamine, is a hormone-like substance produced by the pineal gland and by other tissues (such as the gastrointestinal tissue)<sup>[4]</sup>. It modulates, mostly indirectly, the natural rhythms of body functions<sup>[5]</sup> and can prevent oxidant damage *in vivo*<sup>[6,7]</sup>. Because melatonin is a potent scavenger of free radicals<sup>[8,9]</sup>, we further investigated the effect of melatonin on  $\cdot\text{OH}$  formation in this model.

### MATERIALS AND METHODS

**Chemicals** Sodium salicylate, 2, 3-dihydroxybenzoic acid, 2, 5-dihydroxybenzoic acid,  $\text{C}_{210-7}(1s)-(+) -10$  camphor-sulfonic acid of AR grade were purchased from Sigma. Melatonin, AR grade, kindly gifted by Professor XIA Qi-Geng, was manufactured by Shanghai Chemical Reagent Factory. HPLC-grade methanol was purchased from BDH Laboratory (UK).

**Instruments** Waters 510-liquid chromatograph pump, BAS LC-4C electrochemical amperometric detector. The analytical stainless-steel column was  $4.6\text{ mm}\times 150\text{ mm}$  and packed with  $5\text{-}\mu\text{m}$  Lichrosorb RP-18. CMA auto microdialysis instruments and CAM/12 microdialysis probe were purchased from BAS Co (USA).

**Rats** Sprague-Dawley ♂ rats weighing  $242\pm 17\text{ g}$  ( $n=12$ ) were randomized into 2 groups. One was used as control

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<sup>2</sup> Correspondence to Prof SUN Feng-Yan.

Phn: 86-21-6404-1900, ext 2774. Fax: 86-21-6403-9987.

E-mail: fysun@shmu.edu.cn

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(sc injected with vehicle — isotonic NaCl containing 3 % Tween-20), the other was treated with sc 0.4 % melatonin ( $1 \text{ mL} \cdot \text{kg}^{-1}$ ) at 30 min before ischemia.

**Brain ischemia-reperfusion and microdialysis** Rats were anesthetized with 5 % chloral hydrate ( $300 \text{ mg} \cdot \text{kg}^{-1}$ ). A microdialysis probe was stereotaxically implanted in the left lateral striatum (standard: A 1.8 mm, L 2.2 mm, H 5 mm). The probe was perfused with cerebrospinal fluid at flow rate of  $2 \mu\text{L} \cdot \text{min}^{-1}$  by a microinfusion pump. After 30 min, sodium salicylate  $5 \text{ mmol} \cdot \text{L}^{-1}$  was added in the perfusate. Following a 45-min stabilization period, vehicle or melatonin was injected sc. After a 30-min interval, ischemia was induced<sup>(10)</sup>. The occlusion of MCA lasted for 30 min, followed by reperfusion. Samples of microdialysis were collected through an auto CMA perfusion collector at 15-min intervals, from 15 min before injection to 30 min after reperfusion (total 105 min) throughout the pre-injection, pre-ischemia, intra-ischemia and post-ischemia. The rectal temperature of rats was kept close to  $37^\circ\text{C}$  by a warming lamp over the body.

#### Detection of $\cdot\text{OH}$ by salicylate trapping method

Each sample was injected with  $20 \mu\text{L}$  onto the analytical column. The mobile phase consisted of edetic acid  $0.4 \text{ mmol} \cdot \text{L}^{-1}$ ,  $\text{NaH}_2\text{PO}_4$   $0.2 \text{ mol} \cdot \text{L}^{-1}$ ,  $\text{C}_{210-7}(1s)-(+) \cdot 10$  camphorsulfonic acid  $0.7 \text{ mmol} \cdot \text{L}^{-1}$  and 5 % methanol. The flow rate was  $0.6 \text{ mL} \cdot \text{min}^{-1}$ . The compounds were detected with the electrode set at  $+0.7 \text{ V}$  against an  $\text{Ag}/\text{AgCl}$  reference electrode. Calibration curves were made daily with  $20 \mu\text{L}$  of different concentrations of DHBA standards.

**Statistical analysis** DHBA amounts recorded during and after ischemia were compared to pre-injection values. Within each group, differences were analyzed using a paired *t*-test. Differences between groups (melatonin-treated group and vehicle group) were analyzed by two-way group *t*-test.

## RESULTS

Hydroxyl radical level was elevated during cerebral ischemia and reperfusion. DHBA concentrations in the group of 15 min after ischemia were higher than that in control group, which persisted for 30 min after reperfusion (Fig 1, Tab 1).

The production of DHBA in ischemia and reperfusion was decreased by melatonin ( $P < 0.05$ ) during ischemia 16–30 min, reperfusion 1–15 min and reperfusion 16–30 min (Tab 1).

## DISCUSSION

In the present system of salicylate trapping method coupled with microdialysis, melatonin was found, for the first time, to suppress the formation of  $\cdot\text{OH}$  in rat

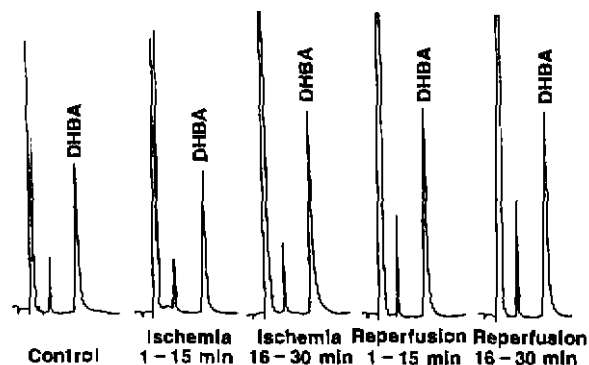


Fig 1. HPLC of DHBA content changes in perfusate during cerebral ischemia and reperfusion.

Tab 1. DHBA contents in perfusate ( $\text{mg} \cdot \text{L}^{-1}$ ) after sc melatonin ( $4 \text{ mg} \cdot \text{kg}^{-1}$ , 30 min before ischemia). <sup>a</sup> $P > 0.05$ , <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs pre-injection; <sup>a</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$  vs vehicle.

	Ratios of DHBA contents to pre-injection	
	Vehicle ( $n = 7$ )	Melatonin ( $n = 5$ )
Pre-injection	1 ( $102 \pm 41 \text{ mg} \cdot \text{L}^{-1}$ )	1 ( $98 \pm 40 \text{ mg} \cdot \text{L}^{-1}$ )
Injection	$1.12 \pm 0.23^a$	$1.01 \pm 0.05^a$
Ischemia		
1–15 min	$1.28 \pm 0.31^a$	$1.18 \pm 0.22^a$
16–30 min	$1.38 \pm 0.26^b$	$1.04 \pm 0.10^{bc}$
Reperfusion		
1–15 min	$1.67 \pm 0.34^c$	$1.03 \pm 0.06^{df}$
16–30 min	$1.36 \pm 0.20^c$	$0.96 \pm 0.18^{df}$

brain during ischemia and reperfusion.

A great deal of free radicals are formed during ischemia and reperfusion, among which hydroxyl radicals are formed mainly through Fenton reaction and NO mechanism<sup>(2)</sup>. Hydroxyl radicals are most reactive, toxic, they play a central role in neural damage during cerebral ischemia and reperfusion. As we mentioned before, melatonin has been found to be a potent scavenger of free radical and can prevent hepatic oxidative damage *in vivo*, which was suggested to relate to the induction of brain glutathione peroxidase<sup>(8)</sup>. Because of its high lipophilicity and diffusibility, melatonin easily enters cells and gains access to all subcellular compartments. While the effect of other traditional oxygen radical scavengers such as superoxide dismutase and catalase may be

limited by their inability to cross the blood-brain barrier. Therefore, we observed the effect of melatonin on the formation of ·OH in rat brain with a temporary MCA occlusion.

The present results further confirmed that melatonin is a potent ·OH quencher *in vivo*. Melatonin is non-toxic and well-tolerated, it easily enters the brain and neurons, all these properties offer a possibility that melatonin could be a practical means for treating the subsequent neural damage following cerebral ischemia and reperfusion.

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褪黑激素降低脑缺血再灌注中羟自由基的生成<sup>1</sup>

李学君, 张玲妹, 顾靖, 张安中, 孙凤艳<sup>2</sup>  
(上海医科大学神经生物学教研室, 医学神经生物学国家重点实验室, 上海 200032, 中国)

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关键词 褪黑激素; 羟自由基; 脑缺血; 微透析

目的: 研究褪黑激素(melatonin)对大鼠脑缺血再灌注中羟自由基生成的影响。方法: 采用栓线法阻塞左侧 MCA 30 min 再灌注模型。通过水杨酸捕获法与微透析技术结合来观察缺血再灌中羟自由基含量的变化。结果: DHBA 水平在缺血 15 min 后显著升高, 持续到再灌注后 30 min 仍维持较高水平。缺血前 30 min 给予 melatonin (4 mg·kg<sup>-1</sup>, sc)显著降低缺血 16 - 30 min 及再灌注 1 - 30 min 时 DHBA 的含量。结论: Melatonin 对大鼠缺血再灌中羟自由基的产生有抑制作用。

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