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Expression of preproopiomelanocortin mRNA and preprodynorphin mRNA in brain of spontaneously hypertensive rats[1]

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KEY WORDS hypertension; preproopiomelanocortin; dynorphins; messenger RNA; *in situ* hybridization; brain; inbred SHR rats; inbred WKY rats

AIM: To compare the expressions of prepropiomelanocortin (POMC) mRNA and preprodynorphin (PPD) mRNA between 16-wk-old spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar-Kyoto rats (WKY). **METHODS**: The expression of POMC mRNA and PPD mRNA were detected with nonradioactive *in situ* hybridization by digoxigenin-labeled RNA probe. **RESULTS**: POMC mRNA mainly was expressed in arcuate nucleus, compared with WKY, SHR had higher level of POMC mRNA (542). PPD mRNA was found in hippocampus, hypothalamus, central gray, nucleus of the solitary tract (NTS), and thoracic spinal cord (T4 – T6). Compared with WKY, PPD mRNA level of SHR decreased in dentate gyrus (2342), NTS (381), and medial preoptic area (467); no difference was observed in arcuate nucleus (263), thoracic spinal cord (750 – 1800) and CA1, CA2, CA3 of hippocampus (1674, 2014, 2626). **CONCLUSION**: Increase of POMC mRNA in arcuate nucleus and decrease of PPD mRNA in dentate gyrus of SHR may be associated with the genesis of spontaneous hypertension.

Preproopiomelanocortin (POMC) is the precursor of β-endorphin (β-END) and many other bioactive peptides including corticotropin (ACTH), and melanocyte-stimulating hormone (MSH). β-END is a potent agonist for μ-opiate receptor and is involved in central cardiovascular regulation[1,2], and altered content of β-END in pituitary was found in SHR as compared with WKY[3].

Preprodynorphin (PPD) is the precursor of dynorphins which are endogenous opiate agonists predominately for κ subtype. In conscious rats, intracerebroventricular injection (icv) of dynorphin A (1 – 13) produced a dose-related pressor effect while intravenous injection (iv) caused a depressor effect[4]. κ Agonist U-50 488H given icv induced an increase in BP and heart rate (HR) in hypertensive rats and a decrease in normotensive rats[5]. The amount of dynorphin A (1 – 8) in hippocampus and hypothalamus of SHR is lower than that of WKY[3].

These results indicated that opioid system took participation in regulation of cardiovascular activity and was changed in essential hypertension. The aim of this study was to investigate whether the biosynthetic activity of β-endorphinergic and dynorphinergic neurons were altered in case of essential hypertension.

MATERIALS AND METHODS

SHR and WKY rats (♂, aged 16 wk) were purchased from Department of Pharmacology, Second Military Medical University. The pSP64 plasmid was kindly gifted from Dr Jong HONG, National Institute of Environmental Health Sciences, USA. Digoxigenin (Dig) RNA labeling and detecting kits were purchased from Boehringer Corp. The brain sections were prepared[6].

RNA probe label The pSP64 plasmid containing preproopiomelanocortin (POMC) cDNA and preprodynorphin (PPD) cDNA were cut by hind Ⅰ and hind Ⅲ, respectively, at 37 ℃ for 2 h, and then served as the template for synthesis RNA was labeled with Dig at uridinetriphosphate (UTP) via a spacer linked to the steroid hapten digoxigenin (Dig-UTP) according to the procedure described on the kit.

Hybridization and probe detection According to our previous report[6], the sections were washed in PBS, then incubated in hybridization buffer containing Dig-labeled RNA probe 0.5 mg·L^{-1} at 37 ℃ for 16 – 24 h, washed again, and incubated in 1 % normal goat serum and 0.3 % Triton X-100 with antibody against Dig alkaline phosphate conjugate (dilution 1:500) at 37 ℃ for 2 h. After washed in PBS, sections were incubated in color-substrate solution at 20 ℃ for 6 – 12 h, then the reaction was ended in Tris-HCl-edetic-acid. Sections were mounted on clean slides, air-dried and cover-slipped. Positive

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neurons were identified by the insoluble blue precipitates in the cytoplasm.

Controls The controls were performed as described in our previous report[6].

Statistics The integral absorbance of the neurons as a measure of the level of POMC mRNA or PPD mRNA expression was measured using Quantimet 570 image analyzer. Three sections of each sample from 5 rats were determined and analyzed by t test between SHR and WKY.

RESULTS

That no precipitate was seen in the controls indicated that the blue predipitates in cytoplasm concerned the specific binding of RNA probe to the tissue mRNA.

POMC mRNA was mostly expressed in arcuate nucleus of hypothalamus in both SHR and WKY. In cortex, such as piriform cortex, there was also few POMC mRNA existed. Compared with WKY, SHR had higher expression of POMC mRNA in arcuate nucleus (Fig 1A, 1B, Plate 1, Tab 1).

Tab 1. Absorbance of POMC mRNA or PPD mRNA positive neurons in SHR and WKY. $n = 5$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **WKY.**

Anatomical regions	Absorbance SHR	WKY
	POMC mRNA	
Arcuate nucleus	542 ± 254[c]	64 ± 37
	PPD mRAN	
Hypothalamus		
arcuate nucleus	263 ± 4[a]	304 ± 199
medial preoptic area	467 ± 126[b]	3 670 ± 1 107
ventromedial nucleus		
dorsal part	1 226 ± 761[a]	1 833 ± 555
ventral part	832 ± 471[c]	1 618 ± 373
Hippocampus		
dentate gyrus	2 342 ± 387[c]	3 912 ± 425
CA1	1 674 ± 404[a]	1 581 ± 188
CA2	2 014 ± 147[a]	2 458 ± 394
CA3	2 626 ± 582[a]	2 582 ± 370
Mesencephalic central gray		
dorsal area	391 ± 384[a]	410 ± 433
ventrolateral area	630 ± 293[a]	824 ± 632
Solitary tract nucleus	381 ± 252[b]	936 ± 547
Thoracic spinal cord (T4 – T6)		
dorsal horn	1 073 ± 563[a]	1 280 ± 327
ventral horn	1 786 ± 108[a]	1 763 ± 219
lateral part of intermediate zone	759 ± 260[a]	1 050 ± 183

In SHR, the highest level of PPD mRNA (absorbance > 2000) were seen in dentate gyrus and CA2, CA3 of hippocampus. Moderate level of PPD mRNA (absorbance: 1000 – 2000) was found in CA1 of hippocampus, ventral and dorsal horn of thoracic spinal cord (T4 – T6), dorsal part of ventromedial hypothalamic nucleus. But in arcuate nucleus, ventral part of ventromedial hypothalamic nucleus, medial preoptic area (MPO), nucleus of solitary tract (NTS), central gray (dorsal, ventrolateral), lateral part of intermediate zone of thoracic spinal cord (T4 – T6), there were less PPD mRNA expressed (absorbance < 1000) (Tab 1).

In dentate gyrus (Fig 1C, 1D), NTS (Fig 1E, 1F), ventral part of ventromedial hypothalamic nucleus and MPO, SHR showed lower expression of PPD mRNA. But in the other nucleus examined, such as central gray, thoracic spinal cord, arcuate nucleus and CA1, CA2, CA3 of hippocampus, no different expression of PPD mRNA was found between SHR and WKY.

DISCUSSION

The present study showed predominant POMC mRNA expression in arcuate nucleus, and the expression in SHR was higher than that in WKY. Anatomical evidence obtained from immunohistochemical studies has demonstrated that β-endorphinergic neurons are mostly located at the arcuate nucleus of hypothalamus[7]. Paraventricular hypothalamic nucleus (PVN) is a major terminal field for β-endorphinergic neurons[8], and microinjecting β-END into PVN induced a greater dose-independent increase in BP and HR in SHR than those in WKY[9]. In the meanwhile, SHR had higher level of β-END in the neurointermediate lobe of pituitary[3], where is the processing site for β-END. Thus, the higher POMC mRNA expression in arcuate nucleus of SHR may be ascribed to the increase of biosynthetic activity of β-endorphinergic neurons.

Our study found lower PPD mRNA expression in dentate gyrus, while no changes in CA1, CA2, CA3 of hippocampus of SHR compared with WKY. Dynorphin is another important peptide in cardiovascular regulation and its central cardiovascular effect, especially in hippocampus, has been reported

by several studies[10,11]. Intrahippocampal injections of dynorphin A (1-8) caused dose-dependent decrease in BP in SHR and WKY, that could be blocked by the selective κ antagonist norbinaltorphimine[10]. Lower dynorphin A (1-8) content was found in SHR hippocampus in the development of hypertension compared with WKY[3]. Moreover, dentate granule cells are the sole source of prodynorphin-derived peptides in the hippocampal formation, and is considered as a central cardioregulatory site in the rat[12]. Therefore, our findings indicated that the biosynthetic activity of dynorphinergic neurons in dentate gyrus were decreased in the case of hypertension in SHR.

NTS and MPO play an essential role in cardiovascular regulation. Our study observed SHR had lower PPD mRNA expression in these sites. Early researches reported that microinjecting dynorphin A (1-13) into MPO or NTS induced hypotension[13,14], and lower dynorphin A (1-8) levels was seen in hypothalamus of SHR[3]. Together with our results, it suggested that the biosynthetic activity of dynorphinergic neurons in these two sites might also be decreased in hypertension.

In summary, the POMC mRNA expression increased in arcuate nucleus and PPD mRNA expression decreased in dentate gyrus and NTS of SHR, these results indicated that the biosynthetic activity of β-endorphinergic neurons increased and that of dynorphinergic neurons decreased in hypertension.

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前阿黑皮原和强啡肽原基因在自发性高血压大鼠脑内的表达[1]

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关键词 高血压；前阿黑皮原；强啡肽类；信使 RNA；原位杂交；脑；近交 SHR 大鼠；近交 WKY 大鼠

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目的：检测前阿黑皮原（POMC）和强啡肽原（PPD）基因在高血压（SHR）和同龄 Wistar-Kyoto 大鼠（WKY）中的表达。**方法**：用地高辛（DIG）标记的 RNA 探针进行原位杂交检测 POMC mRNA 和 PPD

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[1]

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KEY WORDS melatonin; hydroxyl radical; cerebral ischemia; microdialysis

AIM: To study the effect of melatonin on hydroxyl radical (·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 mg·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 (·OH)-mediated processes play an important role[1,2]. Due to its high reactivity, ·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[3] was used to examine the ·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)[4]. It modulates, mostly indirectly, the natural rhythms of body functions[5] and can prevent oxidant damage *in vivo*[6,7]. Because melatonin is a potent scavenger of free radicals[8,9], we further investigated the effect of melatonin on ·OH formation in this model.

MATERIALS AND METHODS

Chemicals Sodium salicylate, 2, 3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, C_{210}-7(1s)-(+)-10 camphorsulfonic 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 mm × 150 mm and packed with 5-μm Lichrosorb RP-18. CMA auto microdialysis instruments and CAM/12 microdialysis probe were purchased from BAS Co (USA).

Rats Sprague-Dawley ♂ rats weighing 242 ± *s* 17 g (n = 12) were randomized into 2 groups. One was used as control

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