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力学；二氢吡啶类

目的：研究间硝苯地平剂量效应的药代动力学. **方法**：兔被随机分成三组，分别静脉注射高、中、低(0.5, 1, 2 mg·kg^{-1})三种剂量的间硝苯地平，用 HPLC 法测定血浆药物浓度. **结果**：间硝苯地平的血药浓度和时间数据经拟合均符合二室模型，主要药动学参数如下(以剂量 1 mg·kg^{-1}为例)：V_d = 0.37 L·kg^{-1}，$T_{\frac{1}{2}\alpha}$ = 6.4 min，$T_{\frac{1}{2}\beta}$ = 84.1 min，AUC = 94.1 mg·min·L^{-1}，Cl = 0.65 L·kg^{-1}·h^{-1}. 各剂量组间的 Cl，$T_{\frac{1}{2}\beta}$经方差分析无显著差异，用单位体重的曲线下面积对剂量进行线性回归存在显著正相关. **结论**：间硝苯地平分布广，消除也迅速；在剂量 0.5－2 mg·kg^{-1}范围内消除动力学呈非剂量依赖性关系.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Mar; 17 (2): 111 - 114

Mapping of preproenkephalin mRNA in brain of spontaneously hypertensive rats[1]

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

AIM: To detect different expression of preproenkephalin mRNA (PPE mRNA) in 16-wk-old spontaneously hypertensive rat (SHR) and age-matched normotensive Wistar-Kyoto rat (WKY). **METHODS**: Nonradioactive *in situ* hybridization was performed using digoxigenin-labeled RNA probe. **RESULTS**: Compared with WKY rats, PPE mRNA levels of 16-wk-old SHR increased in hypothalamic nuclei (>20), amygdaloid nuclei (>23), ventrolateral central gray (21.2), reticular substantia nigra (21.5), interpeduncular nuclei (>21), nucleus of the solitary tract (30.7), rostroventrolateral reticular nucleus (29.1), gigantocellular reticular nucleus (23.9) and thoracic spinal cord (>30); decreased in dorsal central gray (22.7). No difference was found in compact substantia nigra (22.8), dentate gyrus (26.2) and CA1, CA2, CA3 of hippocampus (>25). **CONCLUSION**: PPE mRNA in brain regions involved in modulation of blood pressure may be associated with the genesis of spontaneous hypertension in SHR.

[1] Project supported by the National Natural Science Foundation of China, No 39370235.
Received 1994-11-21 Accepted 1995-03-02

Enkephalin, an endogenous ligand of opioid receptors, is important in the regulation of blood pressure (BP). Intracerebroventricular injection (icv) of μ agonist [D-Ala^2-MePhe^4-Gly^5-ol]-enkephalin (DAGO) and δ agonist [D-Ala^2, D-Leu^5]-enkephalin (DADLE) increased the BP[1]. *In situ* hybridization study showed preproenkephalin mRNA was localized in hypothalamic nuclei, hippocampus, NTS, and spinal cord[2], where the cardiovascular regulation took place.

The icv of μ agonist morphiceptin induced a pressor response in SHR but hypotension in WKY rat, and δ agonist Tyr-D-Thr-Gly-Phe-Leu-Thr (DTLET) icv decreased BP in SHR but increased BP in WKY[3]. Compared with WKY rats, SHR had greater concentration of methionine-enkephalin (Met-Enk) in cortex, pons, and medulla[4], but lower Leu-Enk in suprachiasmatic nucleus[5]. These studies imply that opiate system is disturbed in essential hypertension.

The aim of this study is to determine whether the biosynthetic activity of CNS opiates in brain is altered in case of essential hypertension.

MATERIALS AND METHODS

SHR and WKY rats (aged 16 wk, ♂, n = 5, weighing 268 ± s 6 g and 310 ± s 12 g, respectively) were purchased from Department of Pharmacology, Second Military Medical

University. The pSP64 plasmid was from NIH, USA. Digoxigenin (Dig) RNA labeling and detecting kits were purchased from Boehringer Corp.

Tissue preparation Rats were anesthetized with sodium pentobarbital (45 $mg \cdot kg^{-1}$, ip) and then perfused via aorta with 250 mL of 4 % paraformaldehyde in phosphate-buffer saline 0.1 $mol \cdot L^{-1}$ (PBS, pH 7.2). Brain and thoracic spinal cord were immersed in 30 % sucrose-PBS, and cut frozen at 20 μm. The sections were immersed in cryoprotectant solution[6] and stored at −20 ℃ until use.

RNA probe label The pSP64 plasmid (containing PPE DNA fragment of 960 bp) was cut by SacI at 37 ℃ for 2 h and then served as the template for synthesis of RNA by *in vitro* transcription. During transcription, the synthesized RNA was labeled with Dig at uridine-triphosphate (UTP) via a spacer linked to the steroid hapten digoxigenin (Dig-UTP) according to the procedure described on the kit.

Hybridization and probe detection To avoid RNase contamination, all solutions, glassware, plasticware and paint brush were autoclaved.

According to our previous report[7], the sections were moved into PBS, and then into 4 % paraformaldehyde, finally were rinsed in PBS for 1 h. The sections were incubated with proteinase K (2 $mg \cdot L^{-1}$) in Tris-HCl-EDTA (pH 8.0) for 30 min at 37 ℃, and washed in PBS. To decreased nonspecific background hybridization, sections were treated in 0.25 % acetic anhydride and triethanolamine 0.1 $mol \cdot L^{-1}$, and incubated in 5XSSC and 50 % formamide.

The sections were incubated in hybridization buffer containing the labeled RNA probe 0.5 $mg \cdot L^{-1}$ for 16 − 24 h at 37 ℃, then rinsed in 2XSSC for 15 min, incubated in 2XSSC (containing RNAase 20 $mg \cdot L^{-1}$) for 30 min at 25 ℃, and then rinsed sequentially in 2XSSC and 0.5XSSC.

After buffered in Tris buffer (pH 7.5), sections were incubated in 1 % normal goat serum and 0.3 % Triton X-100 in Tris buffer, and then incubated with antibody against Dig alkaline phosphate conjugate (dilution 1∶500) in the abovementioned solution for 2 h at 37 ℃. After washed in PBS, sections were immersed in buffer-3 (pH 9.5) for 2 min, incubated in color-substrate solution for 6 − 12 h at 20 ℃, then the reaction was ended in Tris-HCl-EDTA. Sections were mounted on clean slides, air-dried and cover-slipped. Positive neurons were identified by the insoluble blue precipitates in the cytoplasm.

Controls The following controls were performed (1) adjacent sections were incubated with hybridization buffer without the Dig-labeled RNA probe. (2) Just before the hybridization, the sections were incubated with 5XSSC containing RNase 20 $mg \cdot L^{-1}$ for 30 min at 25 ℃, then were processed for in situ hybridization as above.

Statistics The optical density (OD) of the neurons as a measure of the level of PPE mRNA expression were determined. The OD reported here was OD of neurons substracted that of the background level. Three sections of each sample from 5 rats were determined and analyzed by t test between SHR and WKY rat.

RESULTS

No precipitate was seen in the sections incubated without Dig-labeled probe or pretreated with RNase before hybridization in controls. These negative results indicated that the blue precipitates in cytoplasm concerned the specific binding of RNA probe to the tissue mRNA.

The highest level of PPE mRNA (>30) was seen in neurons in laminae Ⅲ − Ⅹ of thoracic spinal cord, nucleus of the solitary tract (NTS), and piriform cortex of SHR. In SHR, positive neurons in hypothalamic nuclei including arcuate nucleus, ventromedial area and medial preptic area, rostroventrolateral reticular nucleus (RVL), nucleus of horizontal limb diagonal band, dentate gyrus, and CA1, CA2, CA3 of hippocampus showed moderate level of PPE mRNA (25 − 30). However the dorsolateral hypothalamic area, lateral hypothalamic area, amygdalohip area, amygdaloid nuclei (medial, basolateral), mesencephalic central gray (dorsal, ventrolateral), substantia nigra (compact, reticular), interpeduncular nuclei, gigantocellular reticular nucleus showed less PPE mRNA (<25) (Tab 1).

In most nuclei examined, namely hypothalamic nuclei (Fig 1A, 1B, Plate 3), nucleus of horizontal limb diagonal band, amygdalohip area, amygdaloid nuclei, piriform cortex, ventrolateral central gray, reticular substantia nigra, interpeduncular nuclei, NTS (Fig 1C, 1D), RVL, gigantocellular reticular nucleus and thoracic spinal cord, higher levels of PPE mRNA were seen in SHR than those in WKY. But in dorsal central gray, SHR showed lower level of PPE mRNA compared to WKY rat. There were no differences in compact substantia nigra, dentate gyrus, and CA1, CA2, CA3 of hippocampus (Fig 1E, 1F) between SHR and WKY rat (Fig 1).

DISCUSSION

The changes of regional PPE mRNA level in SHR and WKY brain except NTS are similar to the result of Hoegler[8]. Since the total RNA from

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

Anatomical regions	$10^2\times$ Optical density WKY	SHR
Hypothalamus		
arcuate nucleus	21.3±1.0	29.1±0.9^c
dorsomedial nucleus	18.4±1.8	20.9±0.9^b
ventromedial nucleus	21.9±1.2	25.5±0.6^c
lateral area	18.3±1.0	20.3±1.0^b
medial preoptic area	21.8±1.0	27.4±0.8^c
Horizontal limb diagonal band nucleus	21.2±0.8	26.3±1.0^c
Piriform cortex	24.1±1.8	31.9±2.6^c
Amygdalohip area	18.2±0.5	19.4±1.0^b
Amygdaloid		
medial nucleus	21.1±0.8	24.0±2.6^b
basolateral nucleus	21.6±0.8	23.2±0.8^b
Mesencephalic central gray		
dorsal area	24.5±0.8	22.7±0.5^c
ventrolateral area	19.6±0.8	21.2±1.0^b
Substantia nigra		
compact part	24.0±1.0	22.8±0.9^a
reticular part	20.0±0.5	21.5±1.0^b
Interpeduncular nuclei		
lateral nucleus	21.2±1.2	23.6±1.6^b
central nucleus	20.0±1.0	21.6±0.9^b
Dentate gyrus	25.4±1.0	26.2±0.7^a
Hippocampus		
CA1	25.0±0.8	25.2±0.6^a
CA2	25.4±0.8	26.3±0.8^a
CA3	25.4±0.9	26.0±0.8^a
Solitary tract nucleus	27.4±1.3	30.7±1.1^c
Rostroventrolateral reticular nucleus	26.2±1.9	29.1±2.0^b
Gigantocellular reticular nucleus	21.7±0.8	23.9±0.8^c
Thoracic (T4-6) spinal cord		
Laminae III-V	22.0±0.7	30.8±2.0^c
Laminae VII	24.9±1.2	32.4±1.1^c
Laminae VIII	27.7±1.3	33.9±2.1^c
Laminae IX	30.3±1.9	36.0±2.1^c
Laminae X	26.1±1.1	33.8±1.2^c

whole pon and medulla was used in northern blot, the difference of 2 results may arise from the method used to detect PPE mRNA.

Both of hypothalamus and NTS are critical integration center for BP regulation. Higher level of PPE mRNA was found in these regions of SHR. Based on these findings, it is thought that the biosynthesis activity of PPE is increased in SHR, and enhancement of activities of enkephalinergic neurons may induce an increase in release of enkephalin in SHR. It has been found that microinjection of DAGO into preoptic area and [D-Ala^2-Met^5]-enkephalin into NTS caused dose-dependent pressor responses[9,10]. Therefore, higher levels of PPE mRNA in hypothalamus and NTS of SHR is thought to be related to genesis of spontaneous hypertension of SHR. However, in other brain regions of SHR such as amygdaloid, thoracic spinal cord participating in regulation of BP, higher levels of PPE mRNA were also been found in our study, but the relationship between PPE mRNA level in these regions and regulation of BP remains unknown.

In the present study, no differences of PPE mRNA in hippocampus were found between SHR and WKY. There is extensive evidence showing that cardiovascular function of hippocampus is mainly ascribed to dynorphin and receptor[11,12,13]. Taken together with our autoradiographic results[14] that there was not significant change of δ and μ opioid receptors in rat hippocampus of strains, we suppose that hippocampus enkephalin is less important in the genesis of hypertension in SHR.

In conclusion, the PPE mRNA level is increased at most brain regions in SHR as compared with that of WKY rat. These changes of PPE mRNA level are related to spontaneous hypertension of SHR.

ACKNOWLEDGMENTS To Prof HUANG Deng-Kai for his valuable advice in morphological study, and to Mr LU Shi-Duo for his help in preparation of tissue sections.

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前脑啡肽原信使 RNA 在自发性高血压大鼠脑内的区域定位

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关键词 高血压；前脑啡肽；信使 RNA；原位杂交；脑；近交自发性高血压大鼠；近交 WKY 大鼠

目的：检测前脑啡肽原信使 RNA (PPE mRNA)在 16 周自发性高血压大鼠(SHR)和 Wistar-Kyoto 大鼠(WKY)中的不同表达. **方法**：用地高辛标记的 RNA 探针做非放射性原位杂交. **结果**：与 WKY 相比，SHR 的 PPE mRNA 量在下丘脑，中央灰质腹外侧，孤束核，延髓腹外侧网状核，胸髓等处升高；在中央灰质背侧降低；在黑质致密带，齿状回，海马无变化. **结论**：PPE mRNA 在核团中的变化可能与高血压的发生有关.