BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Jan; 16 (1): 58—61

Phencyclidine receptors in brain and spinal cord of spontaneously hypertensive rats aged 4—16 wk[1]

YIN Xia, ZHU Yan—Hua, XU Shao—Fen (*Department of Neurobiology, State Laboratory of Medical Neurobiology, Shanghai Medical University, Shanghai 200032, China*)

AIM: To study the relationship between the density of phencyclidine [1-(1-phenylcyclohexyl)piperidine hydrochloride, Phe] receptor binding sites in brain and thoracic spinal cord (T4-6) and the development of hypertension in spontaneously hypertensive rat (SHR). **METHODS:** The density of Phe binding sites was determined by autoradiography using [^{3}H]Phe in 4-, 8-, 12- and 16-wk-old rats. **RESULTS:** There were fewer Phe binding sites in the hippocampus and dorsal horn of thoracic spinal cord of SHR at 12 and 16 wk ($P < 0.01$), when hypertension has established; while at 4 wk of age, before the development of hypertension, more Phe binding sites were found in SHR. As blood pressure began to rise at 8 wk, SHR had more Phe binding sites in hippocampus *vs* WKY, but no difference was seen between 2 strains in the dorsal horn of thoracic spinal cord. **CONCLUSION:** Phe receptors might be involved in the genesis of SHR hypertension.

KEY WORDS phencyclidine receptors; hypertension; autoradiography; inbred SHR rat; inbred WKY rat

Our previous study[1] showed that the density of phencyclidine (Phe) receptors decreased in several brain regions of SHR at 16 wk, suggesting a possible involvement of Phe receptors in the central modulation of cardiovascular function. The present study was designed to compare Phe receptor number in the brain and spinal cord of spontaneously hypertensive rats (SHR) with those of Wistar-Kyoto (WKY) rats at various ages during the development of hypertension.

MATERIALS AND METHODS

Rats Male SHR and normotensive WKY rats were obtained from Department of Pharmacology, the Second Military Medical University, aged 4, 8, 12 and 16 wk, were used. The body weight of SHR at the above-stated ages were 61±7, 194±7, 212±6, and 276±4 g, respectively; those of WKY were 85±11, 228±12, 278±9, and 328±9 g, respectively. They were individually housed for at least 7 d before the experiment.

Reagents [^{3}H]Phe (900 TBq·mol^{-1}) and Phe were synthesized and labeled by the School of Pharmacy of our University.

Tissue preparation The rats were decapitated. Brains and thoracic spinal cords (T4-6) were dissected immediately on ice-chilled plastic plate, mounted on chucks using 4 % carboxymethyl cellulose (CMC), and frozen in dry ice. Brain sections (25 μm) of various levels were cut at −18 ℃ in a cryostat and thaw-mounted on gelatin/chrome alum-coated slides. Tissue sections were stored at −20 ℃ for up to 24 h before use.

Radio-binding assay [^{3}H]Phe binding was tested according to our previous study[2]. Tissue sections on slides were incubated in Tris-HCl 5, sucrose 50 mmol·L^{-1} (pH 7.4) and [^{3}H]Phe 10 nmol·L^{-1} at 4 ℃ for 1 h as total binding. Non-specific binding was determined in the presence of unlabeled Phe 100 μmol·L^{-1}. After 1 h of incubation, sections were washed sequentially through 6 rinses of ice-cold Tris-HCl 5, sucrose 50 mmol·L^{-1} and bovine serum albumin (BSA) 0.5 % (pH 7.4) and 5 rinses of distilled water.

[1] Supported by the Science Foundation of Chinese Ministry of Public Health, № 0758112, and by the National Natural Science Foundation of China, № 39370235.
Received 1993-07-15 Accepted 1994-08-09

Autoradiography After rinsing, slides were dried under a stream of hot air and then tightly juxtaposed against ^{3}H-sensitive films (Hyperfilm-^{3}H, Amersham) and stored at 4 ℃ for 35 d. The optical density of each autoradiogram was determined with a computerized microdensitometer. Specific bindings were determined as the total bindings minus the nonspecific binding and the background film density. Optical density values in brain regions were converted to receptor densities (pmol/10 mg tissue) using a standard curve which was made from ^{3}H standards exposed to each film.

Statistical test Significances between densities at binding sites were evaluated using the *t* test (groups, comparisons).

RESULTS

At 4 wk of age, before the development of hypertension, more dense Phe binding sites were shown in hippocampus and thoracic spinal cord ($P<0.01$) of SHR compared to those in WKY rat. At 12 or 16 wk, a period that hypertension has clearly manifested, the opposite results were seen in these regions of SHR which showed lower densities of Phe binding sites *vs* WKY rats. However, at 8 wk, an age when hypertension begins to appear and is still progressing, higher Phe binding sites were seen in hippocampus of SHR and no changes in dorsal horn of thoracic spinal cord between SHR and WKY rat. (Tab 1)

The changes in Phe receptor densities with ages occurred also in periaqueductal gray, substantia nigra, striated cortex (17,18 area), ventral horn and lateral part of intermediate zone of thoracic spinal cord. Lower densities of binding sites appeared in these regions of SHR at 16 wk, while higher densities appeared at 4 wk, when compared with those of WKY. At 8 wk, no changes of Phe receptor density were found in these regions except for striated cortex which higher density was seen in SHR rat ($P<0.01$). At 12 wk, only substantia nigra of SHR rat had lower density of Phe receptor as compared to WKY rat ($P<0.01$). (Tab 1)

Tab 1. Autoradiographic densities (pmol/10 mg tissue) of Phe binding sites in brain and thoracic spinal cord of SHR and WKY rats. $n=5$ rats. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* WKY.

Age/wk	WKY	SHR
Hippocampus		
4	1.95±0.98	3.63±0.40^b
8	2.65±0.22	8.05±0.41^c
12	6.32±0.23	2.11±0.15^c
16	3.88±0.16	1.68±0.03^c
Substantia nigra		
4	3.42±0.89	2.84±0.49^a
8	7.03±1.68	5.99±2.22^a
12	8.37±1.25	4.75±0.28^c
16	3.63±0.23	1.12±0.13^c
Periaqueductal gray		
4	5.27±0.42	3.26±0.24^b
8	7.61±0.77	5.82±2.16^a
12	6.57±1.33	4.97±1.40^a
16	2.54±0.10	0.75±0.14^c
Striated cortex (17,18 area)		
4	4.80±0.83	6.56±0.89^b
8	2.92±0.64	10.15±0.04^c
12	6.42±1.42	4.90±0.61^a
16	5.40±0.52	1.18±0.34^c
Dorsal horn (T4-6)		
4	2.03±0.98	8.88±1.19^c
8	7.09±1.82	8.83±1.83^a
12	8.98±1.08	3.75±0.98^c
16	10.07±1.04	3.07±0.78^c
Lateral part of intermediate zone (T4-6)		
4	2.18±0.41	9.01±1.34^c
8	9.63±2.89	10.11±1.68^a
12	4.45±0.74	2.76±0.54^a
16	10.49±0.51	1.52±0.07^c
Ventral horn (T4-6)		
4	1.73±0.13	7.66±0.96^c
8	9.09±2.11	9.13±2.36^a
12	2.55±0.81	3.00±0.45^a
16	8.47±0.49	3.23±0.47^c

DISCUSSION

SHR is considered to be a suitable model for human essential hypertension. Blood pressure (BP) of SHR approaches borderline

hypertensive level at 8 wk, and definitely hypertensive level as early as 12 wk (20 kPa is considered to be hypertensive in SHR)[15]. We and other authors have all previously found that when given icv or intrathecally (ith) Phe cause a fall in BP and heart rate (HR)[4], and the effect of Phe receptor on modulation of central cardiovascular activity was closely related to noradrenaline (NA)[2,3]. Thus, we suggest that altered levels of Phe receptor in our results are possibly important in the development of hypertension in SHR.

There are neural connections from hippocampus to classical cardiovascular centers and changes of opioid peptides and receptor have been seen in hippocampus of SHR[6,7]. Our data showed an increase in the density of Phe binding sites in 4-wk-old SHR compared to age-matched WKY, but the largest increase was seen at 8 wk, an age when hypertension emerges and is still progressing. The transient rise in Phe receptor number in SHR may be due to a up-regulation of Phe receptors owing to the rise of BP. As hypertension continued processing and became more manifest, the up-regulation of receptors might be prevented by certain mechanisms. The same phenomenon has been observed in opioid receptors in SHR[7]. Further research is warranted to elucidate the mechanism of decreased receptor number with increasing age. However, this response was not so apparent in spinal cord. The reason why hippocampus is more sensitive than spinal cord to the change of BP may be the fact that hippocampus belongs to the limbic system, which is important in emotional behavior, and that the SHR has exaggerated responses to stress[5]. Lower affinity for Phe receptors in spinal cord may be another reason for its later changes in the density of Phe binding site.

In conclusion, our results showed lower density of Phe receptor in SHR at 12 and 16 wk when hypertension was seen in SHR, and higher in SHR at 4 wk when hypertension did not begin in SHR. These changes of Phe receptor during the development of hypertension indicated that Phe receptor could play a role in the development of hypertension.

ACKNOWLEDGMENTS To Prof HUANG Deng-Kai for his valuable suggestions and support in morphological study. To Mr LU Shi-Duo for preparation of tissue sections.

REFERENCES

1 Yin X, Zhu YH, Xu SF. Changes of phencyclidine receptor in discrete brain nuclei and spinal cord in spontaneously hypertensive rats. Chin J Physiol Sci 1994; **10**: 175-9.

2 Iams SG, Wexler BC. Inhibition of the development of spontaneous hypertension in SH rats by gonadectomy or estrabdiol. J Lab Clin Med 1979; **94**: 608-16.

3 Yu CX, Wang QP, Xia Y, Xu SF. Relationship between spinal PCP receptor on cardiovascular effect and noradrenergic system. Acta Physiol Sin 1990; **42**: 331-9.

4 Yu CX, Xu SF. Cardiovascular effect mediated by phencyclidine receptor in central nervous system. Acta Physiol Sin 1990; **42**: 45-52.

5 Bayorh MA, Zukowska-Grojec Z, Palkovits M, Kopin IJ. Effect of phencyclidine (PCP) on blood pressure and catecholamine levels in discrete brain nuclei. Brain Res 1984; **321**: 315-8.

6 Li SJ, Hong JS, Ingenito AJ. Hippocampal levels of Dynorphin A(1-8) in neonatal and 16-wk-old spontaneously hypertensive rats: comparisons with DOCA-salt hypertension. Neurochem Res 1990; **15**: 1141-6.

7 McConnaughey MM, Wong SC, Ingenito AJ. Dynorphin receptor changes in hippocampus of the spontaneously hypertensive rats. Pharmacology 1992; **45**: 52-7.

8 Hallback M, Folkow B. Cardiovascular responses to acute mental "stress" in spontaneously hypertensive rats. Acta Physiol Scand 1974; **90**: 684-98.

58-61 11

4—16周龄高血压大鼠脑与脊髓苯环利定受体的变化

R965.2

殷 萱, 朱燕华, 许绍芬 (神经生物学教研室, 神经生物学国家重点实验室, 上海医科大学, 上海 200032, 中国)

高血压 苯环利定受体 放射自显影

目的：研究高血压大鼠(SHR)脑和胸髓中苯环利定受体密度变化与高血压发生的关系. **方法**：4，8，12，16周大鼠中枢中[^{3}H]Phe 标记的苯环利定受体密度用放射自显影法检测. **结果**：高血压稳定期(12，16周)，SHR 海马和胸髓背角中苯环利定受体明显少于非高血压大鼠(WKY)；而血压升高前，4周的 SHR 则相反；8周血压开始上升时，SHR 海马苯环利定受体增加，而在背角无差别. **结论**：苯环利定受体可能参与了 SHR 的发病过程.

关键词 苯环利定受体；高血压；放射自显影术；近交 SHR 大鼠；近交 WKY 大鼠

61-64 12

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Jan; 16 (1): 61—64

双香豆素对癌细胞的光敏反应及二乙基二硫代氨基甲酸钠的增敏作用[1]

周秀芳，杨宣武，郑荣梁[2]（兰州大学生物系，兰州 730000，中国）

R965

Photosensitization of dicoumarol on tumor cells and enhancement by sodium diethyldithiocarbamate[1]

ZHOU Xiu-Fang, YANG Xuan-Wu, ZHENG Rong-Liang[2] (*Department of Biology, Lanzhou University, Lanzhou 730000, China*)

AIM: To study the photosensitization of dicoumarol (Dic) on tumor cells and any effect by sodium diethyldithiocarbamate (DDC). **RESULTS**: Dic, 40, 80, 120 $\mu mol \cdot L^{-1}$ concentration-dependently inhibited the DNA synthesis (81 %—93 %) and increased the mortality (50 %—70.4 %) of ascitic hepatoma (Hep A) cells exposed in light. These 2 indices were changed slightly by Dic alone even in non-illuminating group. DDC enhanced the photosensitization of Dic group. **CONCLUSION**: Photosensitization of Dic showed strong antitumor activities against Hep A cells. The mortality of Hep A and inhibition of the DNA synthesis in Dic-DDC-light group significantly stronger than in Dic-light group. Photosensitization of Dic was not due to $^1O_2$ and $OH^\cdot$, but closely related to O_2^- and H_2O_2.

KEY WORDS bishydroxycoumarin; diethyldithiocarbamate; photosensitizing agents; free radicals; neoplasm DNA

A **目的**：研究双香豆素(Dic)对肿瘤细胞的光敏反应及二乙基二硫代氨基甲酸钠的增敏作用. **结果**：Dic 40，80，120 $\mu mol \cdot L^{-1}$光敏反应使腹水型肝癌细胞(Hep A)死亡率增加(50 % — 70.4 %)，照光组始终高于避光组30 %左右. 照光组细胞 DNA 合成抑制率(81 % — 93 %)明显高于避光组(19 %—53 %)，且有浓度依赖性. 避光组 Dic 对细胞也有轻微的损伤. **结论**：Dic 光敏反应对 Hep A 细胞有较强抗肿瘤活性，DDC 能增强 Dic 对细胞的光敏杀伤作用. Dic-DDC 照光组细胞死亡率及 DNA 合成的抑制率比单独的 Dic 照光组明显增高. Dic 光敏反应与$^1O_2$和 $OH^\cdot$ 无关，而与 H_2O_2与 O_2^- 密切相关.

[1] Project supported by the National Natural Science Foundation of China. № 38970238.

[2] To whom correspondence should be addressed.

Received 1993-11-05 Accepted 1994-05-31

(主题词在下页)