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Different distributions of opioid receptors in spontaneously hypertensive rats and Wistar-Kyoto rats[1]

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AIM: To compare the densities of opioid receptors in spontaneously hypertensive rats (SHR) with those of normotensive Wistar-Kyoto (WKY) rats in central nervous system which are related to the regulation of BP. **METHODS**: [^{3}H]Etorphine, a nonspecific opioid ligand, was used to determine the distributions of opioid receptors in 16-wk-old SHR and WKY rats by quantitative autoradiography. **RESULTS**: The densities of [^{3}H]etorphine in hippocampus ($P<0.01$), periaqueductal gray, nucleus of the solitary tract, and thoracic (T4 — 6) spinal cord ($P<0.05$) of SHR were lower than those of WKY rats. But in basolateral amygdaloid nucleus ($P<0.01$), habenular nuclei ($P<0.05$), and hypothalamic nuclei including arcuate nucleus ($P<0.01$), higher densities of opioid receptors were found in SHR. No difference existed in interpeduncular nuclei between the 2 groups. **CONCLUSION**: The difference in distributions of opioid receptors is related to the hypertension in SHR.

KEY WORDS etorphine; opioid receptors; hypertension; brain; spinal cord; autoradiography; inbred SHR rats; inbred WKY rats

Plenty of evidences suggested that the endogenous opioidergic system of both central and peripheral origin was involved in the regulation of blood pressure (BP)[1,2]. Decreased dynorphin A(1—8)[3] and leucine-enkephalin concentrations[4] were found in several brain regions of SHR. Question arises as to the relationship between the opioid receptors and the elevated BP of essential hypertension.

SHR is a much better model than the normotensive WKY rat for human essential hypertension[5]. The activity of opioid receptor system during altered BP has previously been determined in brain membranes of SHR[6-9], but there were great variations. The saturable binding of [^{3}H]naltrexone in brain of SHR was about twice that in WKY rat[10], but lower [^{3}H]naloxone binding[6] and dynorphin receptor binding sites[7] were found in the hippocampus of SHR. In order to elucidate the difference in the distributions of opioid receptors in SHR and WKY rat, the present investigation was carried out to compare the densities of opioid receptors in SHR to those of WKY rat in the structures of the central nervous system (CNS) which are related to the regulation of BP. The quantitative autoradiography was used to visualize the dynamic pictures of receptor density changes.

MATERIALS AND METHODS

Rats SHR, ♂, 16-wk-old and age-matched WKY rats were obtained from Department of Pharmacology, the Second Military Medical University, and housed individually for at least 7 d before the experiment.

Measurement of BP Systolic BP (SBP) of SHR and WKY rats were monitored by tail cuff method in

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conscious restrained rats using a BP recorder MRS-Ⅱ (Shanghai Institute of Hypertension). To reduce the influence of stress, the BP was measured 3 times on separate days. The data reported here were those taken just before the rats were killed.

Drugs [^{3}H]Etorphine (1.11 PBq mol^{-1}) and nonlabeled etorphine were synthesized and labeled by the School of Pharmacy, Shanghai Medical University.

Tissue preparation The rats were killed by decapitation. The brains and thoracic spinal cords were quickly mounted on chucks using 4 % carboxymethyl cellulose (CMC) and frozen by dry ice. Corresponded to the stereotaxic atlas of Paxinos and Watson (1986), 5 levels of brain and spinal cord were sectioned at 20 μm in a cryostat at −18 ℃. The sections were thaw-mounted on gelatin/chrome alum-coated slides, and stored at −20 ℃ for up to 24 h before use.

Radiobinding assay Tissue sections on slides were incubated with [^{3}H]etorphine 12 nmol L^{-1} in Tris-HCl 50 mmol L^{-1} (pH 7.4) at 25 ℃ for 45 min as total binding, and nonspecific binding was determined in the presence of unlabeled etorphine 20 μmol L^{-1}. Sections were washed sequentially through 6 rinses of ice-cold Tris-HCl 50 mmol L^{-1} (pH 7.4) and 0.5 % bovine serum albumin (BSA) and 5 rinses of cold distilled water, then dried rapidly under a stream of hot air, and stored at 20 ℃.

Autoradiography The sections were tightly juxtaposed against tritium-sensitive films (Hyperfilm-^{3}H, Amersham) exposed at 4 ℃ for 35 d, developed in Kodak D19 at 19 ℃ for 4 min, and fixed for 10 min. A computerized microdensitometer was used to determine the optical density (OD) of each brain region and the standard of tritium microscale. Specific binding was determined by subtraction of background film density as well as the nonspecific binding from total binding. OD values for brain regions were converted to receptor densities according to the standard curve which was delivered from a series of ^{3}H standards exposed to each film.

Statistical analyses Data were analyzed by t test.

RESULTS

The SBP of SHR (28.2±s 0.9 kPa) was higher than that of WKY rat (14.5±s 1.6 kPa) ($P<0.01$), while the body weight of SHR (282±7 g) was lower than that of WKY rat (331±11 g) ($P<0.01$).

Twenty brain areas of SHR were examined, and showed the highest [^{3}H]etorphine binding sites in the basolateral amygdaloid nucleus (2.87 nmol/g tissue) and posterior hypothalamic area (2.77) relatively; high binding (2.0−2.5) existed in habenular nuclei, hippocampus, posterior cingulate cortex, ventrolateral part of periaqueductal gray (PAG) and intermediolateral cell column of thoracic spinal cord (IML); intermediate level of binding (1.0−2.0) was found in 12 of 20 areas examined, including caudate nucleus, hypothalamus nuclei (except for posterior area), interpeduncular nuclei, dorsal PAG, substantia nigra, superior colliculi, nucleus of the solitary tract (NTS), dorsal and ventral horns of thoracic spinal cord; low density was in striated cortex (Str 17, 18) where opioid receptor density was only 6 nmol/g tissue (Tab 1).

Compared with WKY rat, SHR had fewer binding sites of [^{3}H]etorphine in mesencephalic hippocampus (−30 %), PAG (dorsal −18.9 % and ventrolateral part −39.5 %), posterior cingulate cortex (−26.2 %), striated cortex (−66.7 %), substantia nigra (−48.7 %), NTS (−23.9 %), ventral (−19.2 %) and dorsal (−20.6 %) horn of thoracic spinal cord. Controversial results were obtained in basolateral amygdaloid nucleus (+22.3 %), habenular nuclei (+28.3 %), arcuate nucleus (+15.2 %), lateral (+14.2 %) and medial (+11.1 %) preoptic area, lateral (+15.5 %) and posterior (+17.0 %) hypothalamic area, where higher levels of opioid receptors were found in SHR. But in caudate nucleus, interpeducular nuclei, superior colliculi, and IML, there were no significant differences between SHR and WKY rat (Tab 1, Fig 1, Plate 2).

Tab 1. Receptor densities (nmol/g tissue) from autoradiograms of brain sections from SHR and WKY rat. $n=5$ rats. $\bar{x}\pm s$.
$^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* WKY.

Anatomical regions	WKY	SHR
Telencephalon		
Caudate nucleus	1.23±0.27	1.17±0.13^a
Posterior cingulate cortex	2.60±0.43	2.06±0.19^b
Diencephalon		
Basolateral amygdaloid nucleus	2.23±0.23	2.87±0.33^c
Habenular nuclei	1.70±0.30	2.37±0.50^b
Hypothalamus		
Arcuate nucleus	1.50±0.17	1.77±0.19^c
Lateral hypothalamic area	1.63±0.10	1.93±0.13^c
Lateral preoptic area	1.20±0.07	1.40±0.17^b
Medial preoptic area	1.60±0.13	1.80±0.07^c
Posterior area	2.30±0.27	2.77±0.33^b
Mesencephalon		
Hippocampus	2.73±0.27	2.10±0.30^c
Interpeduncular nuclei	1.87±0.20	1.63±0.20^a
Periaqueductal gray		
Dorsal part	1.63±0.20	1.37±0.10^b
Ventrolateral part	2.93±0.33	2.10±0.30^c
Striated cortex	1.00±0.23	0.60±0.10^c
Substantia nigra	2.87±0.43	1.93±0.17^c
Superior colliculus	1.50±0.27	1.60±0.17^a
Medulla oblongata		
Nucleus of the solitary tract	2.07±0.17	1.67±0.30^b
Thoracic (T4−6) spinal cord		
Dorsal horn	1.93±0.30	1.60±0.17^b
Intermediolateral cell column	2.40±0.10	2.43±0.27^a
Ventral horn	2.30±0.33	1.93±0.13^b

DISCUSSION

Hypothalamus is a critical region for modulation of BP. Increased opioid receptors in SHR hypothalamus in our study were in harmony with other observation[11]. These changes could be due to the up-regulation of opioid receptors in response to the low levels of opioid peptides in SHR hypothalamus[3]. It seemed also possible that there might be other unidentified mechanisms of the increased receptor density. However, high level of hypothalamic opioid receptors was thought to be responsible for the hypertension in SHR. When taken together with previous results which showed lower density of dynorphin receptor[7] in the hippocampus of SHR, the decreased opioid receptor in SHR hippocampus in the present report suggested that fluctuation in the level of hippocampal opioid receptors might be significant in the emotional components of central cardiovascular control.

However, there existed a difference between Martucci's report[10] and ours. The differences might result from the homogenation of whole brain which diminished the differences of receptor densities among discrete brain region. On the other hand, etorphine, a non-specific opioid agonist, binds to all types of opioid receptors, it is hard to determine that in certain brain regions which type of opioid receptor predominates. Further investigation is needed to clarify the changes of each subtype of opioid receptors in SHR.

In summary, SHR showed a higher binding density in hypothalamus and amygdala, and a lower one in hippocampus and PAG *vs* WKY rat. Fluctuations of opioid receptors in these regions involved in cardiovascular regulation suggested that opioid receptors might be critical to hypertension in SHR.

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阿片受体在自发性高血压大鼠和正常大鼠中的不同分布

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目的：比较16周自发性高血压大鼠（SHR）和对照组 Wistar-Kyoto（WKY）大鼠中枢神经系统中与血压调节有关的核团内阿片受体密度的变化。**方法**：用放射自显影方法，运用氚标依托啡作配基检测阿片受体的分布。
结果：在海马（$P<0.01$）、中央灰质、孤束核、胸髓（$P<0.05$）几处，SHR 阿片受体密度较 WKY 大鼠低；而在杏仁核（$P<0.01$）、僵核（$P<0.05$）和下丘脑核群包括弓状核（$P<0.01$），SHR 却有较高的阿片受体密度。
结论：不同分布的阿片受体与自发性高血压大鼠的血压有关。

关键词 依托啡；阿片受体；高血压；脑；脊髓；放射自显影；近交 SHR 大鼠；近交 WKY 大鼠