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Distributions of μ and δ opioid receptors in central nervous system of SHR rats and normotensive WKY rats[1]

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KEY WORDS μ opioid receptors; δ opioid receptors; hypertension; autoradiography; inbred SHR rats; inbred WKY rats

AIM: To compare the distributions of opioid receptor subtypes in central nervous system of spontaneously hypertensive rat (SHR) and normotensive Wistar-Kyoto (WKY) rat.
METHODS: [^{3}H] Ohmefentanyl (OMF), [^{3}H] *N*-methyl-*N*-[7-(I-pyrrolidinyl)-1-oxaspiro (4,5)dec-8-yl] benzeneacetamide (U-69593) and [^{3}H]etorphine after suppression of μ and κ-sites by 15 μmol·L^{-1} each of unlabeled OMF and *trans*-(1R,2R)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl]-benzeneacetamide hydrochloride (U-50 488H) were used as ligands for μ, κ, and δ opioid receptor subtypes in autoradiography, respectively. **RESULTS**: δ receptors had an increase in hypothalamic nuclei, periaqueductal gray, caudate and interpeduncular nuclei, and a decrease in substantia nigra in SHR than in those of WKY rat. μ receptors were less concentrated in basolateral amygdaloid nucleus, habenular nuclei and nucleus of solitary tract of SHR than in those of WKY rat. κ receptor density was not checked out in the present study.
CONCLUSION: Distribution of opioid receptor subtypes is related to hypertension of SHR, and δ opioid receptor is more important than μ opioid receptor in the maintenance of hypertension in SHR.

Opioid receptors are implicated in the regulation of blood pressure (BP)[1-4]. Our previous study[5] has mapped out the distributions of opioid receptors in various brain regions and spinal cords of SHR and WKY rat by autoradiography using a non-selective opioid agonist, [^{3}H]etorphine which interacted equally with μ, δ, and κ receptors.

Ohmefentanyl (OMF) is a highly selective agonist for μ receptor subtype[6]. U-69593 and U-50 488H are specific ligands for κ receptor subtype. Therefore, by using [^{3}H]OMF, [^{3}H]U-69593 and [^{3}H]etorphine after suppression of μ and κ-sites by unlabeled OMF and U-50 488H, the present experiment was carried out to compare the distributions of opioid receptor subtypes in several brain regions which were related to cardiovascular regulation in SHR with those of WKY rat by autoradiography.

MATERIALS AND METHODS

SHR and WKY rats, ♂ (aged 16 wk), obtained from Department of Pharmacology, the Second Military Medical University, were individually housed for at least 7 d before experiment. [^{3}H]OMF (2.07 PBq·mol^{-1}) and nonlabeled OMF were produced by Shanghai Institute of Materia Medica, Chinese Academy of Sciences. [^{3}H]etorphine (1.13 PBq·mol^{-1}) and nonlabeled etorphine were made by School of Pharmacy of Shanghai Medical University. [^{3}H]U-69593 (2.11 PBq·mol^{-1}) was purchased from Amersham Corp. U-50 488H was kindly donated by Du Pont Corp.

Measurement of blood pressure Systolic BP (SBP) of conscious SHR and WKY rats were monitored by tail cuff method 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.

Tissue preparation Rats were decapitated. The brain and thoracic (T4-6) spinal cords were mounted on chucks using 4 % carboxymethyl cellulose (CMC) and frozen in dry ice. Corresponded to the stereotaxic atlas of Paxinos and Watson (1986), 5 levels of the brain and spinal cord were sectioned at 20 μm at −18 ℃. The sections were thaw-mounted on gelatin/chrome alum-coated slides. Tissue sections were stored at −20 ℃ for up to 24 h before use.

Radiobinding assay For μ or k receptors, tissue sections were incubated in 100 μL of Tris-HCl 50 mmol·L^{-1} (pH 7.4) with [^{3}H]OMF or [^{3}H]U-69593 8 nmol·L^{-1} at 25 ℃ for 45 min as total binding, and nonspecific binding was determined in the presence of unlabeled OMF or U-50 488H 10 μmol·L^{-1}, respectively. For δ recep-

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tor, total binding was determined in 100 μL of Tris-HCl (pH 7.4) 50 mmol·L^{-1} with [^{3}H]etorphine 12 nmol·L^{-1} after suppressing μ and κ receptor binding sites by 15 μmol·L^{-1} of OMF and U-50 488H, respectively. Nonspecific binding assay was performed in the same incubation except for the presence of unlabeled etorphine 20 μmol·L^{-1}. At the end of incubation, sections were washed sequentially through 6 rinses of ice-cold Tris-HCl 50 mmol·L^{-1} and 0.5 % bovine serum albumin (pH 7.4) and 5 rinses of ice-cold distilled water, then dried rapidly under a stream of hot air, and stored in 20 ℃.

Autoradiography The sections were exposed to tritium-sensitive films (Hyperfilm-^{3}H, Amersham) at 4 ℃ for 35 d, then 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 also the standard of the tritium microscale. Specific binding was determined by subtraction of background film density and of nonspecific binding from total binding. OD values for brain regions were converted to receptor densities according to the standard curve delivered from a series of ^{3}H-standards exposed to each film.

Statistical analyses The t test was used to compare receptor densities between SHR and WKY rats. The values determined in 3 sections/rat at each anatomical level were averaged, and the averaged data from 5 rats were expressed as $\bar{x} \pm s$.

RESULTS

The SBP of SHR (26.6 ± s 1.7 kPa) was higher than that of WKY rats (17.1 ± 1.1 kPa) ($P<0.01$). The body weight of SHR (267 ± 9 g) was lower than that of WKY rat (320 ± 12 g) ($P<0.01$).

[^{3}H] Etorphine for δ receptor In SHR, binding of [^{3}H]etorphine after suppression of μ and κ sites was most concentrated in habenular nuclei (1.83 nmol /g tissue) and medial preoptic area (1.81). High densities (1.4 − 1.7) occurred in posterior hypothalamic area, periaqueductal gray (PAG, dorsal), lateral preoptic area, and gray matter of thoracic spinal cord. Moderate density area (1.0 − 1.4) included lateral hypothalamic area, arcuate nucleus, posterior cingulate cortex, PAG (ventrolateral), interpeduncular nuclei, nucleus of the solitary tract, superior colliculi, striated cortex, hippocampus, and caudate nucleus. The lowest δ receptor densities (< 1.0) were seen in basolateral amygdaloid nucleus and substantia nigra (Tab 1).

SHR had higher densities of δ opioid receptor in hypothalamic nuclei, PAG, interpeduncular nuclei, superior colliculi and caudate nucleus, but lower in substantia nigra as compared with WKY rats. In habenular nuclei, hippocampus, nucleus of the solitary tract and thoracic spinal cord, no difference was found between 2 strains (Tab 1, Fig 1, Plate 1).

[^{3}H]OMF for μ receptor Compared to δ receptor density, μ receptor density (< 1.0 nmol/g tissue) was much lower in both SHR and WKY rat. However, μ receptor was much concentrated in hypothalamic nuclei (except for lateral preoptic area) and gray matter of thoracic spinal cord of SHR (receptor density > 0.5 nmol/g tissue). Fewer receptor was found in other examined regions (Tab 1).

In most of the regions observed, no difference was found between SHR and WKY rats, except that SHR had lower μ receptor density in basolateral amygdaloid nucleus, habenular nuclei, posterior cingulate cortex, and nucleus of the solitary tract (Tab 1, Fig 2, Plate 1).

[^{3}H]U-69593 for κ receptor The levels of [^{3}H]U-69593 binding density were so low in both SHR and WKY rat that κ receptor density was hardly detectable.

DISCUSSION

In the present study, more σ receptor was found in 11 of 20 examined brain regions including hypothalamic nuclei and most mesencephalic nuclei, and fewer μ receptor were in 4 of 20 areas in SHR *vs* WKY rat. The magnitude of change between μ and δ receptor in SHR could be due to their different role in regulation of BP.

In hypothalamus, microinjection of [D-Ala^2, D-Leu^5] enkephalin (DADLE, δ agonist) into medial preoptic area and anterior area caused increased BP and heart rate (HR)[7,8], and SHR had lower concentration of Leu-enkephalin[9] *vs* WKY rat. Therefore it is possible that the higher δ receptor density in hypothalamic nuclei of SHR in our study is the result of up-regulation of receptor.

Our results are consistent with the study reported by Kujirai[10], but not with the reports which found SHR had more δ or μ receptor in membranes of amygadala or hypothalamus[2,4]. Our result showed SHR just had higher δ receptor density in basolateral amygdaloid nucleus and μ

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Tab 1. δ and μ receptor densities on autoradiograms of brain sections from rats. $n=5$. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* WKY.

Anatomical regions	Receptor density, nmol/g tissue			
	σ receptor		μ receptor	
	WKY rats	SHR rats	WKY rats	SHR rats
Telencephalon				
Caudate	0.74±0.13	1.03±0.17^c	0.18±0.04	0.14±0.07^a
Posterior cingulate cortex	1.10±0.10	1.25±0.23^a	0.45±0.04	0.38±0.05^b
Diencephalon				
Basolateral amygdaloid nucleus	0.48±0.07	0.57±0.16^a	0.55±0.11	0.32±0.07^c
Habenular nuclei	1.67±0.15	1.83±0.13^a	0.91±0.14	0.45±0.12^c
Hypothalamus				
Arcuate nucleus	0.72±0.23	1.26±0.30^c	0.61±0.05	0.70±0.13^a
Lateral hypothalamic area	0.63±0.16	1.35±0.23^c	0.52±0.07	0.59±0.05^a
Lateral preoptic area	0.71±0.11	1.43±0.37^c	0.27±0.05	0.32±0.06^a
Medial preoptic area	1.00±0.17	1.81±0.40^c	0.43±0.08	0.52±0.09^a
Posterior area	0.80±0.10	1.67±0.36^c	0.64±0.11	0.73±0.14^a
Mesencephalon				
Hippocampus	0.91±0.23	1.09±0.21^a	0.28±0.05	0.25±0.09^a
Interpeduncular nuclei	0.80±0.17	1.10±0.26^b	0.36±0.11	0.46±0.08^a
Periaqueductal gray				
Dorsal part	1.10±0.14	1.63±0.37^c	0.30±0.07	0.36±0.10^a
Ventrolateral part	0.83±0.17	1.20±0.30^c	0.29±0.07	0.34±0.08^a
Striated cortex	0.95±0.13	1.17±0.26^a	0.46±0.18	0.34±0.14^a
Substantia nigra	0.93±0.24	0.67±0.13^b	0.38±0.05	0.45±0.08^a
Superior collicul	0.80±0.17	1.13±0.30^b	0.32±0.10	0.36±0.09^a
Medulla oblongata				
Nucleus of the solitary tract	0.93±0.16	1.10±0.20^a	0.27±0.10	0.16±0.06^b
Thoracic spinal cord (T4-6)				
Dorsal horn	1.28±0.32	1.47±0.30^a	0.61±0.12	0.62±0.08^a
Intermediate lateral zone	1.30±0.42	1.33±0.40^a	0.54±0.07	0.59±0.06^a
Ventral horn	1.30±0.28	1.57±0.47^a	0.62±0.07	0.68±0.09^a

receptor density in hypothalamus than WKY rat, but no statistical difference was between them. The difference between those study and ours may be due to the technique used in the study, autoradiography can distinguish minute difference in every minute nucleus at each section, while homogenation of whole amygdaloid complex can diminish the minute difference.

The present study failed to detect κ receptor in SHR and WKY rat. U-69593 is a potent agonist for κ_1 binding sites, low level of κ_1 receptor in rat brain[11] could be responsible for the failture.

In conclusion, our results revealed more δ receptor in hypothalamic nuclei and PAG, and no changes in μ receptor density in hypothalamus and hippocampus in SHR *vs* WKY rat. Such differences in distributions of opioid receptor subtypes may be related to the elevated BP in SHR, and greater changes in δ receptor suggested that δ receptor might be more important in the maintenance of hypertension in SHR.

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μ和δ阿片样受体在自发高血压大鼠和正常血压WKY大鼠中枢神经系统中的分布

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关键词　μ阿片样受体；δ阿片样受体；高血压；放射自显影法；近交SHR大鼠；近交WKY大鼠

目的：比较自发高血压大鼠(SHR)和对照组WKY大鼠中枢神经系统中阿片受体亚型的分布．**方法**：用放射自显影法，选用^{3}H-OMF，^{3}H-U69593分别标记μ和κ受体，用遮盖法以^{3}H-etorphine标记δ受体．**结果**：δ受体密度在SHR下丘脑、中央灰质高于WKY，μ受体密度在SHR杏仁基底外侧核、僵核、孤束核低于WKY，κ受体密度没能检测出．**结论**：阿片受体亚型不同分布与SHR的血压有关，并且δ受体对高血压的维持作用大于μ受体．

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Modulatory effects of gonadorelin on GABA-induced depolarization and GABA-activated current in rat spinal ganglion neurons[1]

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KEY WORDS gonadorelin; GABA; spinal ganglia; microelectrodes; electrophysiology

AIM: To explore the modulatory effects of gonadorelin on GABA-induced depolarization and GABA-activated current in membrane of rat primary sensory neurons. **METHODS**: Intracellular recordings and whole-cell patch clamp techniques were performed on neurons in rat spinal ganglia (SG) preparation and neurons freshly isolated from rat SG, respectively. Drugs were applied by superfusion and/or by bath application. **RESULTS**: In the majority of neurons GABA (10 $\mu mol \cdot L^{-1}$ － 1 $mmol \cdot L^{-1}$) induced a depolarization, which was blocked by bicucullin (100 $\mu mol \cdot L^{-1}$). Pretreatment with gonadorelin (50 $\mu mol \cdot L^{-1}$) decreased the GABA-induced depolarization by 79 ± 22 % ($n = 29$), while gonadorelin elicited no effect or slight depolarization alone. In 6 of 11 cells, GABA-activated currents were also inhibited by pretreatment with gonadorelin (50 $\mu mol \cdot L^{-1}$), while in 5 of 11 cells, there was no change or a slight potentiation. **CONCLUSION**: Gonadorelin exerts an inhibitory effect on GABA-induced depolarization and GABA-activated current in the primary sensory neurons.

GABA is the major neurotransmitter involved in the formation of primary afferent depolarization (PAD) and thus related to the generation of presynaptic inhibition[1,2]. Our previous study revealed that peptide gonadorelin modu-

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