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Effects of κ, σ, and phencyclidine receptors agonists in rat tail arteries of spontaneously hypertensive rats[1]

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ABSTRACT The effects of the *kappa* receptor agonist *trans*-4-dichloro-*N*-methyl-*N*-(2-(1-pyrrolidin) cyclohexyl)-benzeneacefamide methane sulfonate (U-50 488H), etorphine, the *sigma* (σ) receptor agonists (+)-3-(3-hydroxychenyl)-*N*-(1-propyl) piperidine ((+)-3-PPP), 1, 3-di-*o*-tolyl-guanidine (DTG), and the phencyclidine (Phe) receptor agonists Phe, *N*-(1-(2-thienyl) cyclohexyl) piperidine (TCP), and dizocilipine maleate (MK-801) on electrically stimulated constriction (ESC) were investigated in the rat tail arteries (RTA) of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. Etorphine and U-50 488H inhibited the response to ESC in SHR more than that in WKY. The effects of U-50 488H were greater than those of etorphine. The IC_{50} and K_{act} of U-50 488H in SHR were 2.5±2.0 and 0.43±0.22 μmol·L^{-1}, respectively, while the corresponding figures in WKY were 23±15 and 2.3±1.0 μmol·L^{-1}, respectively ($P<0.05$). The inhibitory effects of (+)-3-PPP on ESC in RTA of SHR were weaker than those in WKY. Its IC_{50} and K_{act} in SHR were 11.6±5.4 and 0.87±0.30 μmol·L^{-1}, respectively, while the corresponding figures in WKY were 0.63±0.16 and 0.35±0.18 μmol·L^{-1}, respectively ($P<0.05$). But the inhibitory effect of DTG was very slight and the difference of K_{act} between WKY and SHR was not significant. The enhancing effects of Phe, TCP, and MK-801 in SHR were not at all different from those in WKY at each concentration tested. These results suggested that the sensitivity of *kappa* (κ) receptor in peripheral blood vessels of SHR was higher than that of WKY while that of the σ receptor was quite the contrary, and that the sensitivity of Phe may have little difference.

KEY WORDS arteries; *kappa* receptors; *sigma* receptors; phencyclidine receptors; etorphine; inbred WKY rats; inbred SHR rats

There existed *kappa*, *sigma*, and phencyclidine (Phe) receptors in the peripheral blood vessels, and these opiate receptors were located on the adrenergic nerve terminals which seemed to be distributed mainly in the adventitia and muscularis of the vessels[1-4]. The presynaptic effect of Phe induced an increased NE release and then resulted in enhancing electrically stimulated constriction (ESC), but the effects of κ agonists were contrary to the those of Phe[1,5]. Each σ ligand exhibited a distinct individual inhibitory profile with respect to the preferential actions on either norepinephrine- or serotonin-induced contractions, suggesting that these ligands acted through several mechanisms to inhibit the contractility in RTA[6].

Hypertension was usually associated with some abnormal structure and function of peripheral blood vessels[7]. The present study was to investigate whether there was varied sensitivity of κ, σ, and Phe receptors in the

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peripheral blood vessels of spontaneously hypertensive rats (SHR) in comparison to that of Wistar-Kyoto (WKY) rats *in vitro*.

MATERIALS AND METHODS

SHR and WKY rats ♂ (aged 3 m), weighing 268 ± *s* 29 g were purchased from the Second Military Medical University. Phe hydrochloride was prepared in the School of Pharmacy, Shanghai Medical University. *N*-(1-(2-thienyl) cyclohexyl) piperidine (TCP) was from Beijing Fang-Hua Research Institute. Dizocilpine maleate (MK-801) was a gift from Dr L L Iverson (Merck Sharp Dohme Research Laboratory, Neuroscience Research Center, UK). *Trans*-3, 4-dichloro-*N*-methyl-*N*-(2-(1-pyrrolidin) cyclohexyl)-benzeneacefamide methane sulfonate (U-50 488H) (Sigma). Etorphine and naloxone were synthesized by the Department of Pharmaceutical Chemistry, Shanghai Medical University. (+)-3-(3-Hydroxychenyl)-*N*-(1-propyl) piperidine ((+)-3-PPP) and 1, 3-di-*o*-tolyl-guanidine (DTG) were supplied by Du Pont de Nemours & CO. Dextromethorphan (DM) was kindly gifted by Dr J N Musacchio (New York University Medical Center).

Tissue preparation The rat tail arteries (RTA) were prepared and set up in a 4-ml organ bath (Krebs' solution through 95% O_2 + 5% CO_2, tension 0.5 g) at 37 ℃. The Krebs' solution, kept at room temperature (20 ℃), was composed of NaCl 122.2; KCl 5.4; $CaCl_2$ 3.2; $MgSO_4 \cdot 7H_2O$ 1.2; $NaHCO_3$ 25.6; glucose 11.0; KH_2PO_4 1.2; $Na_2EDTA \cdot 2H_2O$ 0.027 (in mmol·L^{-1}).

The contraction of blood vessel was induced by electric field stimulation (EFS, 25 — 30 V, 10 Hz, trains of 5 pulses, 1 ms per pulse, 2.5 min interval). The preparation was allowed to equilibrate for 5 h prior to receiving EFS while the bath medium was renewed every 20 min. Concentration-response curves were plotted by increasing the bath concentration of ligands cumulatively. The concentration could be induced only when the previous one reached its maximal effect and remained constant. When ligands exhibited their maximal effects, the antagonists (naloxone to κ and σ receptors and DM to Phe receptor) were given.

Statistical analysis

$$\text{Inhibition } \% = \frac{\text{Pre VC} - \text{Post VC}}{\text{Pre VC}} \times 100\ \%$$

$$\text{Enhancement } \% = \frac{\text{Post VC} - \text{Pre VC}}{\text{Pre VC}} \times 100\ \%$$

Prevasoconstriction (Pre VC) meaned the tension (ng) of vasoconstriction before the ligands were given. Postvasoconstriction (Post VC) meaned the tension (ng) of vasoconstriction after the ligands were given.

IC_{50} meaned the concentration for 50% inhibition. EC_{50} meaned the concentration for 50 % enhancement.

95% Fiducial limits = $\bar{x} + t_{0.05(n-1)}$ SE

Statistical significance was evaluated by *t* test.

RESULTS

Inhibitory effects of etorphine and U-50 488H The inhibitory effects of vasoconstrictor responses of entorphine in SHR were greater than that in WKY from 0.01 to 10 μmol·L^{-1}, and the differences were significant both at 0.1 and 10 μmol·L^{-1} ($P<0.05$) (Fig 1 A). Compared to WKY, the IC_{50} (Tab 1) and K_{act} (Tab 2) of U-50 488H in SHR were smaller ($P<0.05$).

Tab 1. Concentration for 50 % inhibition (IC_{50}, μmol·L^{-1}) and 95 % fiducial limits (μmol·L^{-1}) of etorphine, U-50 488H, (+)-3-PPP, DTG and 50 % enhancement (EC_{50}, μmol·L^{-1}) of Phe, TCP, MK-801 on vasoconstriction response of RTA induced by EFS. $n=5$, $\bar{x}\pm s$. $^aP>0.05$. $^bP<0.05$ *vs* WKY.

Ligands	SHR rats	WKY rats
Etorphine	29.2±21.0 (0.01—58.0)	—
U-50 188H	2.5±2.0 (0.03—1.97)	22.8±15.1[b] (1.1±11.5)
(+)-3-PPP	11.6±5.1 (4.9—18.3)	0.63±0.16[b] (0.43—0.83)
DTG	—	—
Phe	11.7±8.5	2.6±1.1[a]
TCP	5.4±1.8	6.6±3.8[a]
MK-801	3.1±0.9	2.2±0.6[a]

Inhibitory effects of σ ligands *In vitro* (+)-3-PPP markedly inhibited the ESC in RTA (Fig 1 C). Its IC_{50} (Tab 1) and K_{act} (Tab 2) in SHR were greater than those in WKY ($P<0.05$).

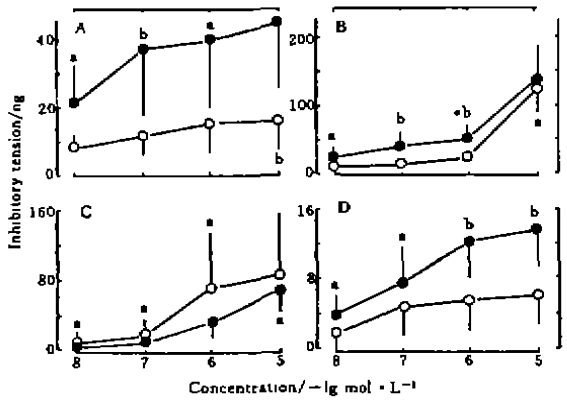

Fig 1. Effects of etrophine (A), U-50 488H (B), (+)-3-PPP (C), and DTG (D) on EFS-induced vasoconstriction in RTA. WKY (○), SHR (●). $n=5$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ *vs* WKY.

The inhibitory effects of DTG were greater at 1 to 10 μmol·L^{-1} in SHR than those in WKY ($P<0.05$), but the inhibitory effect was only slight (Fig 1 D).

Tab 2. K_{act} (μmol·L^{-1}) of eturphine, U-50 488H, (+)-3-PPP, DTG, Phe, TCP, and MK-801 on the EFS-induced vasoconstriction in RTA. $n=5$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ *vs* WKY.

Ligands	SHR rats	WKY rats
Etrophine	0.06±0.04	0.02±0.01[b]
U-50 488H	0.43±0.22	2.33±1.00[b]
(+)-3-PPP	0.87±0.30	0.35±0.18[b]
DTG	0.04±0.02	0.02±0.01[a]
Phe	1.31±0.73	1.34±0.39[a]
TCP	2.11±0.54	2.12±1.22[a]
MK-801	2.49±0.83	2.23±0.93[a]

Enhancing effects of Phe ligands All Phe ligands (Phe, TCP and MK-801) showed enhancing effects on ESC in RTA (Fig 2). From 1 to 10 μmol·L^{-1} the enhancing effects of TCP in SHR were stronger than that in WKY ($P<0.05$) (Fig 2 B). But there were no significant differences of EC_{50} and K_{act} between SHR and WKY (Tab 1,2).

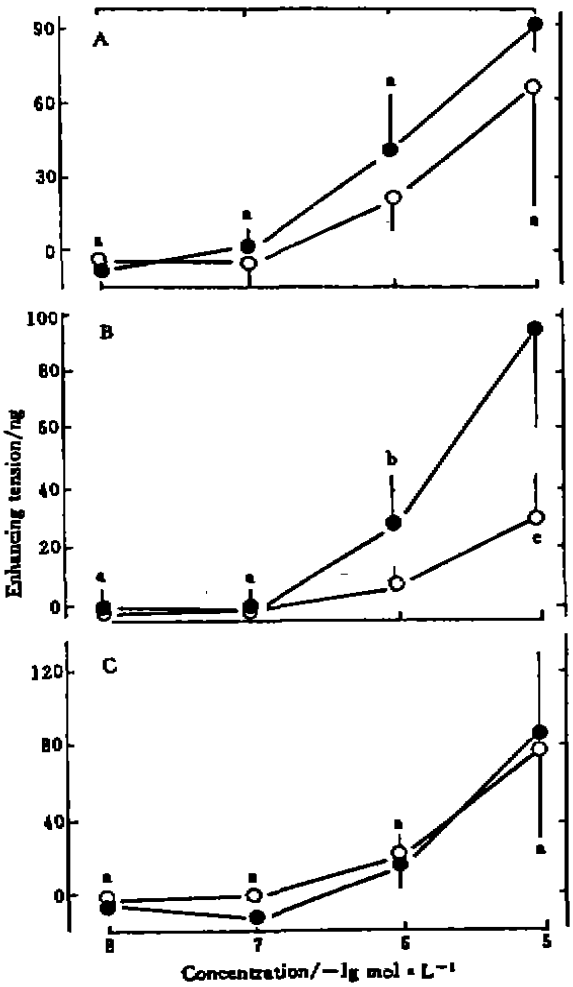

Fig 2. Effects of Phe (A), TCP (B), and MK-801 (C) on EFS-induced vasoconstriction in RTA. WKY (○), SHR (●). $n=5$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* WKY.

DISCUSSION

Our results clearly demonstrated that in comparison to WKY rats, the SHR rats had a greater sensitivity to the κ receptor agonists U-50 488H and etorphine. κ Agonist did not antagonize the NE induced vasocostriction, while phentolamine could abolish the vasoconstriction induced by stimulation or by NE. The inhibitory effect of κ agonist on ESC prob-

ably due to presynaptic inhibition of NE release from nerve terminals[5]. In this case, κ receptor agonists might inhibit the NE release from nerve terminals in SHR more effectively than those in WKY. These findings would, therefore, be consistent with the increased sensitivity of κ receptors on presynapses in peripheral blood vessels of SHR rats. These functional changes of κ receptors might be a sort of counterbalance to hypertension in SHR.

Our results also demonstrated that the sensitivity to σ receptor ligand (+)-3-PPP in SHR was lower in comparison to that in WKY. σ Ligand were probably interfering with the chain of events initiated by α adrenergic or 5-HT_2 serotonergic receptors[6]. Hence further studies are required to determine the mechanism of the decreasing sensitivity of σ receptor in SHR.

It was demonstrated that the sensitivities of Phe ligands were different between SHR and WKY, but not significant at all in their concentrations. It was possible that there were fewer changes in the sensitivities of Phe receptors on presynapses in SHR than that in WKY.

There remains, however, an important issue to be explored. That is whether there are changes of density and affinity of these receptors in SHR. Further studies are required to clarify these problems such as binding radio - assay, etc.

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高血压大鼠尾动脉上 κ，σ 和苯环利定受体激动剂的效应

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摘要 利用电场刺激引起的大鼠尾动脉收缩模型，研究了 κ，σ 和苯环利定(Phe)受体在高血压大鼠(SHR)上的变化. 结果，埃托啡和 U-50 488H 在 SHR 上的抑制作用显著高于非高血压大鼠(WKY). (+)-3-PPP 的结果与上述相反，DTG 的作用很小. Phe，TCP 和 MK-801的增强作用在两者间无显著差别. 提示在 SHR 上 κ 受体的敏感性增高，σ 受体相反，而 Phe 受体的敏感性变化较少.

关键词 动脉；κ 受体；σ 受体；苯环利定受体；埃托啡；近交 WKY 大鼠；近交 SHR 大鼠