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DOWN-REGULATION OF BRAIN AND SPINAL CORD κ-OPIATE RECEPTORS IN SPONTANEOUSLY HYPERTENSIVE, WISTAR–KYOTO NORMOTENSIVE, AND SPRAGUE–DAWLEY RATS BY CHRONIC TREATMENT WITH U-50,488H

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Abstract—The effects of chronic administration of U-50,488H, a κ-opiate receptor agonist, on the binding of [^{3}H]ethylketocyclazocine ([^{3}H]EKC) to κ-opiate receptors on the cerebral cortical and spinal cord membranes of spontaneously hypertensive (SHR), Wistar–Kyoto normotensive (WKY), and Sprague–Dawley (SD) rats were determined. Age-matched (10 weeks old) male rats of each strain were injected twice daily for 7 days with either U-50,488H (25 mg/kg, i.p.) or its vehicle. On day 8, the rats were killed. The cerebral cortex and the spinal cord were isolated for binding studies. The systolic blood pressure and heart rate of SD and WKY rats did not differ but the blood pressure of SHR rats was higher than that of SD and WKY rats. The receptor density (B_{max}) and apparent dissociation constant (K_d) values of [^{3}H]EKC binding to the spinal cord of WKY and SHR rats did not differ. However, the spinal cord of SD rats had higher B_{max} and K_d values than WKY or SHR rats. The cortex of the SD rats had a lower B_{max} value than the other two strains. Treatment with U-50,488H decreased the B_{max} value of [^{3}H]EKC in spinal cord of SD rats, increased the K_d value in SHR rats, and had no effect in WKY rats. Decreases in the B_{max} value were produced in the cortex of all strains of rats, but a greater effect was observed in WKY and SHR rats than in SD rats.

Spontaneously hypertensive (SHR) and normotensive Wistar–Kyoto (WKY) rats have been utilized to study biochemical mechanisms in the regulation of blood pressure. Multiple opiate receptors namely μ, δ, and κ have been implicated in the regulation of blood pressure; however, the results have not always been consistent [1]. This has been the case for several reasons including the animal model used, selectivity of agonists and antagonists, the presence or absence of an anesthetic, and whether or not the animals were ventilated artificially [1].

Previous studies from this laboratory indicate that SHR rats have a higher density of κ-opiate receptors as indicated by a higher B_{max} value for the binding of [^{3}H]ethylketocyclazocine ([^{3}H]EKC) to brain membranes than found in WKY rats [2]. Further evidence of higher density of brain κ-opiate receptors was evidenced by greater analgesic and diuretic responses to U-50,488H, a selective κ-opiate agonist, in SHR rats than in WKY rats [3].

Studies from this laboratory also indicate that chronic administration of U-50,488H to Sprague–Dawley rats causes down-regulation of whole brain and spinal cord κ-opiate receptors [4]. Further studies revealed that the down-regulation of κ-opiate receptors by U-50,488H was induced in cerebral cortex, midbrain, and pons and medulla [5].

The present studies were undertaken to determine whether or not chronic administration of U-50,488H produces similar changes in κ-opiate receptors of cerebral cortex and spinal cord of Sprague–Dawley, WKY and SHR rats.

MATERIALS AND METHODS

Animals. Age-matched (10 weeks old) male SHR rats of the Okamoto-Aoki strain, WKY and Sprague–Dawley (SD) rats were obtained from Charles River, MA. The animals were acclimated to the laboratory environment for at least 4 days before being used. They were housed three to a cage in rooms with controlled temperature (23 ± 1°), humidity (50 ± 10%) and artificial lighting (6:00 a.m. to 6:00 p.m.). Food and water were made available continuously.

Chemicals. U-50,488H [*trans*-3,4-dichloro-*N*-methyl - *N* - (2 - [1 - pyrrolidin]cyclo - hexyl)benzeneacetamide methane sulfonate) was a gift from Drs. John McCall and Philip Von Voigtlander of the Upjohn Co., Kalamazoo, MI. The drug was freshly prepared by dissolving in saline and was injected i.p. in a volume of 1 mL/kg of body weight. [^{3}H]EKC (sp. act. 45 Ci/mmol) was obtained from the National Institute on Drug Abuse, Rockville, MD, through the courtesy of Drs. R. L. Hawks and R. S. Rapaka. [D-Ala^2,MePhe^4,Gly-ol^5]Enkephalin (DAMGO) and [D-Ala^2,D-Leu^5]enkephalin (DADLE) were purchased from the Bachem Chemical Co., Bubendorf, Switzerland.

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Measurement of blood pressure and heart rate. The systolic blood pressure and heart rate were recorded indirectly in unanesthetized rats by the plethysmographic tail-cuff method using a rat tail manometer-tachometer system (Natsume, model KN-210, Peninsula Laboratories, Belmont, CA) as described previously [2, 6–8]. Before measurements, the rats were prewarmed for 15 min in a chamber (40°) to increase the blood flow to the tail. The results were compatible with direct blood pressure recording [9].

Treatment with U-50,488H. The rats from different strains were divided into two groups. One group of rats was injected twice daily with U-50,488H (25 mg/kg, i.p.) and the other with its vehicle for 7 days. The animals were killed on day 8 for determining the binding of [^{3}H]EKC to cerebral cortical and spinal cord membranes.

Determination of the binding of [^{3}H]EKC to rat brain or spinal cord membranes. Preliminary studies indicated that the binding of [^{3}H]EKC to rat brain or spinal cord membranes was greater in Tris–HCl buffer than in potassium phosphate buffer; therefore, Tris–HCl was used in the present studies. The rats were decapitated and their brains and spinal cords quickly removed on ice. The cerebral cortices were isolated and used for the binding studies. The tissue (cerebral cortex or spinal cord) was homogenized in 30 vol. of ice-cold Tris–HCl buffer (0.05 M, pH 7.4) using a Polytron homogenizer (setting 5 for 20 sec). The homogenate was centrifuged at 49,000 *g* for 15 min. The pellet was resuspended in the same buffer and was incubated at 37° for 15 min to remove the endogenous opioids from the receptor sites. After a second centrifugation at 49,000 *g* for 15 min, the pellets were resuspended in the Tris–HCl buffer and used for subsequent binding studies. The binding of [^{3}H]EKC was carried out as described previously [2, 3, 10] using a concentration range of 0.21 to 18.0 nM. The incubations were carried out in the presence of a 100 nM concentration of both unlabeled DADLE and DAMGO to suppress δ and μ opioid recognition sites, respectively. The incubation mixture contained 0.1 mL of buffer in a final volume of 0.3 mL. The binding assays were performed in triplicate at 37° for 30 min. The specific binding was defined as the difference in binding observed in the absence and presence of 10 μM levorphanol. The incubation was terminated by rapidly filtering the contents of the incubation tubes under reduced pressure using Millipore manifold filtration units and Whatman GF/F glass fiber filters. It was followed by two 5-mL washes of the same ice-cold buffer used for the assay. The filters were transferred to liquid scintillation vials containing 10 mL of 3a 70 scintillation fluid (Research Products International, Elk Grove Village, IL). After an overnight equilibration period, the radioactivity in the samples was determined in a Packard Liquid Scintillation Counter (model 4640) with a 54% counting efficiency. The concentration of protein in the samples was determined by the method of Lowry *et al.* [11].

The receptor density (B_{max}) and apparent dissociation constant (K_d) values were determined from the saturation curve and Scatchard analysis. Five rats were used for each treatment group. The means of B_{max} and K_d values were compared by using analysis of variance (ANOVA) followed by Scheffe's test. A value of $P < 0.05$ was considered significant.

Table 1. Blood pressure and heart rate of 10-week-old Sprague–Dawley (SD), Wistar–Kyoto (WKY) normotensive, and spontaneously hypertensive (SHR) rats*

Rat strain	Blood pressure (mm Hg)	Heart rate (beats/min)
SD	151.39 ± 1.14	417.08 ± 4.74
WKY	148.25 ± 2.13	418.95 ± 7.88
SHR	201.60 ± 1.96†	414.66 ± 3.70

* Systolic blood pressure and heart rate were recorded indirectly in unanesthetized rats with the plethysmographic tail-cuff method. Values are means ± SEM, N = 16.

† $P < 0.05$ vs SD or WKY rats.

Effects of U-50,488H and U-69,593 on the binding of [^{3}H]EKC to rat cerebral cortical membranes. Although similarities in the binding sites for [^{3}H]EKC and [^{3}H]U-69,593 have been demonstrated for guinea pig brain, nothing is known about such interaction in the rat brain. The effects of U-50,488H and U-69,593 on the binding of a 1.5 nM concentration of [^{3}H]EKC was determined in rat cortical membranes. Twelve concentrations of the unlabeled drug which ranged from 10^{-9} to 10^{-4} M were used to determine the IC_{50} value. Five rats were used to compute the mean IC_{50} value and its SEM.

RESULTS

Systolic blood pressure and heart rate of SD, WKY and SHR rats. The systolic blood pressure and heart rate of age-matched SD and WKY rats did not differ. However, the systolic blood pressure of SHR rats was significantly greater ($P < 0.05$) than those of SD and WKY rats but the heart rates did not differ (Table 1).

Effects of U-50,488H and U-69,593 on the binding of [^{3}H]EKC on rat cerebral cortical membranes. κ-Opiate agonists, U-50,488H and U-69,593, displaced [^{3}H]EKC from its binding sites with IC_{50} values of 20 ± 3.0 and 238 ± 45 nM, respectively. U-50,488H was thus 10-fold more potent than U-69,593 in competing for the [^{3}H]EKC binding sites ($F1/8 = 24.6$; $P = 0.002$).

Effect of chronic administration of U-50,488H on the binding of [^{3}H]EKC to κ-opiate receptors of cerebral cortex of SD, WKY and SHR rats. A typical saturation curve and Scatchard plot for the binding of [^{3}H]EKC to cerebral cortical membranes of a Sprague–Dawley rat are shown in Fig. 1B. [^{3}H]EKC bound to cerebral cortical membranes of rats at a single high-affinity site. ANOVA of B_{max} values of cerebral cortex of SD, WKY and SHR rats indicated highly significant differences ($F2/12 = 23.10$; $P < 0.0005$) (Fig. 2A). The B_{max} values of [^{3}H]EKC binding in SHR and WKY rats did not differ ($P = 0.92$) but the value in SD rats was only 50% of the other two strains ($P < 0.0005$). K_d values of [^{3}H]EKC

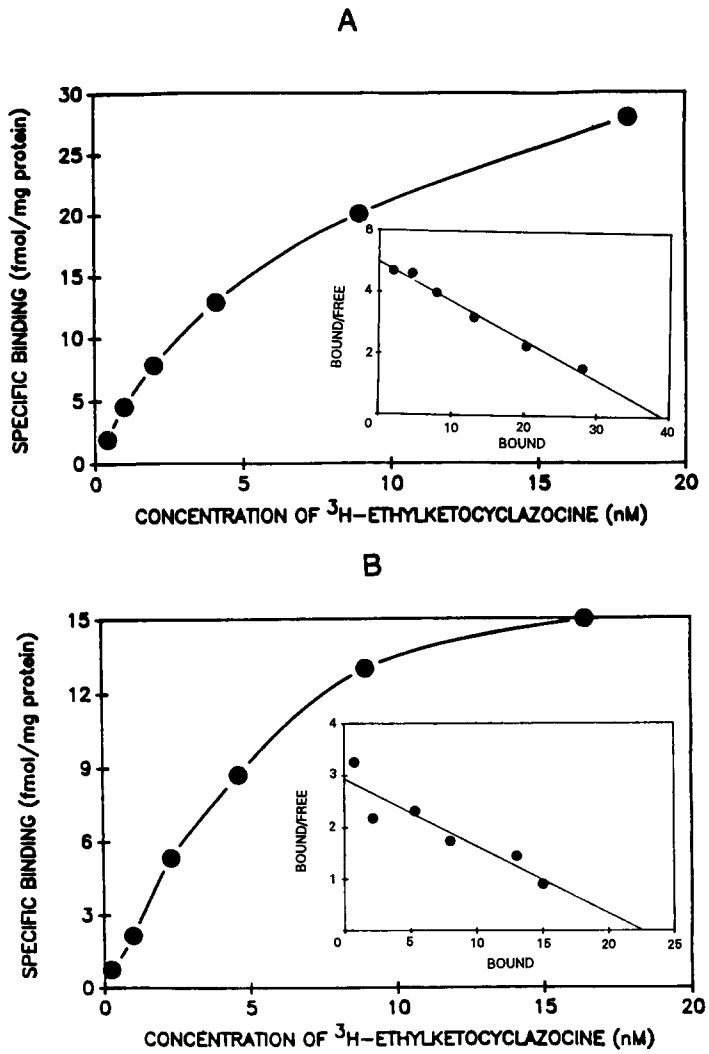

Fig. 1. Typical saturation curve and Scatchard plot of [^{3}H]EKC binding to κ-opiate receptors on spinal cord (A) and cerebral cortical (B) membranes of a Sprague–Dawley rat.

binding to cerebral cortex of SD, WKY and SHR rats also indicated a significant difference ($F2/12 = 5.12$; $P = 0.025$). Further analysis showed that the K_d values for WKY and SHR rats were similar but that for SHR was significantly lower than for the SD rats. Chronic administration of U-50,488H for 7 days decreased the B_{max} value of [^{3}H]EKC significantly in all strains of rats. The decrease in WKY ($F1/8 = 19.05$; $P = 0.002$) and SHR ($F1/8 = 299.75$; $P < 0.0005$) rats was greater than in SD ($F1/8 = 8.78$; $P < 0.018$) rats. The K_d values of [^{3}H]EKC for binding to cortical membranes of vehicle and U-50,488H-treated rats from three strains did not differ. Chronic treatment with U-50,488H had no effect on the K_d values of [^{3}H]EKC in any of the three strains (Fig. 2B).

Effect of chronic administration of U-50,488H on the binding of [^{3}H]EKC to κ-opiate receptors of spinal cord of SD, WKY and SHR rats. A typical saturation curve and Scatchard plot for the binding of [^{3}H]EKC to spinal cord membranes of a Sprague–Dawley rat are shown in Fig. 1A. [^{3}H]EKC bound to spinal cord membranes of rats at a single high-affinity site. The B_{max} values of [^{3}H]EKC of vehicle and U-50,488H-treated rats are given in Fig. 3A. Significant differences were noted in the B_{max} values in the three strains with ANOVA showing $F2/12 = 14.28$; $P = 0.001$. The B_{max} values of [^{3}H]EKC in spinal cord membranes of SHR and WKY rats did not differ ($P = 0.97$) but in SD rats the B_{max} value was almost four times higher ($P < 0.002$) than in SHR and WKY rats. Chronic administration of U-50,488H for 7 days decreased the B_{max} value of [^{3}H]EKC in spinal cord of SD rats by more than 50% ($F1/8 = 7.08$; $P = 0.029$) but it remained unaffected in WKY and SHR rats. The K_d values of [^{3}H]EKC for binding to spinal cord membranes of vehicle and U-50,488H-treated rats are shown in Fig. 3B. Chronic administration of U-50,488H had no effect on the K_d value of [^{3}H]EKC in SD and

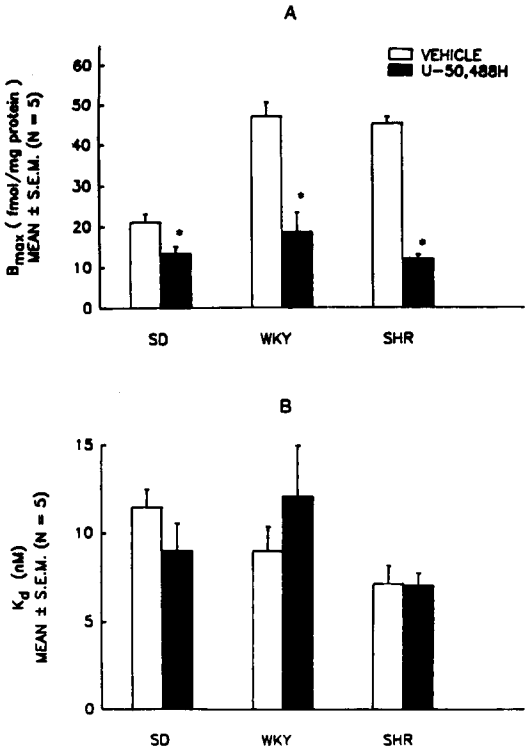

Fig. 2. B_{max} (A) and K_d (B) values of [^{3}H]EKC binding to κ-opiate receptors on cerebral cortical membranes of Sprague–Dawley (SD), Wistar–Kyoto (WKY) and spontaneously hypertensive (SHR) rats treated chronically with U-50,488H (25 mg/kg, i.p.) twice daily for 7 days or its vehicle. Key: * $P < 0.05$ vs the vehicle-treated group.

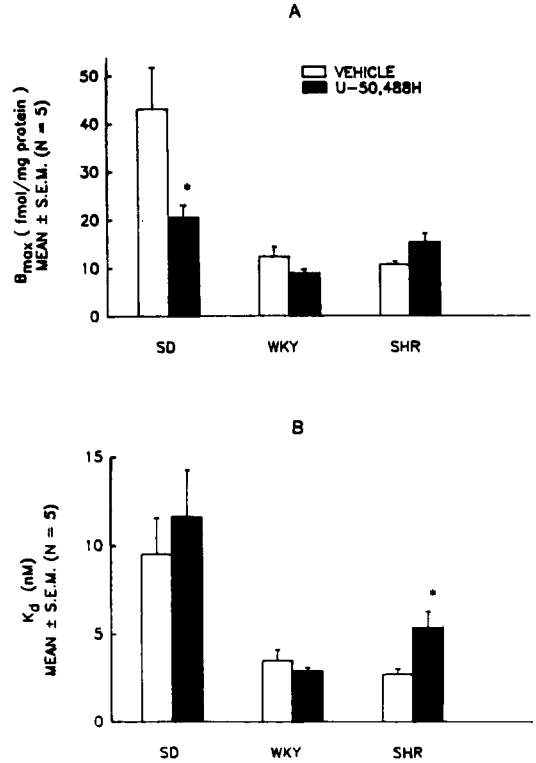

Fig. 3. B_{max} (A) and K_d (B) of [^{3}H]EKC binding to κ-opiate receptors on spinal cord membranes of Sprague–Dawley (SD), Wistar–Kyoto (WKY) and spontaneously hypertensive (SHR) rats treated chronically with U-50,488H (25 mg/kg, i.p.) twice daily for 7 days or its vehicle. Key: * $P < 0.05$ vs the vehicle-treated group.

WKY rats but it increased the K_d value in SHR rats by almost 100% ($F1/8 = 6.90$; $P = 0.030$).

DISCUSSION

The present studies clearly indicate that the densities of κ-opiate receptors in the spinal cord and cerebral cortex of the three strains of rats are different. It appeared that WKY and SHR rats had a similar distribution of κ-opiate receptors but the SD rats had a different distribution. The density (B_{max} value of [^{3}H]EKC) of κ-opiate receptors in the cerebral cortex of SD rats was 50% that of WKY and SHR rats but the K_d values were similar. On the other hand, in the spinal cord the trend was reversed. The density of κ-opiate receptors in the spinal cord of SD rats was 3- to 4-fold higher than in WKY or SHR rats; however, [^{3}H]EKC bound to the spinal cord of SD rats with lower affinity than it did in WKY or SHR rats.

Chronic administration of U-50,488H resulted in the down-regulation of κ-opiate receptors in the cerebral cortex of all three strains of rats. A higher degree of down-regulation occurred in WKY and SHR rats than in SD rats and was due to decreases in the receptor density. It should be noted that the higher the basal B_{max} value, as in the case of WKY and SHR rats, the greater was the effect of U-50,488H. It also appeared that after a certain point, κ-opiate receptors could not be down-regulated further, since in spite of different B_{max} values, following the treatment with U-50,488H the B_{max} values of [^{3}H]EKC in all three strains were stabilized to the same values. The changes in cerebral cortex κ-opiate receptors following U-50,488H treatment in SD rats are consistent with our previous findings [5].

Chronic administration of U-50,488H, on the other hand, produced differential effects on κ-opiate receptors of the spinal cord in the three strains of rats. Consistent with our earlier results, U-50,488H down-regulated (lower B_{max} values) κ-opiate receptors in SD rats [5]. In the spinal cord of WKY rats, U-50,488H did not affect κ-opiate receptors as the B_{max} and K_d values of [^{3}H]EKC remained unaltered. In SHR rat spinal cord, the B_{max} values did not change but the K_d values were elevated significantly. Since the κ-opiate receptor density was already very low in WKY and SHR rats, a further decrease could not be induced in the two strains.

Evidence has been presented for the existence of subtypes of κ-opiate binding sites [12]. Recently, U-69,593 has been suggested to be a more specific agonist for κ-opiate receptors, whereas [^{3}H]EKC

labels μ- and δ-receptors in addition to κ-receptors. Under a μ and δ suppressed condition, [^{3}H]EKC labels only one site. It has been shown that [^{3}H]EKC and [^{3}H]U-69,593 label the same site on guinea pig brain and spinal cord membranes since the profiles of displacement of these ligands by unlabeled agents are similar [13]. Our studies show similar results with rat brain membranes. Thus, the sites studied in the present investigation would correspond to the κ_1-opiate receptors.

In summary, the present studies indicate that SD, WKY and SHR rats have different distributions of κ-opiate receptors in the cerebral cortex and spinal cord. Chronic administration of U-50,488H resulted in down-regulation of κ-opiate receptors in the cerebral cortex and spinal cord of all strains of rats except the WKY rats, the spinal cord of which is resistant to change. A possible reason for failure to induce down-regulation of κ-opiate receptors in the spinal cord of WKY rats may be the low density of κ-receptors.

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