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Brain and Spinal Cord Kappa Opiate Receptors and Pharmacological Responses to U-50,488H in Rats of Differing Ages

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BHARGAVA, H. N., G. A. MATWYSHYN, P. L. REDDY AND VEERANNA. *Brain and spinal cord kappa opiate receptors and pharmacological responses to U-50,488H in rats of differing ages.* PHARMACOL BIOCHEM BEHAV 48(1) 87-91, 1994.—The analgesic and hypothermic responses to U-50,488H (25 mg/kg IP), a kappa opiate receptor agonist, were determined in male Sprague–Dawley rats aged 4, 8, and 24 weeks. In addition, the characteristics of the binding of [^{3}H]ethylketocylazocine (EKC) to kappa opiate receptors in whole brain and spinal cord of rats of three age groups were also determined. Administration of U-50,488H produced an age-related increase in the analgesic response in the rat, i.e., the older rats exhibited a higher intensity of analgesic response than the younger rats. U-50,488H also produced a hypothermic response. The response in 4- and 24-week-old rats was similar, but that in 8-week-old rats was smaller than the rats in the other two age groups. [^{3}H]EKC bound to whole brain and spinal cord membranes of rats at a single high affinity site. The B_{max} value of [^{3}H]EKC in the brain and spinal cord of 24-week-old rats was significantly lower than in 4- and 8-week-old rats; however, the K_d values did not differ. It is concluded that kappa opiate receptor agonist produces age-related increase in its analgesic response and that such effects are not related to the characteristics of kappa receptors in the brain and spinal cord.

Kappa opiate receptors Analgesia Rats Differing ages Brain Spinal cord U-50,488H

CONSIDERABLE attention has been given in recent years not only in understanding the mechanisms in the aging process at the cellular and molecular level, but also in determining the responsiveness of drugs as a function of age (2,13). With increasing age, there is an increase in the use of drugs acting particularly on the central nervous system (CNS) (2,19). Analgesic drugs, both narcotic and nonnarcotic, are used with increasing frequency. The effect of age on the sensitivity to pain has been determined in human subjects (22). Using the radiant heat technique, patients younger than 20 years of age were found to have the greatest degree of sensitivity to pain. The pain sensitivity decreased with age, as evidenced by increased pain threshold in the older age group (22).

The effects of drugs, like morphine, on pain relief has been studied in humans as well as animals of differing ages. In postoperative patients, morphine was found to produce greater pain relief in older patients than in younger ones (1). Similar effects were observed in cancer patients with postoperative pain (9). Morphine also produced greater analgesic effect in older rats in comparison to younger animals (18,23); the only exception was a study in mice where just the opposite effect was noted (25). Studies from this laboratory also indicate that intravenously administered morphine produced greater analgesia in 24-week-old rats in comparison to 8-week-old rats (8).

The mechanisms by which altered responses to morphine are produced in older subjects in comparison to younger ones are not delineated. They may involve either pharmacokinetics of morphine or the distribution of opiate receptors in the CNS. An earlier study from this laboratory clearly indicated that the pharmacokinetic parameters of morphine in serum, namely, area-under-the-concentration-time curve, concentration extrapolated to time zero, half life, elimination rate constant, mean residence time, volume of distribution

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at the steady state and the total body clearance, could not account for differential analgesic responses in rats with differing ages (8).

The binding of [^{3}H] dihydromorphine, a nonselective μ-opiate receptor ligand, to some brain areas, was found to be lower in older than in younger rats (16,17). Similarly, decreased opioid receptors were observed in thymus-derived lymphocytes of aged rats (12). Such observations would be inconsistent with the observed pharmacological effects of morphine in subjects of differing ages.

Opiates, including morphine, produce their effects by acting on a family of receptors, namely μ, δ, and κ receptors. Whereas, μ agonists produce analgesic response by acting at both spinal and supraspinal level, kappa opiate agonists, like U-50,488H, produce analgesia by acting predominantly on spinal pathways (24). Opiates are also involved in thermoregulation. μ Opiate agonists like morphine, produce biphasic response on body temperature. Whereas lower doses produces hyperthermia (10), higher doses produce hypothermia (10,21). On the other hand, κ opiate agonists have been shown to produce only hypothermia (7). If differential effects on morphine-induced pharmacological effects could be seen in rats of different ages, it is possible that κ opiate may also show differential effects. In the present studies, the analgesic and hypothermic effects of U-50,488H, a highly selective agonist at κ opiate receptors, has been determined in rats of differing ages. In addition, the characteristics of brain and spinal cord kappa opiate receptors labeled with [^{3}H]ethylketocyclazocine (EKC) have also been determined in rats of differing ages.

METHOD

Animals

Male Sprague-Dawley rats, ages 4, 8, and 24 weeks old, obtained from Sasco King Animal Co., Oregon, WI, were housed for at least 4 days before being used in a room with controlled temperature (23 ± 1°C), humidity (50 ± 10%) and light (0600–1800 h). The body weights of 4-, 8-, and 24-week-old rats were 141 ± 1, 269 ± 2, and 452 ± 5 g (n = 13), respectively. Food and water were made available continuously.

Chemicals

U-50,488H [trans-3,4, dichloro-N-[2-(1-pyrrolidinyl) cyclohexyl] benzene acetamide] was obtained from The Upjohn Company, Kalamazoo, MI, through the courtesy of Drs. Philip von Voigtlander and John M. McCall, and the National Institute on Drug Abuse, Rockville, MD. The drug was dissolved in distilled water and injected IP in a volume of 1 ml/kg of body weight. [^{3}H]EKC (specific activity 44.95 Ci/mmol) unlabeled D-Ala^2,MePhe^4,Glyol^5-enkephalin (DAMGO) and D-Ala^2,D-Leu^5,enkephalin (DADLE) were supplied by the National Institute on Drug Abuse, Rockville, MD. Unlabeled levorphanol was a gift from Hoffmann-La Roche, Nutley, NJ.

Measurement of Analgesic and Hypothermic Responses to U-450,488H in Rats of Differing Ages

Rats (4-, 8-, and 24-week-old) were injected with U-50,488H (25 mg/kg, IP). The analgesic effect of U-50,488H was measured by using a tail-flick apparatus as described previously (3,4). The tail-flick latencies to thermal stimulation were determined prior to and at various time intervals for a period of 240 min after the drug injection. The basal latencies were found to be approximately 2 s. A cut-off time of 10 s was used to prevent any injury to the tail. The basal response was subtracted from the effect induced by the drug. The curve fitting and integration were done as described previously (4). The analgesic response was expressed as area-under-the-time-response curve (AUC) and was calculated for each rat. The data were expressed as mean AUC_{0-240} min ± SEM The analgesic response in different groups was compared by using the analysis of variance followed by the Scheffe's S test. A value of $p < 0.05$ was considered to be significant. Thirteen rats were used for each age group.

The change in temperature after injecting U-50,488H (25 mg/kg, IP) to rats of differing ages was also determined. The colonic temperature of each rat was recorded before and at various time intervals for a period of 240 min using thermistor probes and a Cole Palmer Digital thermometer. Thirteen rats were used for each age group. The change in temperature was expressed as AUC_{0-240} min. The differences in the hypothermic response to U-50,488H in different groups of rats was determined as described above for the analgesic effect.

Determination of Binding of [^{3}H]EKC to Whole Brain and Spinal Cord Membranes

Membrane preparation. Rats from different age groups were sacrificed. The brains were quickly excised on an ice-cold petri dish. The spinal cord (cervical to lumbar region) was also isolated. The tissue (brain or spinal cord) was homogenized in 30 volumes of ice-cold Tris-HCl buffer (0.05 M, pH 7.4) using a Polytron homogenizer (setting 5 for 20 s). The homogenate was centrifuged at 49,000 × g for 15 min and the pellet resuspended in the same buffer and incubated at 37°C for 45 min to remove the endogenous opioids from their binding sites. After a second centrifugation, the pellet was suspended in K_2HPO_4-HCl buffer (0.05 M, pH 7.4) containing 1 mM EDTA.

Binding assays. The binding of [^{3}H] EKC was performed according to Magnan et al. (12), as adopted earlier (5,7). The binding was carried out in a total volume of 0.5 ml, which contained 0.1 ml of homogenate (300–400 μg protein) and 0.05 M K_2HPO_4 buffer. The binding of [^{3}H] EKC was carried out in a concentration range of 0.5 to 11 nM. The incubations were carried out in the presence of 100 nM each of unlabeled DAMGO and DADLE to suppress μ and δ recognition sites, respectively. All binding assays were done in duplicate at 37°C for 30 min. The specific binding was defined as the difference in binding observed in the absence and presence of 10 μM levorphanol. Binding was terminated by rapidly filtering the contents of the incubation tubes under reduced pressure using a Brandel Cell Harvester (M-24 R) and Whatman GF/B glass fiber filters. The filters were washed twice with 5 ml each of the same ice-cold buffer used for the assay. The filters were transferred to liquid scintillation vials containing 5 ml of SCINT™-A XF scintillation fluid (Packard Instrument Company Inc., Meriden, CT. 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} value) and apparent dissociation constant (K_d value) for the binding of [^{3}H]EKC to brain and spinal cord membranes were determined from the saturation curves and the Scatchard plots using the LIGAND program (15). Values were expressed as mean ± SEM. Five rats were used for each age group. The differences in the B_{max} and K_d values were determined by analysis of variance followed by

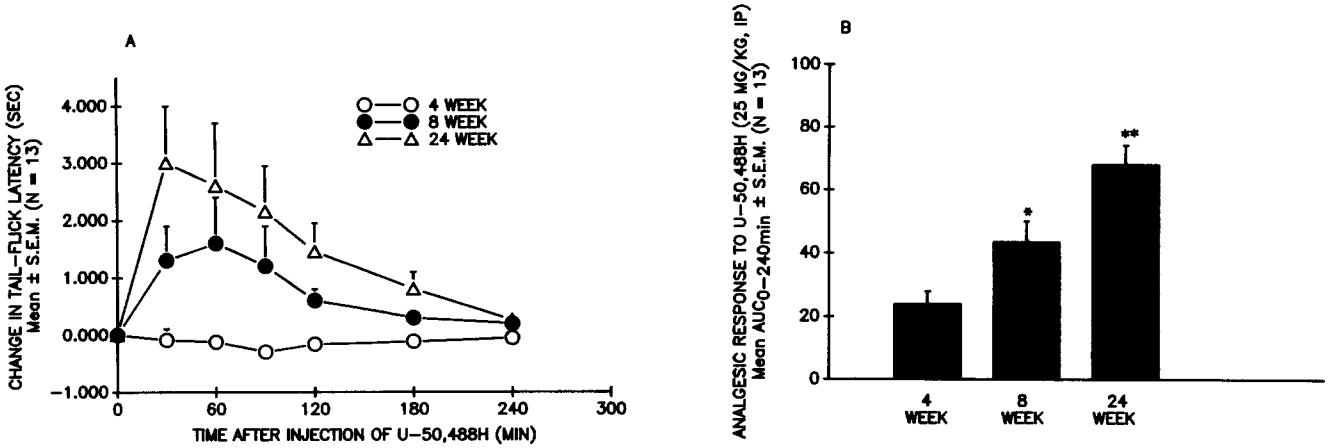

FIG. 1. U-50,488H-induced analgesia in rats of differing ages. Time course of the change in tail-flick latency following the administration of U-50,488H (25 mg/kg, IP) (panel A) and the analgesic response expressed as AUC_{0-240} min (panel B) in rats of differing ages. $*p < 0.05$ vs. 4-week-old rats; $**p < 0.05$ vs. 4- and 8-week-old rats.

Scheffe's S test. A value of $p < 0.05$ was considered to be significant.

RESULTS

Pharmacological Effects of U-50,488H in Rats of Differing Ages

The effect of U-50,488H (25 mg/kg) injected intraperitoneally on the time course of the change in the tail-flick latency of 4-, 8-, and 24-week-old rats is shown in Fig. 1A. Injection of U-50,488H prolonged the tail-flick latencies, the effect being much greater in the older rats compared to the younger rats. Not only the tail-flick latencies were greater in older rats, but the duration of action of U-50,488H was also much longer. The analgesic response to U-50,488H, expressed as AUC_{0-240} min, is shown in Fig. 1B. The analgesic response was least in the 4-week-old rats, highest in 24-week-old rats, and intermediate in 8-week-old rats. Thus, the analgesic response to U-50,488H increased with age of the rats.

The time course of the change in colonic temperature following the injection of U-50,488H (25 mg/kg) in rats of differing ages is shown in Fig. 2A. The maximum decrease in colonic temperature was observed in 24-week-old rats. An almost similar pattern was seen in 4-week-old rats. Rats (8-week-old) exhibited the least hypothermic effect which lasted only for 120 min. In contrast, the hypothermic effect of U-50,488H in 4- and 24-week-old rats lasted for 240 min. The hypothermic response converted into AUC_{0-240} min is shown in Fig. 2B. The hypothermic effect of U-50,488H in 4- and 24-week-old rats did not differ but was significantly greater than that found in 8-week-old rats.

Binding of [^{3}H] EKC to Kappa Opiate Receptors in Brain and Spinal Cord of Rats of Differing Ages

[^{3}H]EKC bound to whole brain and spinal cord membranes at a single high affinity site. The B_{max} and K_d values of [^{3}H] EKC to bind to whole brain membranes of rats of differing

Figure 2

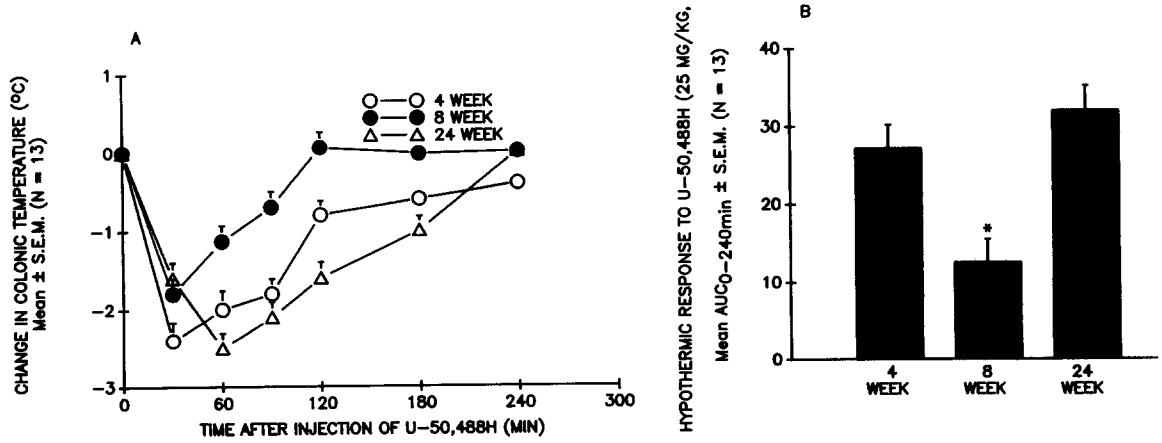

FIG. 2. Effect of U-50,488H on the colonic temperature of rats of differing ages. Time course of the change in colonic temperature of rats following the administration of U-50,488H (25 mg/kg, IP) (panel A) and the hypothermic response expressed as AUC_{0-240} min (panel B) in rats of differing ages. $*p < 0.05$ vs. 4- and 24-week-old rats.

TABLE 1

BINDING CHARACTERISTICS OF [^{3}H]EKC TO κ-OPIATE RECEPTORS IN BRAIN AND SPINAL CORD OF RATS OF DIFFERING AGES

Tissue	Age (Weeks)	B_{max} (fmol/mg Protein) Mean ± SEM	K_d (nM) Mean ± SEM
Brain	4	34.00 ± 2.41	3.22 ± 0.26
	8	28.89 ± 1.44	2.66 ± 0.29
	24	26.86 ± 1.41*	3.20 ± 0.19
Spinal cord	4	20.44 ± 0.85	2.79 ± 0.19
	8	20.10 ± 1.10	3.44 ± 0.28
	24	15.62 ± 1.17*	3.34 ± 0.40

$n = 5$. *$p < 0.05$ vs. 4- and 8-week-old rats.

ages is shown in Table 1. Although the B_{max} values appeared to decrease with age, there was no difference in the values for 4- and 8-week-old rats; however, the B_{max} value of 24-week-old rats was significantly lower than 4- and 8-week-old rats (Table 1). The K_d values of [^{3}H]EKC in the whole brain of rats from three age groups did not differ (Table 1).

The B_{max} and K_d values of [^{3}H]EKC that bind to kappa opiate receptors in spinal cord of rats of differing ages are shown in Table 1. As observed for the brain, the B_{max} values of [^{3}H]EKC in the spinal cord of 4- and 8-week-old rats did not differ. However, the B_{max} value was significantly lower in 24-week-old rats in comparison to 4- or 8-week-old rats (Table 1). The K_d values of [^{3}H]EKC in spinal cord of rats of three age groups did not differ (Table 1).

DISCUSSION

The present studies show that increasing analgesic response to U-50,488H, a kappa opiate agonist, is produced with increasing age of the rat. However, the hypothermic response to U-50,488H does not follow the same pattern. Finally, the κ-receptor density decreases with increasing age.

Opiates produce their analgesic response by interacting with μ, κ, and δ receptors; however, their relative contribution in the elicitation of the response is not clear, because agonists at the three receptors produce the antinociception (12). In general, the analgesic response to opiates is mediated by spinal, as well as, supraspinal structures, κ-opiates being more active at the spinal than supra spinal level (20). It has also been established that the analgesic response of a drug is directly related to its binding to the receptors. If greater response to the same dose of an opiate is produced, then it is possible that there is a greater affinity or the greater density of the target receptors in the spinal cord and/or brain. However, in the present study, the receptor density for κ site decreased in both brain and spinal cord with increasing age. Such an observation would be inconsistent with the enhanced response to U-50,488H with increasing age. However, the direction of change in colonic temperature in response to U-50,488H with increasing age was not the same as for the analgesic response. It is possible that the U-50,488H-induced analgesic and hypothermic responses may involve different mechanisms.

The mechanism by which increased analgesic response to U-50,488H with increasing age occurs is not known. Our previous studies had indicated that IV-administered morphine also produces an enhanced response in older rats than in younger rats (8), and that such effects could not be explained on the basis of differences in the pharmacokinetic parameters of morphine in serum. The opiate receptor density, as measured by the binding of ^{3}H-dihydromorphine, was found to be lower in the brain of older rats in comparison to younger rats. Thus, decreased density of μ opiate receptors in the brain would be inconsistent with the enhanced response to morphine in the older rats.

Previous studies from this laboratory have examined the distribution of morphine in the brain regions and spinal cord of 8- and 24-week-old rats. It was found that the enhanced response to morphine in the 24-week-old rats was related to the higher concentration of morphine in certain brain structures and spinal cord when compared with the younger rats (9).

In summary, the present studies show for the first time that U-50,488H produces greater analgesic response in 24-week-old rats in comparison to younger rats, and that such effects do not parallel the density of κ opiate receptors in brain and spinal cord. However, no consistent pattern was observed for the hypothermic action of U-50,488H with age in the rat.

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