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Kappa Opioid Modulation of Serotonergic Neurotransmission in the Hypothalamus, Hippocampus and Striatum in the Male Rat

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ABSTRACT

Objectives: We have investigated the modulatory effects of κ-opioid receptors on the central serotonergic system in the hypothalamus, hippocampus and striatum.

Materials and Methods: Male Sprague-Dawley rats (n=32) used in the experiments were divided into four groups. Anaesthetized animals were mounted on a stereotaxic apparatus and intracerebroventricularly infused with U-50488H (κ-agonist, 50μg/kg), Nor-BIN (κ-antagonist, 50μg/kg) or U-50488H + Nor-BIN. Control group received saline alone. All animals were decapitated 30 mins after infusions. Hypothalamus, hippocampus and striatum were microdissected and snap frozen. Concentrations of 5-hydroxytryptamin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA, 5-HT metabolite) in these brain regions were determined by HPLC-ECD.

Results: 5-HT and 5-HIAA values in the hypothalamus and hippocampus of U-50488H-treated group were significantly decreased compared to control group ($p<0.01$ and $p<0.05$). Indoleamine levels (ng/gr tissue) in the κ-antagonist group were found to be similar to or slightly lower than the control values ($p<0.05$). Nor-BIN reversed the U-50488H inhibition of 5-HT release in the agonist+antagonist treated group ($p<0.001$). 5-HT levels in the striatum of all three groups were significantly higher than control group ($p<0.01$), 5-HIAA concentrations in the same brain region significantly decreased ($p<0.001$). Ratio of 5-HIAA/5-HT in the hypothalamus, hippocampus and striatum was significantly ($p<0.05$ and $p<0.001$, respectively) lowered by the κ-agonist.

Conclusion: These results suggest that κ-opioid receptors modulate serotonergic neurotransmission in the hypothalamus, hippocampus and striatum.
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Key words: *Serotonin, kappa opioid receptors, U-50488H and Nor-BIN.*

ÖZET

Kappa Opioid Reseptörlerin Sıçan Hipokampus, Hipotalamus Ve Striatum Bölgelerinde Serotonerjik Nörotransmisyonu Modülasyonu

Amaç: Bu çalışma, kappa opioid reseptörlerinin hipotalamus, hipokampus ve striatum'da serotonerjik nörotransmisyon üzerine modülatör etkilerinin araştırılması amacıyla yapıldı.

Gereç ve Yöntem: Deneylerde kullanılan toplam 32 erkek Sprague-Dawley sıçan dört gruba ayrıldı. Anestezi altına alınan sıçanlar stereotaksik cihaza yerleştirildikten sonra, U-50488H (kappa agonist, 50μg/kg), Nor-Binaltorphimine (Nor-BIN, kappa antagonist, 50μg/kg) veya U-50488H + Nor-BIN intraserebroventriküler (ICV) yolla infüze edildi. Kontrol grubunu oluşturan hayvanlara ise aynı yolla serum fizyolojik uygulandı. ICV infüzyonlardan 30 dk sonra tüm sıçanlar dekapite edilerek beyinleri hızla kuru buz üzerine alındı. Mikrodiseksiyon yöntemiyle hipokampus, hipotalamus ve striatum bölgeleri çıkartılarak sıvı nitrojende dondurulduu. Bu beyin bölgelerindeki 5-hydroxytryptamin (5-HT) ve 5-hydroxyindole acetic acid (5-HIAA, 5-HT metaboliti) düzeyleri HPLC-ECD yöntemiyle belirlendi.

Bulgular: 5-HT ve 5-HIAA değerleri (ng/gr doku) U-50488H grubu hipotalamus ve hipokampus bölgelerinde kontrol grubuna göre anlamlı derecede azaldı ($p<0.01$ ve $p<0.05$). Kappa opioid antagonistinin tek başına verildiği grupta indolamin düzeylerinin kontrol grubu değerlerine yakın veya azalmış olduğu gözlendi ($p<0.05$). İki opioid ajanın kombine olarak uygulandığı grupta ise Nor-BIN'in, kappa agonistinin sebep olduğu serotonerjik inhibisyonu anlamlı derecede geri çevirdiği belirlendi ($p<0.001$). Striatumda elde edilen 5-HT düzeyleri her üç grupta da kontrol grubuna göre anlamlı olarak artmışken ($p<0.01$), aynı beyin bölgesinde belirlenen 5-HT metabolit (5-HIAA) değerleri ise oldukça anlamlı derecede azaldı ($p<0.001$).

Sonuç: Bu bulgular, kappa opioid reseptörlerinin hipotalamus, hipokampus ve striatum bölgelerinde serotonerjik nörotransmisyonu modüle ettiklerini göstermektedir.
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Anahtar kelimeler: *Serotonin, kappa opioid reseptörler, U-50488H ve Nor-BIN.*

There is a bulk of evidence to suggest that effects of opioids are mediated, at least in part, by the central serotonergic system (1, 2, 3). It has been reported that morphine enhances brain serotonin (5-hydroxytryptamine, 5-HT) synthesis and metabolism (4). Systemic administration of morphine produced significant increases in both extracellular 5-HT and 5-hydroxyindole acetic acid (5-HIAA; 5-HT metabolite) levels in the diencephalon, hippocampus and striatum in the rat (5). On the contrary, administration of a μ-agonist, diamorphine, has been shown to decrease 5-HT concentrations in specific regions of the hypothalamus (6). Furthermore, morphine increases gamma-aminobutyric acid tone on serotonergic neurons of the dorsal raphe nucleus which results in decreased 5-HT activity (7).

In the literature, most of the reports have examined the role of μ-opioid receptors in the modulation of the central serotonergic neurotransmission (2, 7, 8, 9). There are relatively few studies on the effects of κ-opioid receptors on the

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serotonergic system. U-50488H, a selective κ-receptor agonist, has been reported to have no effect on 5-HT release in rat brain slices containing the dorsal raphe nucleus (10). However, we have previously shown that intracerebroventricular (ICV) administration of another selective κ-opioid agonist, U-69593, significantly reduced concentrations of 5-HT in various nuclei of the hypothalamus in the female rat (6). Activation of κ-opioid receptors suppresses electrically evoked excitatory postsynaptic potentials on 5-HT-sensitive neurones in the rat dorsal raphe nucleus in vitro (11). U-50488H has been shown to inhibit K+-evoked 5-HT release in rat cortical synaptosomes (12). More recently, administration of κ-opioid agonists has been reported to reduce 5-HT content in the rat striatum (13).

Existence of κ-opioid receptors, along with the two other major opioid receptor subtypes (μ and δ) has been reported in various brain areas (14). κ-opioid receptors have been shown in the raphe nucleus and serotonergic axon terminals (15). Furthermore, gene expression of κ-opioid receptors has been reported in a number of brain regions, including the hypothalamus, hippocampus and striatum (16).

In the rat brain, several regions including the hypothalamus, hippocampus and striatum are extensively innervated by the serotonergic neurons located in the raphe nucleus (17, 18). In the central nervous system, 5-HT synthesis requires two enzymes: tryptophan hydroxylase and aromatic-L-aminoacid decarboxylase. Presence of these enzymes has also been shown by autoradiography in the rat brain areas containing serotonergic axon terminals (17).The present study was carried out to examine effects of κ-opioid receptors on the central serotonergic system in the male rat. Site-dependent action of κ-opioids on 5-HT release and turnover was also investigated in the hypothalamus, hippocampus and striatum.

MATERIALS AND METHODS

A total of 32 male Sprague-Dawley rats (Fırat University Biomedical Unit, Elazığ) weighing 230-260g were housed under controlled temperature (21±1 ^{o}C) and light conditions (lights on from 07.00h to 19.00h). Food and water were supplied ad libitum. They were divided into four groups. All experiments were carried out between 09.00-11.00h each morning.

Following chloral hydrate anaesthesia (400 mg/kg I.P.; Botafarma Laboratory, Ankara, Turkey), the rats were mounted on a stereotaxic apparatus and ICV infused with U-50488H (κ-agonist, 50μg/kg/6μl, n=8), Nor-Binaltorphimine (Nor-BIN, κ-antagonist, 50μg/kg/6μl; n=8) or U-50488H + Nor-BIN (n=8). Control group received sterile saline (6μl; n=8) alone. All animals were decapitated 30 mins after ICV manipulations, and their brains rapidly removed on dry ice. Hypothalamus, hippocampus and striatum were microdissected and snap frozen in liquid nitrogen. All experiments were performed in compliance with institutional and international guidelines for laboratory animal care.

Nor-BIN was purchased from Sigma Chem. Co. (St. Louis, USA). U-50488H was provided by Pharmacia-Upjohn (Kalamazoo, Michigan, USA).

Indolamine Analysis: Specific brain regions collected were stored at -80^{o}C prior to analysis. 600μl of 0.1M HCl was added to the samples, along with another 300μl of HCl containing 2ng of 3,4-dihydroxybenzylamine as an internal standard. The samples were homogenised and centrifuged at 3000rpm, 4oC for 10 mins. They were filtered through 0.45 μm microfilters (MFS Inc., USA). Aliquots (20μl) of supernatant were injected on to a reverse phase high performance liquid chromatographic (HPLC) column (S5ODS-250A, 5μm, 4.6mm i.d.x25cm, Supelco Inc.) coupled to an electrochemical detector (ECD, Gilson, France). Concentrations of 5-HT and 5-HIAA (5-HT metabolite) in these brain regions were simultaneously detected. The method has been described in our previous report (19). All brain samples were weighed out on an electronic scale prior to HPLC analysis, and the results were expressed as ng amine/g wet weight tissue.

Statistics: One-way analysis of variance (ANOVA) was performed on the data. Level of significance was set at $p<0.05$.

RESULTS

5-HT levels in the hypothalamus, hippocampus and striatum are shown in Figures 1-3. Concentrations of 5-HT metabolite (5-HIAA) and ratio of 5-HIAA/5-HT are summarised in Tables 1 and 2, respectively.

Table 1. 5-HIAA concentrations (Mean ± SEM) in three brain areas following ICV administration of κ-opioid agonist, antagonist or their combination. a: p<0.01, b: p<0.05, c: p<0.001, d: p<0.005 compared to the control group values, e: p<0.05 compared to the U-50488H group, One-Way ANOVA.

	Brain Regions		
Groups	Hypothalamus	Hippocampus	Striatum
Saline	347.3 ± 92.6	61.6 ± 14.0	228.4 ± 38.9
U-50488H	28.1 ± 8.9 a	26.3 ± 5.1 b	28.5 ± 8.3 c
Nor-BIN	66.9 ± 19.1 b	29.0 ± 5.5 b	54.7 ± 14.8 d
U-50+Nor-BIN	48.1 ± 5.3 e	22.2 ± 5.3	21.2 ± 3.5

Table 2. 5-HIAA/5-HT ratio (Mean ± SEM) in three brain areas following ICV administration of κ-opioid agonist, antagonist or their combination. a: p<0.05 b: p<0.001 compared to the control group; c: p<0.01 compared to the U-50488H-treated rats alone, d: p<0.05 compared to the Nor-BIN-treated animals, One-Way ANOVA.

	Brain Regions		
Groups	Hypothalamus	Hippocampus	Striatum
Saline	0.668 ± 0.171	0.414 ± 0.160	1.655 ± 0.182
U-50488H	0.093 ± 0.048 a	0.191 ± 0.028 a	0.102 ± 0.031 b
Nor-BIN	0.122 ± 0.025 a	0.206 ± 0.043	0.289 ± 0.072 c
U-50+Nor-BIN	0.092 ± 0.008	0.079 ± 0.009 d	0.053 ± 0.009 d

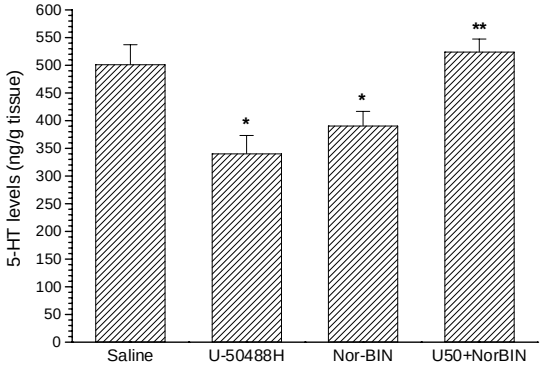

Figure 1. 5-HT concentrations (Mean ± SEM) in the hypothalamus of the male rat following ICV administration of κ-opioid agonist, antagonist or their combination. ***:** p<0.05 compared to the control group values, ******: p<0.05 compared to the U-50488H group, One-Way ANOVA.

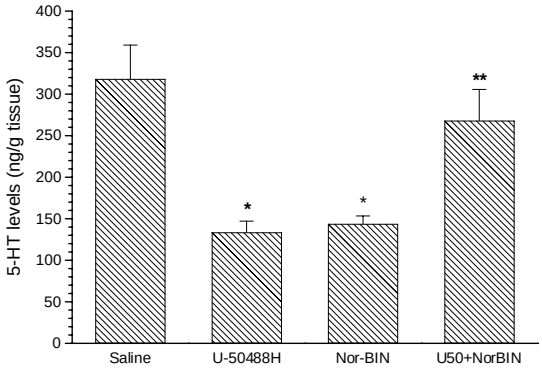

Figure 2. 5-HT concentrations (Mean ± SEM) in the hippocampus of the male rat following ICV administration of κ-opioid agonist, antagonist or their combination. ***:** p<0.05 compared to the control group values, ******: p<0.01 compared to the U-50488H group, One-Way ANOVA.

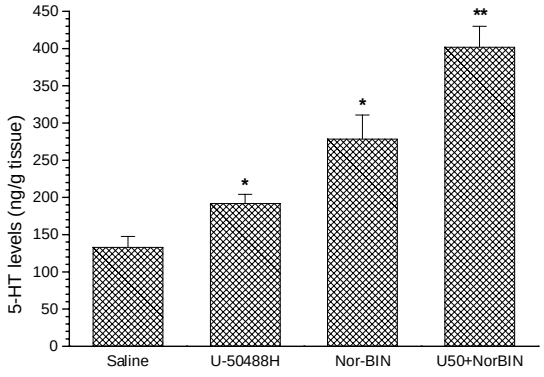

Figure 3. 5-HT concentrations (Mean ± SEM) in the striatum of the male rat following ICV administration of κ-opioid agonist, antagonist or their combination. ***:** p<0.01 compared to the control group values, ******: p<0.05 compared to the U-50488H group, One-Way ANOVA.

Ratio of 5-HIAA/5-HT in the hypothalamus, hippocampus and striatum was significantly (p<0.05 and p<0.001, respectively) lowered by the κ-agonist. Although administration of Nor-BIN elevated the ratio in all three brain areas, the increase was statistically significant (p<0.05) in only the striatum compared to those rats receiving U-50488H alone. Co-administration of Nor-BIN with U-50488H failed to antagonise the inhibitory effects of this κ-agonist on the 5-HIAA/5-HT ratio in the hypothalamus, hippocampus and striatum.

DISCUSSION

Previously, μ- and δ-opioid receptor agonists have been shown to inhibit 5-HT release from rat hippocampal slices in vitro (8). However, κ-opioid receptor agonist, U-50488H was less (about 50%) effective in suppressing K+-evoked release of 5-HT under similar conditions. However, it has been reported that U-69593 (a selective κ-agonist) had no significant effect on spontaneous release of 5-HT in the rat hippocampus monitored by microdialysis (3). In the present study, the same κ-receptor agonist found to be significantly effective in reducing the concentrations of 5-HT and its metabolite in the hippocampus. The ratio of 5-HIAA/5-HT was also lowered. Although a selective κ-antagonist resulted in similar decreases when applied alone, it antagonised the inhibitory effects of U-50488H following combination of the two agents. Thus, the present findings are taken to suggest that κ-opioid receptors exert an inhibitory effect on the serotonergic neurotransmission in the hippocampus.

The hypothalamus receives extensive serotonergic projections originating from the midbrain raphe nuclei (17). Serotonergic fibres have been visualised in various nuclei of the hypothalamus, and their anatomical distribution overlaps with hypothalamic factor-releasing neurons (17, 20). The highest basal mRNA levels for κ-opioid receptors are found in the hypothalamus in the rat (16). There have been very few attempts to examine effects of κ-opioid receptors on 5-HT release in the hypothalamus. We have previously shown that ICV administration of U-69593 significantly decreased indolamine levels in the medial preoptic area, suprachiasmatic nucleus, arcuate nucleus and median eminence of the hypothalamus (6). Tifluadom, a κ agonist had no significant effect on the indoleamine release and/or turnover in specific hypothalamic areas in the female rat (21). In the present experiments, U-50488H significantly reduced concentrations of 5-HT and 5-HIAA in the total hypothalamus in the male rat. The inhibitory effects of the κ-agonist on the indolamine levels were reversed by Nor-BIN following its co-administration of with U-50488H. These findings indicate that κ-opioid receptors modulate the serotonergic release and turnover in the hypothalamus, and their influence is inhibitory.

In the striatum (9) and raphe magnus (22), 5-HT concentrations were increased following administration of morphine. Effects of κ opioids on serotonergic system in the striatum are not well-studied. A recent microdialysis study has shown that systemic administration of salvinorin A, a potent and highly selective κ-opioid agonist had no significant effect on 5-HT concetrations in the nucleus accumbens in the rat (23). In the present study, 5-HT concentrations were elevated in the striatum by both U-50488H and Nor-BIN or their combination. This is somewhat surprising since 5-HIAA levels and ratio of 5-HIAA/5-HT were significantly lowered by κ-agonist and increased by κ-antagonist alone. It might be that control group neurotransmitter levels rapidly metabolised to 5-HIAA in this brain region.

It has been suggested that effects of morphine on 5-HT synthesis and release are regionally selective (9). In order to examine area dependent effects of κ-opioid receptors, indolamine concentrations in three different brain regions were determined in our study. In view of our inconclusive findings in the striatum, the effects of κ-opioids on the serotonergic system may be site-dependent. It appears that the κ-opioid agonist lowers 5-HT synthesis by inhibiting its release from the nerve terminals as both 5-HT and 5-HIAA levels were reduced in parallel in the hypothalamus and hippocampus. This inhibition is probably exerted through their action at pre-synaptic nerve terminals (11).

In conclusion, our results suggest that κ-opioid receptors modulate serotonergic neurotransmission in the hypothalamus and hippocampus. This effect of κ-opioids may be site-dependent in different brain regions. Furthermore, inhibition of 5-HT synthesis and release in the hypothalamus implicates that κ-opioid receptors may affect secretion profiles of various hypothalamic hormones by serotonergic mediation.

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