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Regulation of Histamine Release by *Kappa* Opioid Receptor in Rat Cortical Slices

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ABSTRACT

It has been shown that there are several subtypes of κ opioid receptor. We examined ligand binding profiles and the effects of various opioid agonists on high potassium-stimulated release of [^{3}H] histamine. We have evaluated the properties of non-μ, non-δ binding of [^{3}H] DIP ([^{3}H] diprenorphine), a nonselective opioid antagonist, in rat cortex membranes. Binding to μ and δ sites was inhibited by the use of an excess of competing selective agonists (DAMGO, DPDPE) for these sites. (−) Ethylketocyclazocine (EKC), DIP and bremazocine inhibited [^{3}H] DIP binding. However, arylacetamides (U69593 and U50488H) gave little inhibition Replacement of sodium by NMDG and the addition of guanine nucleotide influenced the inhibitory potency of (−) EKC, an agonist for κ_1-and κ_2-binding site, but not of bremazocine. This result suggests that bremazocine can be an antagonist at this binding site. Also, we have examined the opioid modulation of K^+ (30mM) -induced [^{3}H] histamine release in rat frontal cortex slices labeled with l-[^{3}H] histidine. The [^{3}H] histamine release from cortex slices was inhibited by EKC in a concentration-dependent manner. However, the δ receptor selective agonists, DPDPE and deltorphine II, μ receptor agonists, DAMGO and TAPS, κ_1-agonists, U69593 and U50488H, and ε-agonist, β-endorphin, did not. The concentration-response curve of EKC was shifted to right in the presence of naloxone, nor-binaltorphimine and bremazocine, respectively. These results suggest that κ_2 opioid receptor regulates histamine release in the fromtal cortex of the rat.

Key Words: Kappa opioid, Receptor binding, Histamine Release, Rat, Cortex

Abbrebiations: DIP, diprenorphine; EKC, ethylketocyclazocine; DAMGO, Tyr-D-Ala-Gly-(Me) Phe-(Gly-ol); DPDPE, [D-Pen^2, D-Pen^5] enkephalin; U69593, (5a, 7a, 8b)-(-)-N-Met-N-(7-[1-pyrolidinyl]-1-oxaspiron[4,5]-Dec-8-yl) benzenacetamide; nor-BNI, nor-binaltorphimine;

INTRODUCTION

The existence of three major types of opioid receptor, i.e., μ, δ and κ, in mammalian brain is now well accepted (for review see Simon and Giannini, 1993). The concept of the multiplicity of opioid recptors has veen enlarged because of many pharmacological and biochemical data suggesting the presence of subtypes of opioid receptors.

As with opioid μ and δ receptors are Attali *et al.*, 1984), it nas been proposed that there are multiple kappa receptors in mammaliam brain (Pfiffer *et al.*, 1981; Attali *et al.*, 1982). In neural membrane receptor binding study, the κ_1 receptor

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is of high affinity and predominates in guinea pig brain, and is sensitive to U69593. The κ_2 receptor is of low affinity, predominates in rat brain and is U69,593-insensitive (Zukin *et al.*, 1988). Thus it has been postulated that there are distinct physilolgical roles of κ opioid subtypes. In contrast to κ_1 opioid receptor which shows high affinity to arylacetamides (for example U69593) as an agonist, and nor-BNI as an antagonist, the functional evidence of existence of κ_2 opioid receptors has not been fully elucidated largely because of lack of selective ligands at this site. Recently, Kim and Cox (1993a) reported the regulatory role of κ_2 site on norepinephrine release in rat cortex.

Opioids have been demonstrated to regulate the release of a number of neurotransmitters by interaction with presynaptic receptors in central and pepheral nervous system (for review see Illes, 1989). Endogenous opioids derived from both proenkephalin and prodynorphin are present in rat cortex (Yang *et al.*, 1977; Cone *et al.*, 1983). The histaminergic projection of the mesencephalic reticular formation and mammillary body to the cortex is shown to be under opioid receptor control (Itoh *et al.*, 1988; Nishibori *et al.*, 1985). Thus, it seems likely that endogenous opioids exert a significant role in regulation of histamine function in cortex. The sedative dffect of κ opioid agonists was related with the inhibition of histamine release (Wollemann *et al.*, 1993). However, clear understanding of the role of opioids on histaminergic neuron function remains to be established. Gulat−Marnay *et al.*, (1990) initially reported that various κ opiod agonists, including arylacetamides (U69593 and U50488H), inhibited high potassium-stimulated release of histamine from rat cortex slices in vitro. These authers therefore suggested that κ opioid receptors were able to exert a inhibitory effect on histamine release in rat cortex. In the meanwhile, this result could be challenged by the fact that there are little κ_1 opioid receptor in rat cortex in neural membrane and autoradiographic receptor binding (Kim and Cox, 1991; Unterwald *et al.*, 1991). Developoment of novel ligands with greater selectivity at κ opioid receptors and the subsequent refinement of the concept of receptor multiplicity has allowed us to reexamine the opioid receptor-mediated inhibition of histamine release in cortex.

In an attempt to examine the roles of κ opioid receptor subtypes in regulating the release of histamine in the cortex of the rat, we compared ligands binding profiles and the effects of various opioid agonists on high potassium-stimulated release of [^{3}H] histamine.

MATERIALS AND METHODS

Chemicals

Chemicals and reagents were obtained from following sources: [^{3}H] diprenorphine (46.5 Ci/mmol) and L-[2,5-^{3}H] histidine (52Ci/mmol) were purchased from Amersham (Arlington Heights, IL); DAMGO, DPDPE and bremazocine from Research Biochemicals, Inc. (Natick, MA), EKC methane sulfonate from Sterling Winthrop (Rensselaer, NY); U69,593 and naloxone hydrochloride from Sigma Chemical Co. (St. Louis, MO).

Preparation of Neural Membranes

Male Sprague-Dawley rats (250~300 g) were sacrificed by decapitation, and their brains removed to ice. Cortices were collected in modified Krebsd-Hepes buffer (NaCl, 118 mM; KCl, 4.8 mM; $CaCl_2$, 2.5 mM; $MgCl_2$, 1.2 mM; Hepes, 25 mM; pH adjusted to 7.4) and chilled on ice. In some experiments, NaCl was replaced by NMDG (N-Methyl-D-Glucamine). NMDG has previously been used as a Na^+ substitute in other neurotransmitter release study (Connolly and Limbird, 1983). Each tissue was homogenized in 10 vol of buffer with a Polytron homogenizer at a setting of 70. The homogenate was centrifuged at 27,000×g for 15 min at 4℃, and resuspension in ice-cold buffer. Membranes were stored frozen at −70℃ at a concentration of 2% weight/volume.

Binding assay

Membranes were thawed at room temperature and homogenized by 10 strokes in a teflon glass homogenizer. For κ receptor assays, DAMGO (1 μM) and DPDPE (1 μM) were added to each tube in order to block μ and δ opioid sites. These con-

centrations of site selective displacing ligands were selected from an analysis of their potencies as displacers of each labeled ligand. After all additions, the final membrane concentration in the assay tube was 1% w/v, corresponding to about 300~500 μg protein per sample. All drug solutions were prepared in Krebs-Hepes buffer, pH 7.4. Final volume in each tube was 500 μl. Triplicate samples of membrane suspension were preincubated with or without non-radioactive displacer at 37℃. Radiolabeled ligand (0.5 nM of [^{3}H]DIP) was then added and the incubation continued 20 min unless otherwise stated. The incubation was terminated by the addition of four ml ice-cold buffer and rapid filtration through glass fiber filters (Type G-7; Inotech, Zürich, Switzerland) under reduced pressure by using cell harvester (Inotech). The filters were washed with an additional four ml buffer, transferred to scintillation vials, soaked in an half ml each absolute ethanol, and counted in 3 ml scintillation coctail (Aqualuma plus; Lumac LSC, Olen, Belgium) at an efficiency of about 40%. Non-specific binding was defined as the fraction of bound radioligand that remained in the presence of 1 μM DAGO, 1μ M DPDPE and 10 μM naloxone. Specific binding to membranes was always less than 4% of added radioligand.

Analysis of binding data

Saturation and displacement data were analyxed by the use of the computer program LIGAND (Munson and Rodbard, 1980). This program utilizes a non-linear least squares curve fitting algorithm, and assumes the simultaneous contribution of one or more independent binding sites.

[^{3}H] Histamine release

Male Sprague-Dawley rats (200~300 g) were sacrificed by decapitation and the cortex dissected from each brain. The tissue was chopped into 250 μm slices with a Mcllwain mechanical tissue chopper (The Mickle Laboratory Engineering Co., Surrey, England). The chopped cortices were dispersed in oxygenated modified Krebs-HEPES buffer (KHB: 25 mM HEPES-sodium salt, 100 mM NaCl, 5 mM KCl 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 10 mM glucose, 0.1 mM ascorbic acid) by trituration through a plastic transfer pipet. The washed slices were incubated with 20 ml of KHB containing 30 mM of K^+ for twice at 5 min interval to enhance the synthesis of histamine (Verdiere *et al.*, 1975). Then, slices were incubated with 330 μM L-[2,5-^{3}H] histidine (Amersham, Arlington Heights, IL) at 37° C for 30 min. After ten rinses with drug-free KHB, the slices were rinsed once more in buffer containing 30 nM thioperamide to block presynaptic H_3-receptor-mediated inhibition of histamine release. Thioperamide was subsquently present in all the incubation media. Equal aliquots of tissue slice suspensions were transferred to nylon mesh baskets and incubated in 2 ml of oxygenated KHB in 24 well tissue culture plates for 10-min periods. After determination of base-line release, the tissues were transferred to wells containing 30 mM K^+ in KHB for a further 10-min incubation. Finally, the tissues were transferred to wells containing 0.2 N HCI for 45 min to extract the [^{3}H] histamine present in tissue.

Competitive antagonists were added 10 min prior to the 10 min baseline release period and were present throughout the experiment; opioid agonists were present during the 10 min baseline release and stimulation periods.

High potassium-stimulated release of [^{3}H] histamine was defined as the amount of raioactivity in the medium containing 30 mM K^+, minus the amount released into control buffer containg 5 mM K^+ (base-line release), expressed as a percentage of fractional release relative to the calculated total tissue content of [^{3}H] histamine at the initiation of each release period. Drug or peptide treatments were tested in quadruplicate in each experiment, and each experiment was performed at least 3 times.

The differences in the inhibition curves produced by opioid agonists in the presence or absence of antagonist were tested as follow; Plots of probit conversions of the percent inhibition of stimulatd release of [^{3}H] histamine against the log of the concentration of each agonist in the presence and absence of three concentrations of competitive antagonist were tested for deviations from parallelism, using the program ALLFIT (de Lean *et al.*, 1988). pA_2 values were calculated from log (DR-1) v. log. antagonist concentration plots (Schield plots) when the slope of the plot did not

differ significantly from unity as described by Tallarida and Murray (1986).

RESULTS

Receptor binding characteristics of κ opioid ligands in rat cortex membranes

Competition of κ opioid ligands in [^{3}H]DIP binding: In rat cortex membrane preparations, when μ- and δ-opioid receptors are blocked [^{3}H] DIP binding was inhibited by DIP, (−) EKC and bremazocine, respectively, but not by U69 (Fig. 1). In each case, the data were better fit by a single-site model than by more complex model. This data shows the existence of κ_2 opioid receptor in this preparation.

Effects of sodium replacement on ligand binding: When sodium in the modified Krebs buffer was replaced by an equimolar concentration of NMDG, Ki values of (−) EKC, an agonist for κ_1 and κ_2 opioid receptors, was decreased by a factor of about an half, however, that of DIP, a nonselective opioid antagonist, was not changed. It was interesting to note that Ki value of bremazocine was not changed as in the case of DIP (Table 1).

Effects of GTPγsS on ligand binding: We tested the effects of 2 concentrations of GTPγS, a nonhydrolyzable guanine necleotide analogue, on the ability of κ ligands to compete against [^{3}H] DIP binding in rat cortex membranes. Firstly, in order to confirm that GTPγS had no effect on antagonist affinity for the site which [^{3}H] DIP labeled in this experimental condition, we constructed competition curves for DIP in the presence and absence of 50 or 100 μM of GTPγS.

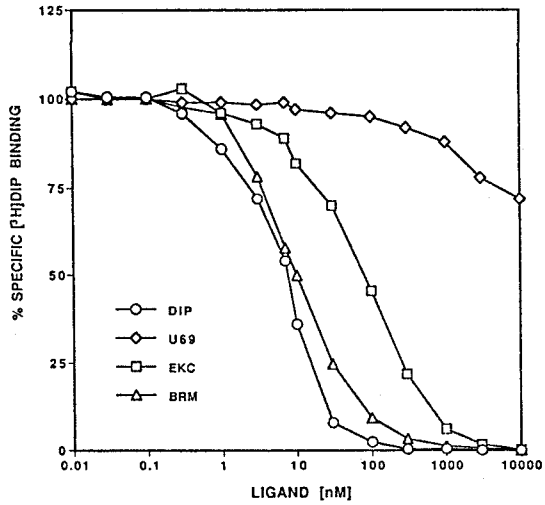

Fig. 1. Comparison of inhibitory potency of diprenorphine (DIP), U69,593 (U69), bremazocine (BRM) and (−) ethylketo-cyclazpcine (EKC) on the [^{3}H] DIP binding in the presence of excess amount of DAMGO and DPDPE as a blocking ligands for μ and δ opioid receptors in rat cortical membrane.

Table 1. The effects of GTPγS and Na^+ on the Ki values of κ-opioids at the binding site for [^{3}H] diprenorphine in the presence of DAMGO (1 μM) and DPDPE (1 μM) in rat cortex membranes

Ligands	Ki (nM)			
	control	$-Na^+$	+GTPγS(50 μM)	+GTPγS(100 μM)
Diprenorphine	9.5±0.8	8.2±0.7	9.7±1.0	9.8± 1.1
(−)EKC	75.8±6.8	40.2±3.5[a]	83.6±8.7	519.0±46.7[a]
Bremazocine	5.2±0.4	5.7±0.5	6.1±0.7	5.8± 0.6
U-69,593	>10,000	ND	ND	ND

EKC: Ethylketocyclazocine. ND: Not determined. Ki values were derived by the computer program LIGAND. Each value represents the mean of three independent experiments with SEM. All experiments were conducted at 37° C in the presence of DAMGO (1 μM) and DPDPE (1 μM). Modified Krebs-Henseleit buffer containing 125 mM NaCl was used with GTPγS (50 or 100 μM). For experiments in which the bining assays were conducted in sodium-free buffer, NaCl was replaced with iso-osmolar N-Methyl-D-Glucamine. In each case, the data were better fit by a single-site model than by more complex model. a: Significantly different from the control value ($p>0.05$).

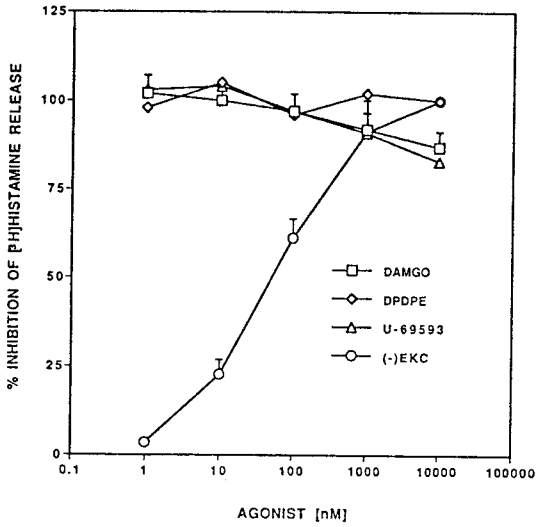

Fig. 2. Influences of DAMGO, DPDPE, U69 and (−) EKC on high K^+- stimulated release of [^{3}H] histamine in rat cortex.

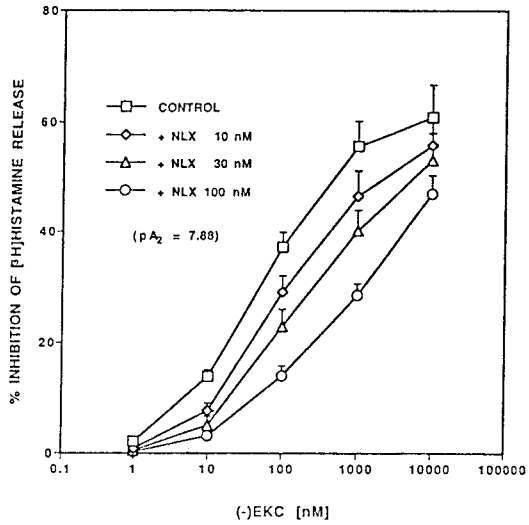

Fig. 3. Antagonism of inhibitory effect of (−) EKC on high K^+- induced release of [^{3}H] histamine by a selective opioid antagonist, naloxone.

There was no significant changes in the affinity DIP for this site (Table 1). Having ascertained that the affinity of antagonist, DIP, was not affected by the addition of GTPγS, we used this antagonist to radiorabeled opioid binding site and examined the pattern of ligand competition produced in the presence and absence of 2 doses of GTPγS. The addition of 100μM GTPγS caused no change in the Ki value of bremazocine (Table 1). This data with the insensitivity to sodium replacement showed that bremazocine can be an antagonist at this site, κ_2 opioid receptor. Fifty μM of GTPγS, however, did not influence the Ki value of any ligand teseted.

The knowledge of agonistic and antagonistic binding characteristics of (−) EKC and bremazocine from above described data, lendered us to examine the functional role of this sites in same portion of brain, cortex.

Influences of various opioids on high potassium-stimulated release of [^{3}H] histamine in rat cortex slices

Effects of opioid agonists: In order to examine the responsible opioid receptor subtype in regulation of [^{3}H] histamine in rat cortex, we compared the influences of several opioid agonists. The baseline release of radioactivity in cortex slice preparations preloaded with [^{3}H] histidine in modified Krebs buffer containing 5 mM K^+, over a period of 10 min, was usually less than 5~7% of the radiolabel present in the tissue. High K^+ (30 mM) in the incubation medium stimulated the release of 28.7±3.1% of total [^{3}H] NE of rat cortical slices. DAMGO, DPDPE and U69 and, agonist for μ, δ or κ_1 opioid receptor, respectively, did not influence the 30 mMK^+-stimulated release of [^{3}H] histamine in this preparation. Among the tested agonists, only (−) EKC inhibited the [^{3}H] histamine release in dose-dependent manner (Fig. 2). At a concentration of 10 μM, (−) EKC produced a maximum inhibition of stimulated [^{3}H] histamine of about 60%. The concentration of (−) EKC producing 50% of the maximum inhibitory effect of (−) EKC under these condition was about 56 nM.

Influences of opioid antagonists on the inhibiktory effect of (−) EKC: (−) EKC known as an agonist for goth of κ_1 and κ_2 opioid receptor, we tested the sensitivity of inhibition by (−) EKC to naloxone, a nonselective antagonist, nor-BNI, a selective κ opioid antagonist which possess higher

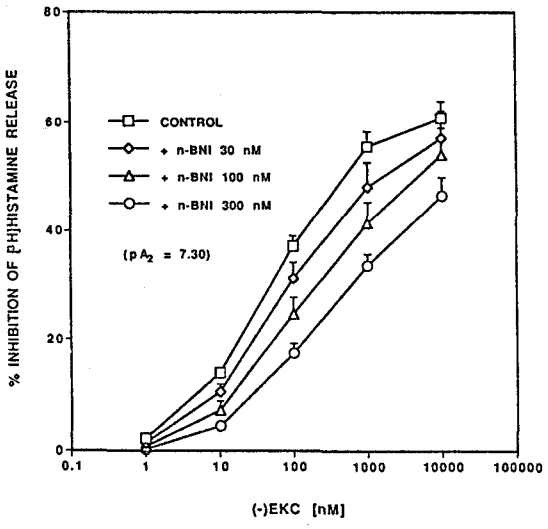

Fig. 4. Antagonism of inhibitory effect of (−) EKC on high K^+-stimulated release of [^{3}H] histamine by a selective κ opioid antagonist, nor-binaltorphimine (nor-BNI).

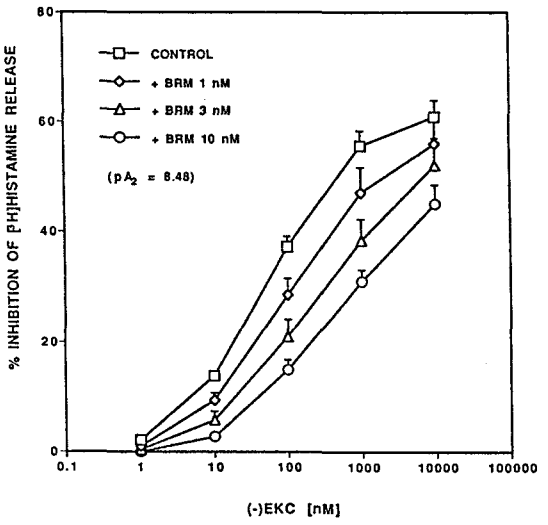

Fig. 5. Antagonism of ingibitory effect of (−) EKC on high K^+-induced release of [^{3}H] histamine by bremazocine.

affinity for κ_1 site, and bremazocine, an agonist at κ_1 site, also an antagonist at κ_2 site. All three antagonists shifted the concentration-response curves of (−) EKC to the right; curves in the presence of antagonist were parallel ($p>0.05$) to curves in the absence of antagonist (Fig 3, 4 and 5). The rank order of degree of antaonism was bremazocine (8.4 ± 0.2)>naloxone (7.8 ± 0.8)>nor-BNI (7.3 ± 0.1) (Fig. 3, 4 and 5).

DISCUSSION

It has been widely accepted that guanine nucleotides and Na^+ ion decrease opioid agonist binding without affecting antagonist binding (Childers and Snyder, 1980; childers, 1988). Taking advantage of the use of radiolabeled antagonist, we employed [^{3}H] DIP as a ladiorabeled ligand in binding experiment. In our study, firstly, we compared several known κ opioid ligands in their fashion of competition with [^{3}H] DIP at the κ opioid receptors in rat cortex membranes. Secondly, we examined the sensitivities of ligand competition with [^{3}H] DIP to dodium replacement and to the addition of GTPγS. When μ- and δ-opioid receptors are blocked [^{3}H] DIP binding was inhibited by DIP, (−) EKC and bremazocine, respectiveluy, but not by U69. These data shows that there are opioid binding sites which has ligand selectivity similar to κ_2 opioid receptor reported in elsewhere (Kim and Cox, 1991; Nock *et al.*, 1990). The sensitivity of opioid receptors to sodium was first demonstrated by Simon *et al.* (1973) and pert *et al.*, (1973). Opioid receptors are much more sensitive to regulation by sodium than by other monvalent cations, althogh all cations have variable effects on opioid binding (Simantov *et al.*, 1976). The effects of sodium was ususally attributed to a reduction of opioid agonist affinity (Simon *et al.*, 1975), brought about largely through an increase in the rate of dissociation (Blume, 1978). (−) EKC is presumed to be an agonist at κ_1 and κ_2 receptors (Petit *et al.*, 1991; Kim and Cox, 1993). Replacement of sodium by NMDG decreased Ki values of (−) EKC. In accordance with other reports, this data shows that (−) EKC acts as an agonist for κ_2 opioid receptor. However, the degree of decrease of Ki value was less than the value for the κ_1 sites in our unpublished report. This suggests that κ_2 site is less sensitively regulated by sodium ion

than κ_1 site. The differential sensitivity of different types of opioid receptors are well documented (Childers, 1988). It is well established that the transduction systems for opioid receptors require agonist-induced association of the receptor with a guanine nucleotide binding protein (G protein) (Childers, 1988). In the presence of guanine nucleotide and Na^+, agonist dissociation rate is increased, and the affinity of the receptor for agonists is reduced (Blume, 1978; Childers *et al.*, 1980). Antagonist binding, however, is not regulated by guanine nucleotide (Pert *et al.*, 1973; Puttfarken *et al.*, 1986; Werlling *et al.*, 1986). It is intersting to note that competition of bremazocine, a benzomorphane derivative reported as an agonist at κ_1 sites and an antagonist for κ_2 sites, with [^{3}H]DIP at these sites was not influenced by neither replacement of sodium nor addition of GTPγS. This data implies that bremazocine behaves as an antagonist for κ_2 sites in rat cortex. Tiberi and Magnan (1989), also, reported the insensitivity of [^{3}H] bremazocine binding to a non-hydrolysable analogue of GTP, guanyl-5'-yl imidophosphate (Gpp(NH)p), in saturation experiment in guinea pig spinal cord neural membranes. Antagonistic action of bremazocine can be seen in other functional assay. Kim and Cox (1993) reported that the inhibition of potassium-stimulated release of norepinephrine was inhibited by low concentrations of bremazocine in rat cortex.

Opioids exert many of their physiological actions thruogh modulation of release of certain neurotransmitter that play regulatory role in various neuronal systems. These regulations were achieved by activation of one or more type(s) of opioid receptors. For example, all three major types of opioid receptors rehulate the release of norepinephrine from guinea pig cortex (Werlling *et al.*, 1987), whereas κ receptor mediates presynaptic regulation of dopamine release from guinea pig striatum (Werlling *et al.*, 1988). Localization of histaminergic nerve terminals and κ_2 receptor might role as a modulator of histaminergic transmission, In the present study, high potassium-stimulated release of [^{3}H] histamine in our experiment. These data suggest that histaminergic neurotransmission is not regulated by any other opioid receptors but only κ_2. It has been shown that bremazocine was most potent among three tested antagonists, and nor-BNI was more potent than maloxone as an inhibitor of radiorabeled ligand binding to κ_2 sites (Tiberi and Magnan, 1990). In our study, there are differences in the degree of antagonism of the inhibitory effect of (−) EKC by three opioid antagonists. The pA_2 value for bremazocine against (−) EKC was much higher than that of any other antagonist against this rug. The rank order of the degree of antagonism was bremazocine > nor-BNI > naloxone. These data implicated the κ_2 opioid rehulation of histamine release again. While, Gulat-Marnay *et al.*, (1990) reported that high potassium-stimulatd release of [^{3}H] histamine from rat cortex slices was inhibited by various opioid agonists that selective agonists for κ_1 receptor and agonist for κ_1 and κ_2 receptor, also, the inhibition by ketocyclazocine, an agonist for κ_1 and κ_2 opioid receptors, was antagonized by nor-BNI with IC50 value of about 20 nM. Although some differences are exist in experimental methods, we cannot explain this discrepancy. On the while, it is generally accepted that there are little binding sites for arylacetamides in rat cortex, especially in the presence of sodium (Kim and Cox, 1991). And, it has been reported that the IC50 value of nor-BNI for an agonist acting at κ_1 receptors is about 1 nM in guinea pig cortex (Kim and Cox, 1993)

In conclusion, our data suggests that the regulation of high potassium-stimulated release of [^{3}H] histamine is mediated by κ_2 opioid receptor in rat cerebral cortex. And, this may explain the sedative dffect of κ opioid agonists in experimental animals and human.

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=국문초록=

백서 대뇌피질에서 Opioid Kappa수용체의 Histamine유리조절기능에 관한 연구

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Opioid-수용체에는 μ, δ그리고 κ의 세가지 주된 형이 존재함이 알려져 있는 바, 최근 수용체 동정 기법과 선택적인 약물의 개발로 인해 그 아류들이 존재함이 보고되고 있다. 그러나 opioid κ_2-수용체의 기능에 대해서는 이 수용체에 있어서의 선택적인 효현제 또는 길항제가 밝혀져 있지 않으므로써 현재까지 잘 알려져 있지 않다. 본 연구에서는 백서 대뇌피질 세표막표본에 opioid μ와 δ-수용체를 과량의 DAMGO와 DPDPE로 봉쇄한 후 수종 κ수용체 결합자의 [^{3}H] idprenorphine (DIP) 결합억제효과와 이에 대한 sodium과 GTYγS의 영향을 관찰하여 이를 지표로 각 ligand의 수용체에서의 작용양상을 검토하여 이를 토대로 동일 표본에서 [^{3}H]diprenorphine([^{3}H]DIP) 결합은 DIP, ethylketocyclazocine 그리고 bremazocine에 의해 효과적으로 억제되었으나, κ_1수용체 효현제인 U69593에 의해서는 억제되지 않았다. Opioid κ_1 및 κ_2-수용체 효현제인 EKC의 Ki치는 배양액내 NaCl을 NMDG로 알려진 GTPγS 100μM 첨가에 의해 증가되었다. 그러나 Bremazocine과 DIP의 Ki치는 soduium 제거 또는 GTPγS 100μM 첨가에 의해 증가되었다. 그러나 Bremazocine과 DIP의 Ki치는 sodium 제거 또는 GTPγS 첨가에 의해 영향받지 않았다. 1-[^{3}H] histidine을 미리 부하한 대뇌피질 절편에서 30mM K^+에 의한 [^{3}H] histamine의 유리는 (−)EKC에 의하여 영향받지 않았다. (−)EKC의 histamine유리 억제효과는 naloxone, norbinaltorphimine 또는 bremazocine에 의하여 각각 억제되었다. 본 실험 성적은 백서 대뇌피질에서 histamine의 유리는 이 부위에 존재하는 opioid κ_2-수용체에 의하여 조절됨을 시사한다.