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Regulation of [^{3}H]Norepinephrine Release by Opioids in Human Cerebral Cortex

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To investigate the receptors mediating the regulation of norepinephrine (NE) release in human cerebral cortex slices, we examined the effects of opioid agonists for μ-, δ-, and κ-receptors on the high potassium (15 mM)-evoked release of [^{3}H]NE. [^{3}H]NE release induced by high potassium was calcium-dependent and tetrodotoxin-sensitive. [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and deltorphin II (Delt II) inhibited the stimulated release of norepinephrine in a dose-dependent manner. However, Tyr-D-Ala-Gly-(Me)Phe-Gly-ol and U69,593 did not influence the NE release. Inhibitory effect of DPDPE and Delt-II was antagonized by naloxone, naltrindole, 7-benzylidenaltrexone and naltriben. These results suggest that both δ_1 and δ_2 receptors are involved in regulation of NE release in human cerebral cortex.

Key Words: Opioid receptors, Norepinephrine release, Human cerebral cortex

INTRODUCTION

Opioids exert many of their physiological actions through modulation of release of neurotransmitters that play regulatory roles. Since the inhibition of the electrical depolarization-induced release of [^{3}H]norepinephrine ([^{3}H]NE) by morphine in the rat cortical slices was reported (Hayes et al, 1983), subsequent studies have shown opioid inhibition of NE release in the terminal fields of various species (for review see Montel et al, 1974). There are species differences in the types of opioid receptors mediating this action (Limberger et al, 1986; Werling et al, 1987; Illes, 1989; Werling et al, 1989; Kim & Cox, 1993).

In human cerebral cortex, it has been reported that opioids regulate the release of certain neurotransmitters. Opioid δ and κ receptors inhibit the stimulated release of acetylcholine (Feuerstein et al, 1996). Electrically stimulated release of NE was inhibited by nociceptin/Orphanin FQ (Rominger et al, 2002). We have previously demonstrated the inhibition of high-potassium-evoked release of [^{3}H]NE by [D-Pen^2, D-Pen^5]enkephalin (DPDPE) in human cerebral cortex slices (Kim et al, 2000). Recently, we reported the existence of two subtypes of δ opioid receptors, δ_1 and δ_2, in human cerebral cortex (Kim et al, 2001).

To characterize the responsible receptors for regulation of NE release in human cerebral cortex, we examined the effects of agonists for μ, δ (δ_1 and δ_2) and κ opioid receptors on the high potassium-stimulated release of [^{3}H]NE in human cerebral cortex slices.

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METHODS

Subjects

Parts of temporo-basolateral region of cerebral cortex were surgically removed from 8 female and 8 male patients (age 24.7±5.6 years). The subjects underwent neurosurgery for severe epilepsy that was not controlled by antiepileptic drugs. Prior written consent from the patient and clearance from the Ethics Committee were obtained for the use of a part of cerebral cortex of epileptic patients. After premedication with atropine, fentanyl, and flunitrazepam, anesthesia was induced by thiopental and maintained with nitrous oxide and isoflurane.

Measurement of [^{3}H]NE release

The human cerebral cortex tissues (300 mg/plate) were chopped into 220 μM slices as described previously (Werling et al, 1997). The chopped tissues 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, pH 7.4). Then, slices were incubated with 15 nM [^{3}H]NE (Specific Activity, 30~50 Ci/mmol) at 37°C for 15 min. After two rinses with drug-free KHB, the slices were rinsed once

ABBREVIATIONS: NE, norepinephrine; DPDPE, [D-Pen^2, D-Pen^5] enkephalin; Delt II, deltorphin II; DAMGO, Tyr-D-Ala-Gly-(Me) Phe-Gly-ol; U69, U69,593; NTB, naltriben; BNTX, 7-benzylidenaltrexone.

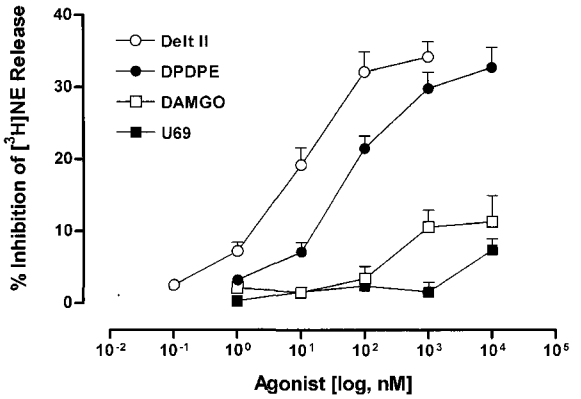

Fig. 1. Effects of Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAMGO), U69,593 (U69), [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and deltorphin II (Delt II) on the high K^+-stimulated-release of [^{3}H]NE in human cerebral cortex slices. Percentage of inhibition of the fractional release of [^{3}H]NE evoked by 15 mM K^+ in the absence of opioid was plotted against log concentration of opioid agonist. Values represent the mean±SEM from 4 independent experiments on different individuals with four replicates for each treatment.

more in buffer containing 1 μM each of desipramine and yohimbine. Equal aliquots (400 μl) of tissue slice suspensions were transferred to nylon mesh baskets, and incubated for 10 min in 2 ml of oxygenated KHB in 24 well tissue culture plates period for determination of base-line release. Then, the tissues were transferred to wells containing 15 mM K^+ in KHB for a further 10 min incubation. Finally, the tissues were transferred to wells containing 0.2 N HCl to extract the [^{3}H]NE present in tissue for 45 min. The tritium content in 400 μl of releasate was determined by liquid scintillation counter.

Competitive antagonist was added 10 min prior to the base-line release period; opioid agonists were present during the 10 min baseline release and stimulation periods. Potency of agonists was expressed in IC_{50} value (negative log of the molar concentration required to produce 50% inhibition of [^{3}H]NE release, when maximal inhibitory effect of a given agonist was regarded as 100%). IC_{50} values were calculated from plot of probit conversion of the percent inhibition of stimulated release of [^{3}H]NE against log concentration of each agonist. Parallelism was tested by the use of the program ALLFIT (De Lean A, Munson, 1988).

Drugs and chemicals were obtained from the following sources: L-[7,8-^{3}H]norepinephrine was from Amersham (Arlington Heights, IL); Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAMGO), (5a,7a,8b)-(-)-N-Met-N-(7-[1-pyrolidinyl]-1-oxaspiron [4,5]-Dec-8-Yl)benzenacetamide (U-69,593), deltorphin II (Delt-II), [D-Pen^2, D-Pen^5]enkephalin (DPDPE), desipramine HCl and yohimbine HCl were from Sigma (St Louis, MO); naloxone, naltriben and naltrindole were from Research Biochemicals, Inc. (Natick, MA); 7-benzylidenaltrexone (BNTX) was from Tocris (U.K.).

RESULTS

The base-line release of preloaded [^{3}H]NE was 3.4±0.2% (n=16) of total content of human cerebral cortex slices in KHB containing 5 mM K^+. High K^+ (15 mM) in the incubation media stimulated the release by 18.3±1.6% (n=16) above the base-line release. Stimulated [^{3}H]NE release from the tissues was attenuated by removal of calcium (1.2±0.3%, n=4) and addition of 1 μM tetrodotoxin (1.1±0.2%, n=4). The release of [^{3}H]NE by high K^+ was inhibited by DPDPE, a δ_1 opioid agonist, and Delt II, a δ_2 opioid agonist, in a concentration-dependent manner. DAMGO, a selective μ opioid agonist, and U69,593, a selective κ opioid agonist, showed little or no influence on the K^+-stimulated release (Fig. 1). The maximal inhibitory effects of DPDPE and Delt II were similar (32.7±2.3 and 34.2±2.8%, respectively), but IC_{50} values were significantly different (27.6±1.4 and 9.5±0.8 nM, respectively, $p<0.05$).

Table 1. Influence of opioid antagonists on the inhibitory effect of DPDPE and Delt II on the K^+ (15 mM)-stimulated release of [^{3}H]NE from human cerebral cortex slices

	DPDPE		Delt II	
Treatment	Maximal effect (%)	IC_{50} (nM)	Maximal effect (%)	IC_{50} (nM)
Saline	32.7±2.3	27.6±1.4	34.2±2.8	9.5±0.8
Nal, 10 nM	30.4±3.1	195.2±21.7*	33.4±3.6	320.9±21.7*
NTI, 3 nM	30.7±2.7	105.1±12.5*	32.1±2.7	332.5±26.7*
BNTX, 10 nM	32.5±2.8	187.1±14.3*	35.2±3.2	213.6±22.5*
NTB, 30 nM	33.2±3.6	117.9±9.7*	33.4±3.3	633.3±35.1*

IC_{50}s were calculated from plots of probit conversion of the percent inhibition of stimulated-release of [^{3}H] norepinephrine ([^{3}H]NE) by log concentration of each agonist. Values for IC_{50}s and maximal effects denote the mean±S.E.M. from 4 independent experiments. *: Significantly different from the saline-treated control value of DPDPE or Delt II alone ($p<0.05$). Delt II: deltorphin II. Nal: naloxone. NTI: naltrindole. BNTX: 7-benzylidenaltrexone. NTB: naltriben.

In order to identify the opioid receptor subtypes involved in the inhibition of K^+-stimulated release of [^{3}H]NE in human cerebral cortex slices, we examined the influences of competitive opioid antagonists for opioid receptors on the effects of DPDPE and Delt II. None of the antagonists tested induced significant change in spontaneous or stimulated efflux of radioactivity by itself. The inhibitory effect of DPDPE and Delt II was inhibited by naloxone (Nal, 10 nM; a non-selective opioid antagonist), naltrindole (NTI, 3 nM; a non-selective antagonist) for δ opioid receptors, 7-benzylidenaltrexone (BNTX, 10 nM; a selective antagonist for δ_1 opioid receptors), and naltriben (NTB, 30 nM; a selective δ_2 receptor antagonist). Concentration response curves for the inhibition of K^+-stimulated [^{3}H]NE release by DPDPE and Delt II were shifted to right, however maximal effects were not affected (Table 1).

DISCUSSION

The distribution of opioid d receptors in human temporal cortex was demonstrated using positron emission tomography (Smith et al, 1999) and in situ hybridization (Peckys et al, 1999). Proenkephalin mRNA signals are predominant in the temporal cortex supporting an intrinsic functional role of δ receptors (Hurd, 1996). Subtypes of δ opioid receptors have been demonstrated based on the relative affinities and potencies of putative subtype-selective drugs. Ligands that are selective for δ_1 sites include DPDPE and BNTX (Jiang et al, 1991; Sofuoglu et al, 1993). Delt II and

NTB are among the selective ligands for δ_2 sites (Jiang et al, 1991; Sofuoglu et al, 1993). We reported the existence of both δ_1 and δ_2 receptors in human cerebral cortex (Kim et al, 2002). In human temporal cortex, opioid δ_1 receptors regulate the acetylcholine release (Feuerstein et al, 1998), but the roles of δ_2 receptors have not been reported. In the present study, the high K^+-stimulated [^{3}H]NE release was inhibited by Delt II as well as DPDPE. The inhibitory effect of DPDPE and Delt II was inhibited by naloxone, NTI, BNTX and NTB. Our results suggest that the release of [^{3}H]NE release is regulated by both δ_1 and δ_2 opioid receptors.

Noradrenergic pathways in the cerebral cortex have been suggested to play roles in various animal behaviors including memory consolidation (Zornetzer et al, 1978), anxiety (Redmond et al, 1979) and sleep (Aston-Jones et al, 1981). Lesions of the locus ceruleus noradrenergic system result in deficits in sustained attention (Carli et al, 1983). Our results suggest that opioids may influence human behaviors by modulating of neurotransmission of noradrenergic system via δ_1 and δ_2 receptors in cerebral cortex. Delta opioid receptors were increased in focal areas of the ipsilateral temporal cortex (Madar et al, 1997) in epileptic patients, suggesting a role of δ receptors in seizure activity. Our results may reflect the pathologic upregulated δ receptors, because we used tissues obtained from temporal lobe of epilepsy patients.

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