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Opioid receptor regulation of 5-hydroxytryptamine release from the rat hippocampus measured by in vivo microdialysis

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The modulation of serotonin (5-HT) release by opioid receptors in the hippocampus of the awake, unrestrained rat was evaluated by use of in vivo microdialysis. The hippocampus was perfused with Ringer's solution (2 μl/min), and extracellular levels of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were estimated by assaying their concentration in the dialysate by HPLC-ECD. Addition of potassium (K^+, 60 and 120 mM) to the perfusate evoked a concentration-dependent release of 5-HT, but did not alter extracellular 5-HIAA levels. Co-perfusion of morphine (0.1 to 10 μM) with K^+ (120 mM) produced a concentration-dependent reduction of 5-HT release. Naltrexone (0.03 to 3 mg/kg, i.p.), a relatively selective μ-opioid receptor antagonist, blocked in a dose-dependent manner the morphine (10 μM)-induced inhibition of 5-HT release. Naltrexone alone did not alter significantly either extracellular 5-HT levels or the release of 5-HT evoked by K^+. Neither co-perfusion with [D-Pen^2, D-Pen^5]-enkephalin (DPDPE, 1 to 10 μM), an agonist selective for δ-opioid receptors, nor with U-69593 (10 μM), an agonist selective for κ-opioid receptors, modified the K^+ (120 mM)-evoked release of 5-HT. These findings indicate that μ-opioid receptors modulate the physiological release of 5-HT from serotonergic neurons in the rat hippocampus.

INTRODUCTION

Specific opioid receptors appear to modulate the release of specific neurotransmitter substances in the central nervous system. For example, stimulation of δ-opioid receptors inhibits both K^+-stimulated and electrically stimulated release of [^{3}H]dopamine from rat striatal slices, but does not inhibit the release of [^{14}C]acetylcholine[15,16,19]. In contrast, stimulation of κ-opioid receptors in rat striatal slices inhibits the neuronal release of [^{14}C]acetylcholine, but not that of [^{3}H]dopamine. In rat cerebral cortical slices, neuronal release of [^{3}H]norepinephrine has been shown to be modulated by μ-opioid receptors[16,19].

The role of opioid receptors in the regulation of serotonin (5-HT) release, however, remains unclear at this time. Hagan and Hughes[6] reported that opioid receptors were not involved in the electrically stimulated release of [^{3}H]5-HT from rat cortical slices. Dewar et al.[3] also were unable to demonstrate opioid receptor mediation of K^+-stimulated release of [^{3}H]5-HT from slices of rat globus pallidus. In contrast, Passarelli and Costa[17] reported that stimulation of μ-, δ- and κ-opioid receptors in rat hippocampal slices inhibited K^+-stimulated release of [^{3}H]5-HT. In this latter study, [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol]enkephalin (DAMGO), a μ-opioid receptor agonist, and DPDPE, δ-opioid receptor agonist, were more efficacious than U50488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzene-actamine), a κ-opioid receptor agonist, as inhibitors of [^{3}H]5-HT release. All of these studies have involved the use of in vitro preparations, and the results of such studies might be influenced significantly by the nature of the artificial environment in which they must be conducted.

In vivo microdialysis, in combination with high performance liquid chromatography (HPLC) and electrochemical detection (ECD), permits the study of neuro-

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transmitter release from neurons in brains of awake, unrestrained animals. Using this approach, we have recently shown that α_2-adrenergic receptors on hippocampal serotonergic nerve terminals regulate K^+-stimulated release of 5-HT in vivo[24]. These findings are in accordance with those reported with hippocampal brain slices in vitro[12]. The objective of the present study was to determine whether opioid receptors regulate 5-HT release from serotonergic neurons in the rat hippocampus. The hippocampus was chosen because it is an area of the rat brain innervated by serotonergic neurons[13] and because radioligand binding studies have demonstrated the presence of μ-, κ- and δ-opioid receptor binding sites[14]. To evaluate opioid receptor function three agonists were used, morphine (μ-selective), DPDPE (δ-selective) and U-69593 (5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]phenyl-benzeneacetamine, κ-selective). In addition, the actions of naltrexone, an antagonist similar to naloxone in its selectivity for μ-opioid receptors, was studied.

MATERIALS AND METHODS

Animals

The experiments were performed on male, Wistar rats (250–350 g and 8 to 12 weeks old, Shizuoka Laboratory Animal Center, Hamamatsu, Japan). The animals were housed in a room with a 12-h light (07.00–19.00)–dark (19.00–07.00) cycle and were given free access to food and water.

Brain microdialysis

Rats were anesthetized with ketamine (100 mg/kg, i.p.), and an 8-mm dialysis-probe guide cannula was stereotaxically implanted into the hippocampus so that a concentric dialysis probe with a 3-mm tip would be positioned with its tip at the following co-ordinates with relation to the bregma and dural surface of the brain[18]: rostral-caudal, −5.8 mm; lateral −4.8 mm. ventral −7.0 mm, from the bregma and the dural surface. The guide cannula was attached to the skull with dental cement. Two days after the surgical implantation of the guide cannula, a dialysis probe was inserted into the guide cannula and the probe was then perfused continuously (2 μl/min) with Ringer's solution of the following composition (mM): NaCl 147, KCl 4, $CaCl_2$ 2.3, pH 6.4. The perfusion medium was delivered by a 1-ml syringe mounted on a microinfusion pump (CMA/100, Carnegie Medicin, Stockholm, Sweden) through a coiled teflon tube and returned through a similar coiled teflon tube that allows the rat to move about freely. In preliminary experiments it was found that the levels of 5-HT in the dialysate were high immediately after insertion of the probe but decreased to constant values within 3 h. Therefore, collection of the perfusate was started 3 h after implantation of the probe. Nine successive 40-μl samples were collected at 20-min intervals in vials that contained 10 μl of ice-cold acetic acid (0.05 N). The samples were injected immediately into a high performance liquid chromatograph.

To stimulate 5-HT release, KCl (either in a concentration of 60 or 120 mM) was administered through the perfusion system for two 10-min periods of time. In order to maintain the osmolarity of the perfusion fluid when either 60 or 120 mM KCl was added, the existing NaCl was replaced with the KCl. The first administration of KCl (S_1, 40–50 min, Figs. 2 and 3) occurred at the beginning of the fourth 20-min collection period and the second administration at the beginning of the seventh (S_2, 100–110 min, Figs. 2 and 3) collection period. In control experiments, the S_2/S_1 ratio was found to be approximately 1.0 under these experimental conditions. Morphine and other opioid receptor agonists were added to the perfusion solution at the time of the second administration of KCl (S_2). Naltrexone, an opioid receptor antagonist, was injected intraperitoneally 20 min prior to the second KCl-perfusion period (S_2). At the end of each experiment, the rat was sacrificed, and its brain was

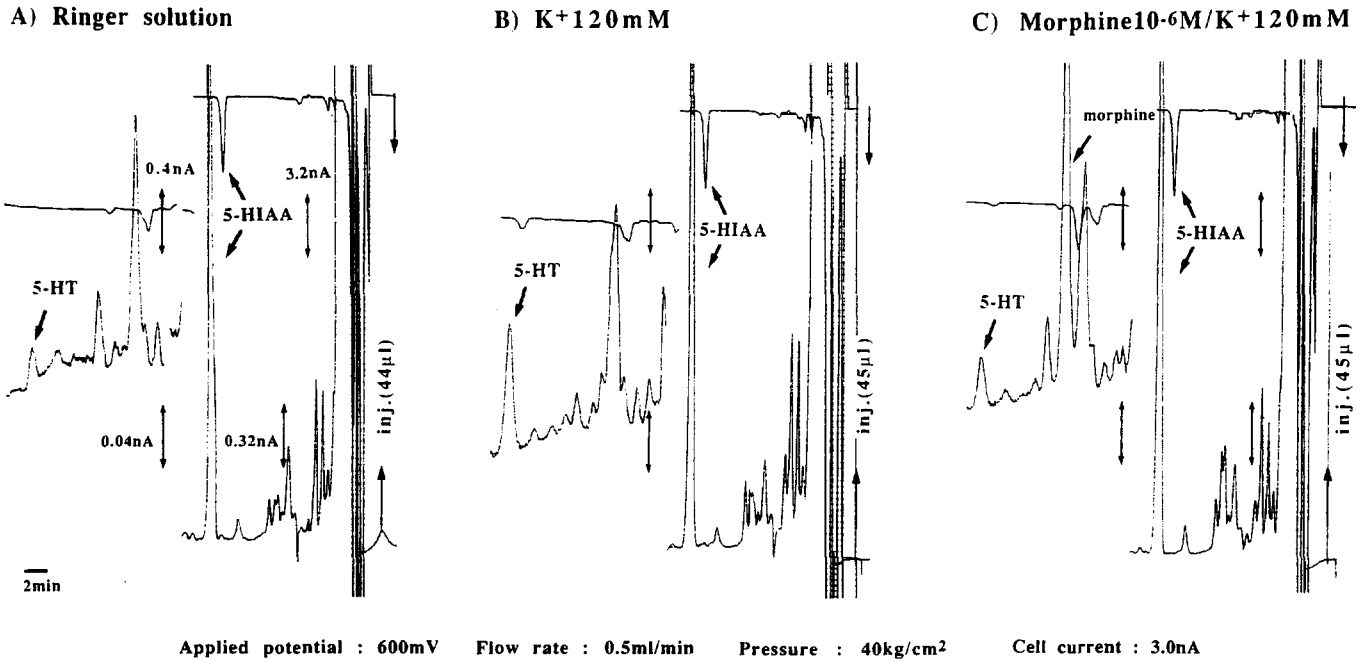

Fig. 1. Chromatograms of samples of dialysate collected during perfusion of the rat hippocampus with Ringer's solution under basal conditions (A), after K^+ stimulation (120 mM, B) or after K^+ stimulation in the presence of morphine (1 μM, C). HPLC-ECD was carried out under the following conditions: flow rate, 0.5 ml/min; pressure, 40 kg/cm^2; applied potential, 600 mV, and cell current, 3.0 nA. Simultaneous tracings at various levels of amplification were made in order to measure peaks for 5-HT and 5-HIAA. Note that K^+ stimulation increases the 5-HT, but not the 5-HIAA peak (B) whereas co-perfusion with morphine (1 μM) reduces markedly the release of 5-HT induced by K^+.

removed, sectioned and examined histologically to determine the precise insertion site of the dialysis probe.

Determination of 5-HT and 5-HIAA

5-HT and its metabolite, 5-HIAA, were separated and measured by use of a HPLC system coupled to an ECD system as described previously[11]. The HPLC-ECD system consisted of a pump (P-500, Irika Kogyo, Kyoto, Japan) coupled to a reversed-phase column (EiCOM, MA-50DS, ODS, 5 μm particle size) and an electrochemical detector (Irika, E-502). Shown in Fig. 1 are typical chromatograms obtained with samples of dialysate collected before (Fig. 1A) and after the administration of KCl, 120 mM, (Fig. 1B) or after the simultaneous administration of KCl, 120 mM, and morphine, 1 μM, (Fig. 1C). Note that morphine produces a peak that does not overlap with those peaks produced by either 5-HT or 5-HIAA.

Drugs

The following compounds were used: 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Co. Ltd., St. Louis, MO, USA), morphine sulphate (Takeda Pharmaceutical Co., Osaka, Japan), naltrexone hydrochloride (DuPont Pharmaceuticals, Garden City, NY, USA), [D-Pen^2, D-Pen^5]-enkephalin (Sigma), U-69593 (The Upjohn Co., Kalamazoo, MI, USA). and ketamine hydrochloride (Sankyo Co. Ltd., Tokyo, Japan).

Calculations and statistical analysis

5-HT in dialysates is expressed as a percentage of the absolute amount of 5-HT in the dialysate collected during the third 20-min collection period, i.e. the period immediately before addition of KCl to the perfusate. The amount of 5-HT released during K^+ stimulation is expressed as the amount in the dialysate collected during the fourth collection period (S_1) less the average of the amounts collected during the third and fifth collection periods or the amount collected during the seventh collection period (S_2) less the average of the amounts collected during the sixth and eighth collection periods. In vitro recoveries for some microdialysis probes were measured in order to determine the recovery efficiency of the probe membranes. To measure recovery, probes were placed in Ringer's solution that contained both 5-HT, 100 pg/μl, and 5-HIAA, 100 pg/μl, and perfused at a flow rate of 2 μl/min at 37 ± 1 °C. Mean in vitro recoveries (the concentrations of the 5-HT or 5-HIAA in the dialysate expressed as a percentage of the concentrations of these compounds outside of the dialysis probe) ± S.E.M. of 8 determinations were 14.9 ± 2.7% for 5-HT and 11.7 ± 1.9% for 5-HIAA.

All results are given as means ± S.E.M. Statistical comparisons were carried out using analysis of variance followed by Student's *t*-test. Values of P less than 5% were considered significant.

RESULTS

Effect of K^+ on 5-HT release

When introduced into the perfusion buffer, KCl increased the concentration in the dialysate of 5-HT, but not that of 5-HIAA, in a concentration-dependent manner (Fig. 2). The mean basal output of 5-HT from serotonergic neurons in the rat hippocampus was 18.0 ± 1.9 fmol/40 μl of dialysate and that of 5-HIAA was 4.6 ± 0.8 pmol/40 μl of dialysate. KCl was introduced into the perfusion medium for 10-min periods at the beginning of the fourth (S_1) and of seventh (S_2) 20-min collection periods. The mean increase in 5-HT in the dialysate with 60 mM KCl was 80.3 ± 30.1% over basal output levels during S_1 and 61.9 ± 11.2% during S_2 (Fig. 2A, $n = 5$). The mean S_2/S_1 ratio in these experiments was 1.07 ± 0.31. The mean increase in 5-HT in the dialysate with 120 mM KCl was 241.6 ± 36.2% over basal output levels during S_1 and 260.8 ± 64.6% during S_2 (Fig. 2A, $n = 4$). The mean S_2/S_1 ratio in these experiments was 1.05 ± 0.17. Neither concentration of KCl caused an appreciable change in 5-HIAA levels over basal output values during either S_1 or S_2 (Fig. 2B). Although both concentrations of KCl gave S_2/S_1 ratios close to unity, the higher concentration of KCl was chosen for experiments with the opioid agonists, morphine, DPDPE and U-69593, and with naltrexone since with this concentration there was less variability within individual animals and among different animals.

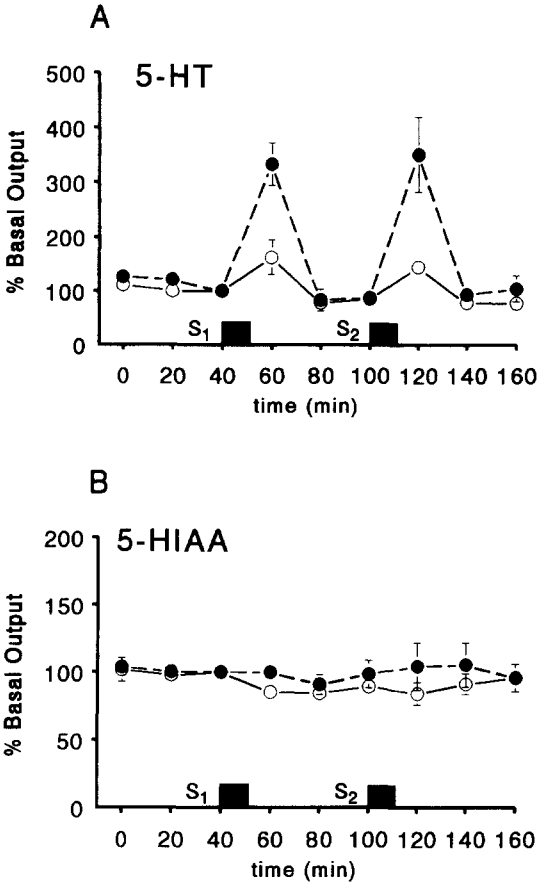

Fig. 2. Effects of low (60 mM) and high (120 mM) KCl upon extracellular 5-HT (A) and 5-HIAA (B) in rat hippocampus during in vivo microdialysis. Ordinate: amount of 5-HT or 5-HIAA in dialysate expressed as a percent of the amount in the third collection period, the period immediately before the first administration of KCl. Abscissa: time beginning with the first and ending with the ninth collection period. Dialysates were collected starting 180 min after insertion of the dialysis probe. S_1 and S_2, 10-min periods during which the concentration of KCl was increased in the Ringer's perfusion buffer. Open circles, 60 mM KCl; solid circles, 120 mM KCl. Each point represents the mean of 4–5 experiments. Vertical bars represent the S.E.M.

Effect of opioid agonists on K^+-evoked 5-HT release in the hippocampus

Morphine, but not DPDPE or U-69593, inhibited the K^+-induced release of 5-HT when added to the

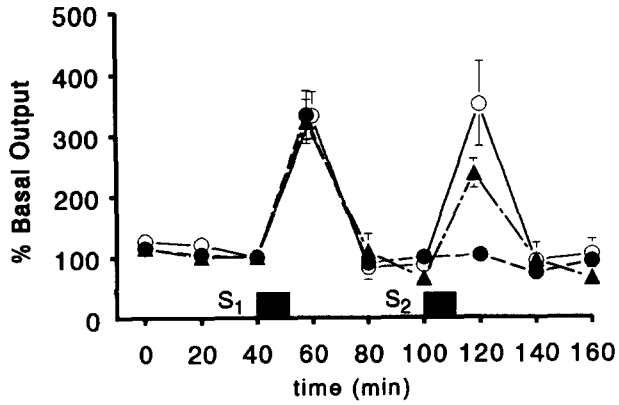

Fig. 3. Inhibition by morphine of hippocampal 5-HT release elicited by KCl, 120 mM. Antagonism by naltrexone. Experimental conditions were the same as in Fig. 2A. Open circles, control experiments. Solid circles, morphine, 10 μM, co-perfused with the second administration of KCl (S_2); solid triangles, rats were treated with naltrexone, 3 mg/kg, i.p. 20 min before co-perfusion of morphine and KCl (S_2). Each point represents the mean of 4–6 determinations. Vertical bars represent the S.E.M.

perfusion medium simultaneously with the second stimulation with KCl (S_2). Morphine, an agonist relatively selective for μ-opioid receptors, inhibited 5-HT release in concentrations that ranged from 0.1 to 10 μM (Figs. 3 and 4A). At a concentration of 0.1 μM, morphine produced a 20.8 ± 8.6% inhibition of release whereas at a concentration of 10 μM the inhibition of release was nearly total (89.3 ± 8.0%). When co-perfused with KCl at concentrations of 1 and 10 μM, DPDPE, an agonist highly selective for δ-opioid receptors, caused no inhibition of 5-HT release (Table I).

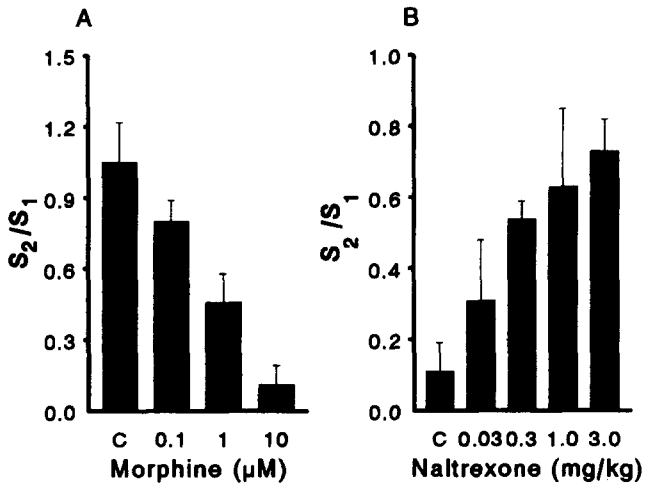

Fig. 4. Concentration-dependent effects of morphine on K^+-evoked release of 5-HT (A) and antagonism by naltrexone of the effects of morphine, 10 μM, on K^+-evoked release of 5-HT (B) during in vivo microdialysis of the rat hippocampus. Ordinate: S_2/S_1 ratios. Abscissa: concentrations of either morphine (A) or naltrexone (B). In some experiments (B), rats were injected intraperitoneally with various doses of naltrexone 20 min before co-perfusion of morphine and KCl (S_2). Each point represents the mean of 4–6 determinations. Vertical bars represent the S.E.M.

TABLE I

Effects of DPDPE and U69593 on the K^+-evoked release of 5-HT

The opioid agonist was administered simultaneously with the second administration of KCl (S_2). Each value represents the mean of n experiments ± S.E.M.

Experimental group	*Ratio (S_2/S_1)*	*n*
Control	1.05 ± 0.17	4
DPDPE 1 μM	0.94 ± 0.09	4
DPDPE 10 μM	1.07 ± 0.22	7
U-69593 10 μM	1.04 ± 0.20	4

Similarly, U-69593, an agonist highly selective for κ-opioid receptors, caused no inhibition of release when co-perfused with KCl at the high concentration of 10 μM during S_2 (Table I). None of the three agonists, when administered without the concomitant administration of KCl, altered the spontaneous release of 5-HT. These results suggest that the modulation by opioids of neuronal 5-HT release in the rat hippocampus is mediated solely by μ-opioid receptors.

Antagonism by naltrexone of the morphine-induced inhibition of 5-HT release

To determine further whether the inhibitory actions of morphine on 5-HT release were mediated by opioid receptors, the actions of morphine were determined in rats treated with naltrexone, an opioid antagonist relatively selective for μ-opioid receptors, in doses that ranged from 0.03 to 3 mg/kg. No concentration of naltrexone caused a change in the release of 5-HT during the period immediately prior to the co-perfusion of morphine with KCl (S_2). Pretreatment with naltrexone did, however, prevent the inhibition of 5-HT release by morphine, 10 μM, in a concentration-dependent manner (Figs. 3 (solid triangles) and 4B). The response to morphine was almost completely blocked by naltrexone, 3 mg/kg. These observations are consistent with the hypothesis that morphine inhibits hippocampal 5-HT release by acting at a μ-opioid receptor.

DISCUSSION

In the present study K^+ evoked large increases in extracellular 5-HT levels in the hippocampus of awake, unrestrained rats that were reflected by increased concentrations of 5-HT in the dialysate during in vivo microdialysis. These increases in 5-HT were related to the concentration of KCl in the perfusion medium. During in vivo microdialysis of the hippocampus of chloralose-anesthetized rats, Sharp et al.[20] found similar increases in extracellular 5-HT levels in the rat

hippocampus during electrical stimulation of the dorsal raphe nucleus. They demonstrated a good correlation between the frequency of electrical stimulation and the amount of 5-HT in the dialysate. These changes in 5-HT levels were accompanied by small increases in the levels of 5-HIAA, the major metabolite of 5-HT in the brain, in the dialysates. 5-HIAA levels, however, were not correlated with the frequency of dorsal raphe nucleus stimulation or with 5-HT levels in the perfusates. No changes in 5-HIAA levels occurred with K^+ stimulation of serotonergic neurons in the rat hippocampus in the present study despite large increases in 5-HT levels. These findings are consistent with the hypothesis that 5-HIAA levels reflect the turnover of the total 5-HT pool in the brain rather than the stimulated release of neuronal 5-HT[5,7].

The action of morphine and other opioid agonists upon 5-HT release and upon 5-HIAA levels in various areas of the rat central nervous system is quite complex. A number of studies have suggested that either acute or chronic administration of morphine increases 5-HT release and turnover in the rat brain[4,21,23]. Matos et al.[9,10] have reported that systemic administration of morphine has highly variable effects upon extracellular levels of 5-HT in the dorsal spinal cord as measured by in vivo microdialysis. These investigators reported that the systemic administration of morphine increased 5-HT levels in only half of the rats studied and that DAMGO, administered intracerebroventricularly, increased 5-HT levels in only one third of the animals. They also found that 5-HIAA levels either decreased or remained unchanged after morphine administration. In contrast, Wood[22] found that morphine caused marked increase in the 5-HIAA content of the dorsal spinal cord. This action of morphine was dose-dependent and was antagonized by naloxone. This effect of morphine, however, was not shared by other opioid agonists that were known to stimulate either μ- or κ-opioid receptors. The present study and a previous study[17] both found that opioid agonists inhibited the release of 5-HT during stimulation of serotonergic neurons in the rat hippocampus. Our study also found no effect of opioid agonists or of naltrexone upon hippocampal extracellular 5-HIAA levels.

The present study suggests that μ-opioid receptors modulate K^+-evoked release of 5-HT from serotonergic neurons in the rat hippocampus as determined by in vivo microdialysis. Morphine produced a concentration-dependent inhibition of 5-HT release; and this action was antagonized by naltrexone, an antagonist relatively selective for μ-opioid receptors. Naltrexone alone had no effect upon the spontaneous release of 5-HT which suggests that endogenous opioids do not exert a tonic regulation of 5-HT release under resting conditions.

Autoradiographic studies have demonstrated the presence of binding sites for ligands relatively selective for δ- and κ-opioid receptor sites, as well as binding sites for μ-opioid receptor-selective ligands, in the rat hippocampus[2,8]. According to these reports, μ-opioid receptors predominate in this area of the rat brain, although δ- and κ-opioid receptors are also present. In the present study, DPDPE, a δ-opioid receptor-selective agonist, and U-69593, a κ-opioid receptor-selective agonist, did not modify K^+-evoked 5-HT release from neurons in the rat hippocampus even when administered in concentrations considerably higher than those required to produce maximum effects in other systems. Thus, despite the presence of δ- and κ-opioid receptor binding sites in the rat hippocampus, the μ-opioid receptor would appear to be the primary opioid receptor involved in the regulation of 5-HT release in the rat hippocampus in vivo.

Several investigators have reported that 5-HT release is not modulated by opioid receptors in the rat brain. Hagen and Hughes[6] found the opioid agonists did not modify the electrically stimulated release of [^{3}H]5-HT from rat cerebral cortical slices; and Dewar et al.[3] reported that opioid agonists did not modify K^+-evoked release of 5-HT from slices of the rat globus pallidus. The differences between these observations and those of the present study might depend upon the areas of the brain that were studied. A similar regional difference in the regulation of 5-HT release by dopamine has been found. Benkirane et al.[1] reported that dopamine, through stimulation of a D_1 receptor, regulates the release of [^{3}H]5-HT from neurons in the substantia nigra but not from neurons in the hippocampus. These investigators also reported no effect of opioid agonists upon [^{3}H]5-HT release in the substantia nigra.

In contrast to the present study, Passarelli and Costa[17] reported that μ-, δ- and κ-agonists inhibited to various degrees the release of [^{3}H]5-HT from slices of rat hippocampus. The effects of DAMGO in their study were those of a typical μ-opioid receptor agonist in that they were blocked competitively by naloxone. The modulation by DPDPE of [^{3}H]5-HT release was unusual, however, in that naloxone produced a noncompetitive antagonism of the inhibitory action of that drug. U-50488H, a κ-opioid receptor-selective agonist, produced a small inhibition of K^+-evoked [^{3}H]5-HT release that was reversed by naloxone. The differences between the present study and that of Passarelli and Costa[17] might be a result of differences in experimental approach. In the in vitro brain slice study, the concen-

trations of [^{3}H]5-HT used to label endogenous 5-HT stores (0.1 mM) were relatively large, and the [^{3}H]5-HT might have been taken up by and subsequently released from non-serotonergic cells, as well as from serotonergic neurons. Another possible explanation for the differences between the two studies might be that in vivo microdialysis, in which release of the actual endogenous neurotransmitter is measured, might reveal a functionally significant population of receptors different from that population of receptors seen in vitro with brain slices labeled with an exogenous radioligand. Nevertheless, both studies demonstrate that in the rat hippocampus μ-opioid receptors modulate 5-HT release.

The current findings are consistent with the hypothesis that specific opioid receptors modulate the release of specific neurotransmitter substances in the central nervous system. δ-Opioid receptors appear to mediate the release of dopamine from the rat striatum[15,16,19]. κ-Opioid receptors appear to modulate the neuronal release of acetylcholine in the rat striatum[15,16]. It has been suggested that μ-opioid receptors modulate the neuronal release of norepinephrine in the rat cerebral cortex[16,19]. The present study indicates that μ-opioid receptors also modulate the release of 5-HT in the rat hippocampus.

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