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κ_1- and κ_2-opioid receptors mediating presynaptic inhibition of dopamine and acetylcholine release in rat neostriatum

[1]Anton N.M. Schoffelmeer, Francois Hogenboom & Arie H. Mulder

Research Institute Neurosciences Vrije Universiteit, Department of Pharmacology, Free University, Medical Faculty, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands

1 The effects of selective opioid receptor agonists and antagonists on N-methyl-D-aspartate (NMDA, 10 μM)-induced release of [^{3}H]-dopamine and [^{14}C]-acetylcholine (ACh) from superfused neostriatal slices were studied to investigate the possible occurrence of functional κ-opioid receptor subtypes in rat brain.

2 The κ receptor agonists (−)-ethylketocyclazocine ((−)-EKC), U69593 and the endogenous opioid peptide dynorphin A_{1-13} caused a naloxone-reversible inhibition of NMDA-induced [^{3}H]-dopamine release, with pD_2 values of about 9, 8.5 and 8.2, respectively, whereas both the μ agonist Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol (DAMGO) and the δ agonist D-Pen^2-D-Pen^5-enkephalin (DPDPE) were ineffective in this respect. The inhibitory effect of submaximally effective concentrations of dynorphin A_{1-13}, U69593 and (−)-EKC on NMDA-induced [^{3}H]-dopamine release were not changed by the δ_1/δ_2-opioid receptor antagonist naltrindole (up to a concentration of 1 μM), but reversed by the κ receptor antagonist nor-binaltorphimine (nor-BNI), with an IC_{50} as low as 0.02 nM, indicating the involvement of U69593-sensitive κ_1-opioid receptors.

3 NMDA-induced [^{14}C]-ACh release was reduced in a naloxone-reversible manner by DPDPE (pD_2 about 7.2), dynorphin A_{1-13} (pD_2 6.7) and EKC (pD_2 6.2), but not by U69593 and DAMGO. The inhibitory effect of a submaximally effective concentration of DPDPE, unlike those of dynorphin A_{1-13} and (−)-EKC, on NMDA-induced [^{14}C]-ACh release was antagonized by naltrindole with an IC_{50} of 1 nM, indicating the involvement of δ-opioid receptors in the inhibitory effect of DPDPE. On the other hand, the inhibitory effects of dynorphin A_{1-13} and (−)-EKC on [^{14}C]-ACh release were readily antagonized by nor-BNI with an IC_{50} of about 3 nM. A 100 fold higher concentration of nor-BNI also antagonized the inhibitory effect of DPDPE, indicating the involvement of U69593-insensitive κ_2-opioid receptors in the inhibitory effects of dynorphin A_{1-13} and (−)-EKC.

4 Although naloxone benzoylhydrazone (NalBzoH), displaying high affinity towards the putative κ_3-opioid receptor, antagonized the inhibitory effects of dynorphin A_{1-13} and (−)-EKC on [^{3}H]-dopamine and [^{14}C]-ACh release as well as that of U69593 on [^{3}H]-dopamine release, it displayed a low apparent affinity (IC_{50} about 100 nM) in each case.

5 In conclusion, whereas activation of κ_1-opioid receptors causes presynaptic inhibition of NMDA-induced dopamine release, κ_2 receptor activation results in inhibition of ACh release in rat neostriatum. As such, this study is the first to provide unequivocal *in vitro* evidence for the existence of functionally distinct κ-opioid receptor subtypes in the brain.

Keywords: κ_1-opioid receptors; κ_2-opioid receptors; dopamine release; acetylcholine release; neostriatum

Introduction

Opiates and opioid peptides activate a family of μ-. δ- and κ-opioid receptors and receptor subtypes in the brain (Reisine, 1995), which has distinct pharmacological profiles, anatomical distributions and functions (Mansour & Watson, 1993; Uhl *et al*., 1994). Pharmacological studies have shown that benzomorphan compounds such as bremazocine and (−)-ethylketocyclazocine ((−)-EKC) produce their antinociceptive and sedative actions through an interaction with κ receptors (Martin *et al*., 1976). These receptors exhibit a high affinity for peptides derived from prodynorphin (particularly dynorphin A_{1-13}), one of three endogenous opioid peptide precursors (Khatchaturian *et al*., 1993). In addition to their role in sensory processing, the κ receptors mediate a spectrum of distinctive functions, including water balance, food intake, gut motility, temperature control and a variety of endocrine functions. Moreover, in man activation of these receptors causes dysphoria and psychotomimesis, leading to suggestions regarding a potential role in psychosis (Pfeiffer *et al*., 1986).

Ligand-receptor binding studies suggested the possible existence of at least two subtypes of the κ-opioid receptor in the brain. The κ_1-receptor binding sites were shown to have a very high selectivity and affinity for arylacetamide-like agonists such as U69593 and U50488 and the κ antagonist nor-binaltorphimine (nor-BNI, Portoghese *et al*., 1991), and to display a high affinity towards (−)-EKC and related compounds. On the other hand κ_2 receptor binding sites were defined as being U69593-insensitive and to show about a 100 fold lower affinity to nor-BNI and (−)-EKC (Zukin *et al*., 1988; Clark *et al*., 1989). In addition, the existence of U69593-insensitive κ_3 receptor binding sites, displaying a high affinity to benzomorphans, the antagonist naloxone benzoylhydrazone (NalBzoH) and the μ receptor agonist Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol (DAMGO), has also been proposed (Clark *et al*., 1989; Brooks *et al*., 1996). There are even proposed subdivisions of κ_1 and κ_2 receptors, based on ligand-receptor binding profiles (Nock *et al*., 1990; Rothman *et al*., 1990). Soon after the cloning of the μ and δ receptor (Evans *et al*., 1992; Kieffer *et al*., 1992; Thompson *et al*., 1993), a cDNA corresponding to the κ_1-opioid receptor was identified (Meng *et al*., 1993; Yasuda *et al*., 1993). Cloning of the κ_2 receptor has thusfar not been successful, whereas that of a κ_3-related protein has recently been obtained (Pan *et al*., 1995).

Previous studies have provided functional neurochemical evidence for the occurrence in rat brain of multiple opioid

[1] Author for correspondence.

receptors mediating inhibition of neurotransmitter release (Mulder & Schoffelmeer, 1993) and changing the functioning of ion channels of neuronal membranes (North, 1993). It is well established, for example, that activation of κ-opioid receptors results in presynaptic inhibition of dopamine release in the neostriatum and other brain regions, as demonstrated *in vitro* (Schoffelmeer *et al*., 1988; Heijna *et al*., 1990; Mulder *et al*., 1991) and *in vivo* (Spanagel *et al*., 1992). However, evidence for the existence of pharmacologically and functionally distinct subtypes of neurotransmitter release-inhibitory κ-opioid receptors has been lacking until now. Therefore, we investigated the effects of agonists and antagonists with high affinity for the multiple κ receptor binding sites on [^{3}H]-dopamine and [^{14}C]-acetylcholine (ACh) release from rat superfused neostriatal slices. Since excitation of striatal dopaminergic and cholinergic neurones primarily involves glutamate-mediated activation of N-methyl-D-aspartate (NMDA) receptors, we studied opioid receptor-mediated inhibition of NMDA-induced neurotransmitter release.

Methods

Superfusion of striatal slices and addition of drugs

All experiments were approved by the Animal Care Committee of the Free University of Amsterdam.

Male Wistar rats (160–200 g body weight; Harlan, Zeist, The Netherlands) were decapitated and neostriatal slices ($0.3 \times 0.3 \times 2$ mm) were prepared with a McIlwain tissue chopper, then incubated with radiolabelled neurotransmitters and superfused essentially as described previously (Schoffelmeer *et al*., 1988). In short, slices were washed twice with 5 ml Mg^{2+}-free Krebs-Ringer bicarbonate medium containing (mM): NaCl 121, KCl 1.87, KH_2PO_4 1.17, $CaCl_2$ 1.22, $NaHCO_3$ 25 and D-(+)-glucose 10 (pH 7.4) and subsequently incubated for 15 min in this medium, containing 0.1 μM [^{3}H]-dopamine and [^{14}C]-choline under an atmosphere of 95% O_2–5% CO_2 at 37°C, in order to label selectively dopaminergic and cholinergic nerve terminals. After being labelled, the slices were washed and transferred to each of 24 chambers of a superfusion apparatus (about 4 mg tissue per chamber; 0.2 ml volume) and superfused (0.25 ml min^{-1}) with medium gassed with 95% O_2–5% CO_2 at 37°C. The superfusate was collected as 10 min samples after 40 min of superfusion ($t = 40$ min). Calcium-dependent neurotransmitter release was induced during superfusion by exposing the slices to medium containing (a half-maximally effective concentration of) 10 μM NMDA for 10 min at $t = 50$ min. Opioid receptor agonists were added 10 min and antagonists 20 min before exposure of the slices to NMDA. More prolonged exposure of the slices to the opioids did not further enhance their effects (data not shown). These drugs were present until the end of the experiment, i.e. until $t = 80$ min. In each experiment quadruplicate observations were made.

Calculation of release data

The radioactivity remaining at the end of the experiment was extracted from the tissue with 0.1 N HCl. The radioactivity in superfusion fractions and tissue extracts was determined by liquid-scintillation counting. The efflux of radioactivity during each collection period was expressed as a percentage of the amount of radioactivity in the slices at the beginning of the respective collection period. The NMDA-induced release of neurotransmitter was calculated by subtracting the spontaneous efflux of radioactivity from the total overflow of radioactivity during stimulation and the following 10 min. Since the release of both neurotransmitters returned to basal levels during the next 10 min period, a linear decline from the 10 min interval before to that 20–30 min after the onset of stimulation by NMDA was assumed for calculation of the spontaneous efflux of radioactivity. The spontaneous efflux of radioactivity from neostriatum slices labelled with [^{3}H]-dopamine and [^{14}C]-ACh was 0.32–0.36% min^{-1} and 0.22–0.25% min^{-1}, respectively, of total tissue radioactivity. The evoked release was expressed as % of the content of radioactivity of the slices at the start of the stimulation period.

In order to calculate the apparent pD_2 values of opioid receptor agonists and IC_{50} values of antagonists, the release data were fitted by means of the non-linear curve-fitting programme ALLFIT. Statistical analysis of the data was carried out with a two-way analysis of variance, followed by Duncan's multiple range test, by use of SPSS/PC + V2.0 (SPSS, Inc). Drug effects are expressed as percentage of respective control values.

Materials

[^{14}C]-choline (15 mCi mmol^{-1}) and [^{3}H]-dopamine (47 Ci mmol^{-1}) were purchased from the Radiochemical Centre (Amersham). N-methyl-D-aspartate, naloxone, D-penicillamine^2-D-penicillamine^5-enkephalin (DPDPE), Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol (DAMGO), dynorphin A_{1-13} and nor-binaltorphimine (nor-BNI) were obtained from Bachem. The following drugs were kindly donated: (−)-ethylketocyclazocine (EKC) by Stirling-Winthrop, (5α,7α,8β-(−)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]phenyl-benzeneacetamide) (U69593) by Upjohn and naloxone benzoylhydrazone (NalBzoH) by Dr GW Pasternak.

Results

Striatal [^{3}H]-dopamine and [^{14}C]-ACh release: opioid receptor agonism

[^{3}H]-dopamine and [^{14}C]-ACh release, induced by exposure of the neostriatal slices to a half-maximally effective concentration (10 μM) of NMDA for 10 min, amounted to 4.53 ± 0.08% and 2.94 ± 0.06% ($n = 152$) of total tissue radioactivity, respectively. Preliminary experiments revealed that this concentration of NMDA did not induce neurotransmitter release in the absence of Ca^{2+} in the superfusion medium (data not shown).

The κ receptor agonists (−)-EKC, U69593 and dynorphin A_{1-13} caused a 30–40% reduction in the NMDA-induced release of [^{3}H]-dopamine with apparent pD_2 values of 9.0 ± 0.2, 8.5 ± 0.1 and 8.2 ± 0.2, respectively, whereas the μ receptor agonist DAMGO and the δ receptor agonist DPDPE were ineffective (Figure 1a). (−)-EKC and dynorphin A_{1-13} caused a similar inhibitory effect on NMDA-induced [^{14}C]-ACh release, albeit with lower apparent pD_2 values (6.2 ± 0.1 and 6.7 ± 0.1, respectively, $P < 0.01$ vs [^{3}H]-dopamine release), whereas U69593 did not affect neurotransmitter release in this case at all (Figure 1b). Figure 1b also shows that DPDPE caused a 40% reduction in NMDA-induced [^{14}C]-ACh release with an apparent pD_2 value of 7.2 ± 0.3, whereas the μ-opioid receptor agonist DAMGO was ineffective.

At their maximally effective concentrations the opioid receptor agonists also caused a slight (although not significant) decrease (by 10–15%) in the spontaneous efflux of radioactivity.

Striatal [^{3}H]-dopamine and [^{14}C]-ACh release: opioid receptor antagonism

As shown in Figure 2a, 1 μM naloxone completely antagonized the inhibitory effects of submaximally effective concentrations of (−)-EKC (3 nM), U69593 (10 nM) and dynorphin A_{1-13} (30 nM) on NMDA-induced [^{3}H]-dopamine. Similarly, 1 μM naloxone prevented the inhibition of NMDA-induced [^{14}C]-ACh release by 3 μM (−)-EKC, 0.3 μM DPDPE and 1 μM dynorphin A_{1-13} (Figure 2b). The δ receptor antagonist naltrindole antagonized the inhibitory effect of 0.3 μM DPDPE on NMDA-induced [^{14}C]-ACh release with an IC_{50} of

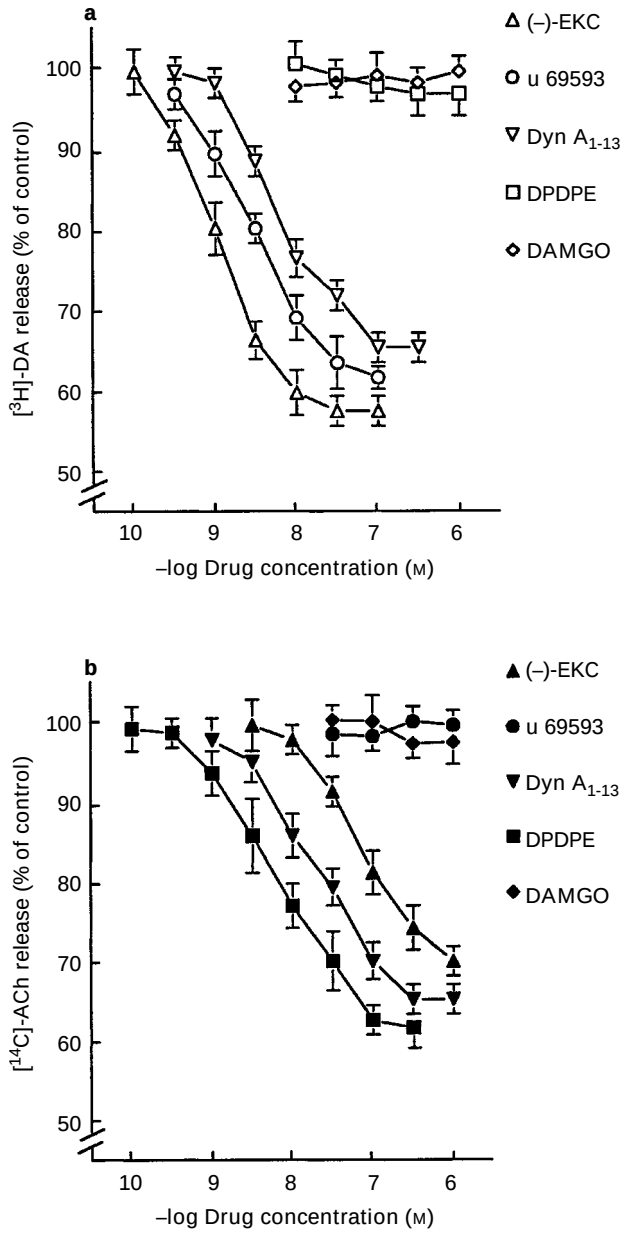

Figure 1 Inhibitory effects of various opioid receptor agonists on NMDA (10 μM)-induced [^{3}H]-dopamine (DA) release (a) and [^{14}C]-ACh release (b) from superfused rat neostriatal slices. The data points represent means of 12–16 observations obtained in 3–4 separate experiments; vertical lines show s.e.mean.

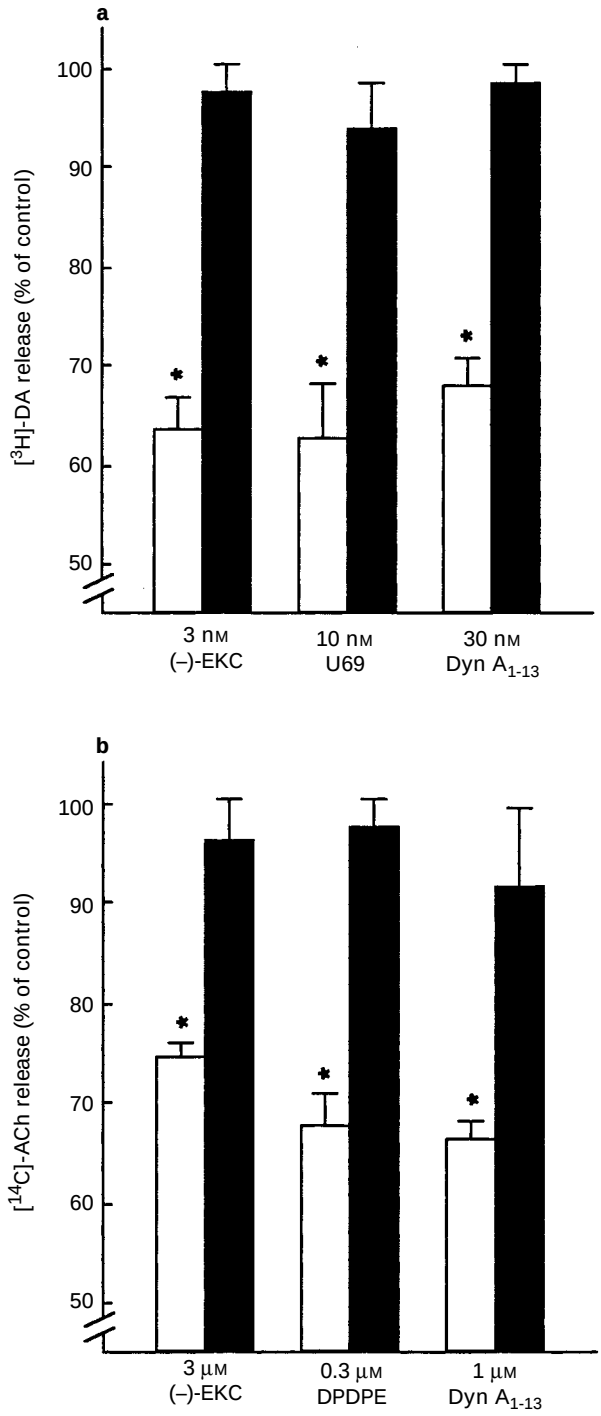

Figure 2 Antagonism by naloxone of the inhibitory effect of κ receptor agonists on NMDA (10 μM)-induced [^{3}H]-dopamine (DA) release (a) and [^{14}C]-ACh release (b) from superfused rat neostriatal slices. Data are means of 12 observations obtained in 3 separate experiments: vertical lines show s.e.mean. Open columns: inhibition by the indicated (submaximally effective) agonist concentrations; solid columns; opioid agonist effects in the presence of 1 μM naloxone. Naloxone alone did not affect neurotransmitter release. *$P<0.01$ vs control release in the absence of opioids.

1.1 ± 0.2 nM. However, the inhibitory effects of 1 μM dynorphin A_{1-13} and 3 μM (−)-EKC on [^{14}C]-ACh release, as well as those of 30 nM dynorphin A_{1-13}, 10 nM U69593 and 3 nM (−)-EKC on [^{3}H]-dopamine release were not affected by naltrindole (Figure 3).

The κ antagonist nor-BNI appeared to be extremely potent in reversing the inhibitory effect of a submaximally effective concentration of (−)-EKC (3 nM), U69593 (10 nM) and dynorphin A_{1-13} (30 nM) on NMDA-induced [^{3}H]-dopamine release with an IC_{50} as low as 0.02 ± 0.01, 0.03 ± 0.01 and 0.02 ± 0.01 nM, respectively. Nor-BNI also readily antagonized inhibitory effects of (−)-EKC and dynorphin A_{1-13} on NMDA-induced [^{14}C]-ACh release with an IC_{50} of 3.0 ± 0.1 and 3.2 ± 0.1 nM, respectively ($P<0.01$ vs [^{3}H]-dopamine release). On the other hand, the inhibitory effect of DPDPE on evoked [^{14}C]-ACh release was antagonized by about 100 fold higher concentrations of nor-BNI than observed with (−)-EKC or dynorphin A_{1-13} as agonist (IC_{50} 210 ± 20 nM, $P<0.01$ vs IC_{50} values with (−)-EKC or dynorphin A_{1-13} as agonist, Figure 4). A similar low apparant affinity of nor-BNI was observed when antagonism of the inhibitory effects of other δ-opioid receptor agonists such as Met-enkephalin and D-Ala^2-D-Leu^5-enkephalin was investigated (data not shown). Finally, as shown in Figure 5, the κ_3 receptor antagonist NalBzoH did not discriminate between the inhibitory effects of dynorphin A_{1-13} and (−)-EKC on the release of either neu-

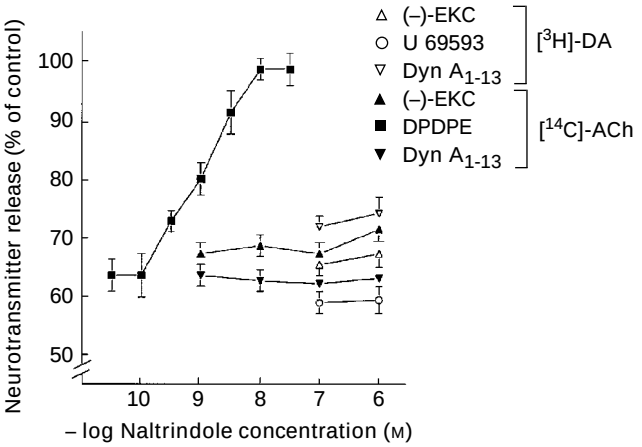

Figure 3 Antagonism by naltrindole of the inhibitory effects of opioid receptor agonists on NMDA (10 μM)-induced [^{3}H]-dopamine (DA) and [^{14}C]-ACh release from superfused rat neostriatal slices. Data are means of 12 observations obtained in 3 separate experiments; vertical lines show s.e.mean. The slices were exposed to the submaximally effective agonist concentrations indicated in Figure 2. Naltrindole alone did not affect neurotransmitter release.

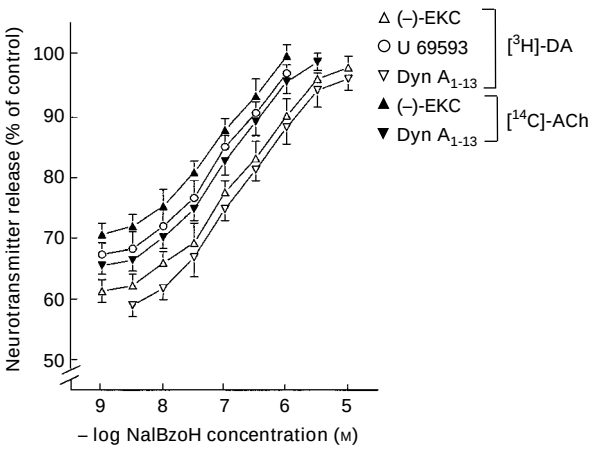

Figure 5 Antagonism by naloxone benzoylhydrazone (NalBzoH) of the inhibitory effects of opioid receptor agonists on NMDA (10 μM)-induced [^{3}H]-dopamine (DA) and [^{14}C]-ACh release from superfused rat neostriatal slices. Data are means of 12 observations obtained in 3 separate experiments; vertical lines show s.e.mean. The slices were exposed to the submaximally effective agonist concentrations indicated in Figure 2. NalBzoH alone did not affect neurotransmitter release.

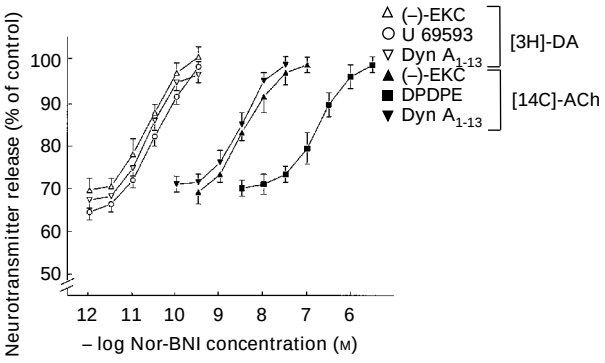

Figure 4 Antagonism by nor-binaltorphimine (nor-BNI) of the inhibitory effects of opioid receptor agonists on NMDA (10 μM)-induced [^{3}H]-dopamine (DA) and [^{14}C]-ACh release from superfused rat neostriatal slices. Data are means of 12–16 observations obtained in 3–4 separate experiments; vertical lines show s.e.mean. The slices were exposed to the submaximally effective agonist concentrations indicated in Figure 2. Nor-BNI alone did not affect neurotransmitter release.

rotransmitter but antagonized their inhibitory effects, like that of U69593 on [^{3}H]-dopamine release, with the relatively high IC_{50} of about 100 nM.

Discussion

The possible existence of at least two (U69593-sensitive and -insensitive) subtypes of the κ-opioid receptor in the brain has so far been primarily based on ligand-receptor binding studies (Zukin *et al*., 1988; Clark *et al*., 1989; Nock *et al*., 1990; Rothman *et al*., 1990). Moreover, whereas the putative κ_1 receptor and a κ_3 receptor-related protein were recently cloned (Meng *et al*., 1993; Yasuda *et al*., 1993; Pan *et al*., 1995), a distinct primary structure of the putative κ_2 receptor has not yet been established. Although ligand-receptor binding data and the identification of the genes that encode for distinct receptor binding sites provide evidence for the existence of receptor multiplicity, it is crucial to demonstrate unequivocally the functional relevance of the proposed receptor classification. Considering the possible existence of κ_1 and κ_2 receptors, the present study is the first to provide such functional evidence.

Our data show that only those opioids displaying a high affinity towards κ-opioid receptor binding sites, such as (−)-EKC, U69593 and the endogenous opioid peptide dynorphin A_{1-13}, cause inhibition of NMDA-induced [^{3}H]-dopamine release from rat neostriatal slices, in agreement with the involvement of κ-opioid receptors as previously described (Schoffelmeer *et al*., 1988; Heijna *et al*., 1990; Mulder *et al*., 1991). Moreover, the very high affinity of the κ_1 receptor-selective antagonist nor-BNI (Zukin *et al*,. 1988; Clark *et al*., 1989; Portoghese *et al*., 1991) for these receptors observed in our present study strongly suggests the occurrence in rat neostriatum of U69593-sensitive κ_1-opioid receptors mediating presynaptic inhibition of dopamine release. On the other hand, with regard to the inhibitory effect of higher concentrations of (−)-EKC and dynorphin A_{1-13} on NMDA-induced [^{14}C]-ACh release, our functional experiments revealed the involvement of κ_2-opioid receptors. This conclusion is based on the following observations. (1) The selective μ-opioid receptor agonist DAMGO did not affect NMDA-induced [^{14}C]-ACh release, indicating the absence of release-inhibitory μ receptors. (2) In contrast to the inhibitory effect of the δ-opioid receptor selective agonist DPDPE on [^{14}C]-ACh release, the inhibitory effects of (−)-EKC and dynorphin A_{1-13} were not reversed by the δ_1/δ_2-opioid receptor antagonist naltrindole (Sofuoglu *et al*., 1991). (3) Whereas the κ receptor antagonist nor-BNI (Portoghese *et al*., 1991) appeared to reverse the inhibitory effect of DPDPE, this antagonistic effect occurred at 100 fold higher concentrations of nor-BNI than observed with (−)-EKC or dynorphin A_{1-13} as agonist. (4) The apparent affinity of the κ-opioid receptor ligands studied here were considerably lower at the receptors mediating inhibition of [^{14}C]-ACh release than at those mediating inhibition of [^{3}H]-dopamine release, in agreement with the involvement of receptors with a low affinity κ_2 and high affinity κ_1 binding site, respectively (Zukin *et al*., 1988). (5) Our observations that the potent κ_1 receptor selective agonist U69593 (Zukin *et al*., 1988; Clark *et al*., 1989) did not affect NMDA-induced [^{14}C]-ACh release (whereas this compound strongly inhibited the NMDA-induced release of [^{3}H]-dopamine with a high apparent affinity), is fully consistent with the involvement of the proposed U69593-insensitive κ_2 receptor subtype. (6) Since the putative U69593-insensitive κ_3 receptor binding site has been characterized by displaying a high affinity towards benzomorphans, such as (−)-EKC and the antagonist NalBzoH (Clark *et al*., 1989), the low apparent affinity of these ligands for the [^{14}C]-

ACh release-inhibitory receptors strongly argues against the involvement of this putative receptor subtype. Moreover, DAMGO was shown to have a high affinity for the κ_3 receptor binding site (Clark *et al*., 1989), whereas the opioid peptide did not affect NMDA-induced [^{14}C]-ACh release in the present study.

Taken together, our data provide neurochemical evidence for the existence of pharmacologically and functionally distinct κ_1 and κ_2 receptors in rat neostriatum, as predicted by previous ligand-receptor binding studies. In this respect, our functional *in vitro* study suggests that in rat neostriatum activation of κ_1-opioid receptors causes inhibition of dopaminergic neurotransmission, whereas that of κ_2 receptors causes inhibition of the activity of cholinergic interneurones. It may therefore be of interest to develop highly selective κ_2 receptor (ant)agonists, which are not yet available. From a pathophysiological point of view, selective κ_2-opioid receptor agonists might be of relevance to ameliorate, for example, extrapyramidal disorders involving hyperactivity of neostriatal cholinergic interneurones, such as Parkinsonian symptoms and neuroleptic-induced symptoms resembling Parkinson's disease and neuroleptic-induced tardive dyskinesias (Lehman & Langer, 1983).

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References

BROOKS, A.I., STANDIFER, K.M., ROSSI, G.C., MATHIS, J.P. & PASTERNAK, G.W. (1996). Characterizing κ_3 opioid receptors with a selective monoclonal antibody. *Synapse*, **22,** 247–252.

CLARK, J.A., LIU, L., PRICE, M., HERSH, B., EDELSON, M. & PASTERNAK, G.W. (1989). Opiate receptor multiplicity: Evidence for two U50488-sensitive kappa$_1$ subtypes and a novel kappa$_3$ subtype. *J. Pharmacol. Exp. Ther.*, **251,** 461–468.

EVANS, C., KEITH, D., MORRISON, H., MAGENDZO, K. & EDWARDS, R. (1992). Cloning of a delta-opioid receptor by functional expression. *Science*, **258,** 1952–1955.

HEIJNA, M.H., PADT, M., HOGENBOOM, F., MULDER, A.H. & SCHOFFELMEER, A.N.M. (1990). Opioid receptor-mediated inhibition of dopamine and acetylcholine release from slices of rat nucleus accumbens, olfactory tubercle and frontal cortex. *Eur. J. Pharmacol.*, **181,** 267–278.

KHACHATURIAN, H., SCHAFER, M.K.H. & LEWIS, M.E. (1993). Anatomy and function of the endogenous opioid systems. In *Opioids I*. ed. Herz, A. pp. 471–497. Berlin: Springer.

KIEFFER, B., BEFORT, K., GARERIAUX-RUFF, C. & HIRTH, C. (1992). The delta-opioid receptor: Isolation of a cDNA by expression cloning and pharmacological characterization. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 12048–12052.

LEHMAN, J. & LANGER, S.Z. (1983). The striatal cholinergic interneuron: synaptic target of dopaminergic terminals. *Neuroscience*, **10,** 1105–1120.

MANSOUR, A. & WATSON, S.J. (1993). Anatomical distribution of opioid receptors in mammalians: an overview. In *Opioids I*. ed. Herz, A. pp. 79–105. Berlin: Springer.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effects of morphine and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.*, **197,** 517–532.

MENG, F., XIE, G., THOMPSON, R., MANSOUR, A., GOLDSTEIN, A., WATSON, S. & AKIL, H. (1993). Cloning and pharmacological characterization of a rat kappa opioid receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 9954–9958.

MULDER, A.H., BURGER, D.M., WARDEH, G., HOGENBOOM, F. & FRANKHUYZEN, A.L. (1991). Pharmacological profile of various κ-agonists at κ-, μ-, and δ-opioid receptors mediating presynaptic inhibition of neurotransmitter release in the rat brain. *Br. J. Pharmacol.*, **102,** 518–522.

MULDER, A.H. & SCHOFFELMEER, A.N.M. (1993). Multiple opioid receptors and presynaptic modulation of neurotransmitter release in the brain. In *Opioids I*. ed. Herz, A. pp. 125–144. Berlin: Springer.

NOCK, B., GIORDANO, A.L., CICERO, T.J. & O'CONNOR, L.H. (1990). Affinity of drugs and peptides for U69593-sensitive and -insensitive kappa opiate binding sites: the U69593-insensitive site appears to be the beta endorphin-specific epsilon receptor. *J. Pharmacol. Exp. Ther.*, **254,** 412–419.

NORTH, R.A. (1993). Opioid actions on membrane ion channels. In *Opioids I*. ed. Herz, A. pp. 773–797. Berlin: Springer.

PAN, Y.X., CHENG, J., XU, J., ROSSI, G., JACOBSON, E., RYAN-MORO, J., BROOKS, A.I., DEAN, G.E., STANDIFER, K.M. & PASTERNAK, G.W. (1995). Cloning and functional characterization through antisense mapping of a kappa$_3$-related opioid receptor. *Mol. Pharmacol.*, **47,** 1180–1188.

PFEIFFER, A., BRANTL, V., HERZ, A. & EMRICH, H. (1986). Psychotomimesis mediated by κ opiate receptors. *Science*, **233,** 774–776.

PORTOGHESE, P.S., GARZON-ABURBEH, A., NAGASE, H., LIN, C.E. & TAKEMORI, A.E. (1991). Role of the spacer in conferring κ opioid selectivity to bivalent ligands related to norbinaltorphimine. *J. Med. Chem.*, **34,** 1292–1296.

REISINE, T. (1995). Opiate receptors. *Neuropharmacology*, **34,** 463–472.

ROTHMAN, R.B., BYKOV, B., DECOSTA, B.R., JACOBSON, A.E., RICE, K.C. & BRADY, L.S. (1990). Interaction of endogenous opioid peptides and other drugs with four kappa opioid binding sites in guinea pig brain. *Peptides*, **11,** 311–317.

SCHOFFELMEER, A.N.M., RICE, K.C., JACOBSON, A.E., VAN GELDEREN, J.G., HOGENBOOM, F., HEIJNA, M.H. & MULDER, A.H. (1988). μ-, δ- and κ-opioid receptor-mediated inhibition of neurotransmitter release and adenylate cyclase activity in rat brain slices: studies with fentanyl isothiocyanate. *Eur. J. Pharmacol.*, **154,** 169–178.

SOFUOGLU, M., PORTOGHESE, P.S. & TAKEMORI, A.E. (1991). Differential antagonism of delta opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: evidence for delta opioid receptor subtypes. *J. Pharmacol. Exp. Ther.*, **257,** 676–680.

SPANAGEL, R., HERZ, A. & SHIPPENBERG, T.S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathways. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 2046–2050.

THOMPSON, R.C., MANSOUR, A., AKIL, H. & WATSON, S.J. (1993). Cloning and pharmacological characterization of a rat μ opioid receptor. *Neuron*, **11,** 1–20.

UHL, G.R., CHILDERS, S.R. & PASTERNAK, G.W. (1994). An opiate receptor gene family reunion. *Trends Neurosci.*, **17,** 89–93.

YASUDA, K., RAYNOR, K., KONG, H., BREDER, C.D., TAKEDA, J., REISINE, T. & BELL, G.I. (1993). Cloning and functional comparison of κ and δ opioid receptors from mouse brain. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 6736–6740.

ZUKIN, R.S., EGHBALI, M., OLIVE, D., UNTERWALD, E. & TEMPEL, A. (1988). Characterization and visualization of rat and guinea pig κ_1 and κ_2 opioid receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **85,** 4061–4065.

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